Abstract

Integration of Metabolism and Survival

PP-1

The metabolic switch in liver methionine metabolism

T. K. Korendyaseva, V. A. Volkov, D. N. Kuvatov, M. V. Martinov, V. M. Vitvitsky and F. I. Ataullakhanov
Laboratory of Physical Biochemistry, National Research Center for Hematology, Moscow, Russia. E-mail: ruah@hc.comcor.ru

Methionine (Met) is an essential amino acid and the only substrate for synthesis of S-adenosylmethionine (AdoMet) that is the main substrate for multiple intracellular methylases. There are two modes of Met metabolism in liver. In case of its dietary restriction Met can be metabolized via conservative remethylation cycle. In case of Met excess (high [Met]) it is mostly converted to cysteine via transsulfuration pathway. Mathematical modeling of methionine metabolism in liver (Martinov et al. 2000) predicts that transition from Met conservation to Met consumption happens in narrow [Met] range and is accompanied by sharp 10-fold increase in [AdoMet] and by significant increase in the rate of Met consumption. To test model predictions we analyzed the dependence of [AdoMet] and the rate of Met consumption on [Met] in suspension of freshly isolated mouse hepatocytes. [Met] varied from 40 to 400 μM. In the narrow [Met] range from 80 to 120 μM [AdoMet] sharply increased by eight times, while Met consumption rate increased by six times in [Met] range from 40 to 150 μM. This data confirms the existence of the metabolic switch in liver metabolism triggered by Met concentration.

PP-2

Effects of hyperthermia on mitochondrial respiration and NAD(P)H fluorescence

R. Zukiene[1], P. Cizas[1], S. Maslauskaite[1], R. Baniene[2] and V. Mildaziene[1]
[1]Department of Biology, Vytautas Magnus University, Kaunas, Lithuania, [2]Institute for Biomedical Research, University of Medicine, Kaunas, Lithuania. E-mail: r.zukiene@gmf.vdu.lt

Hyperthermia has high potential as a cancer treatment modality. That implies the need to determine the kinetic response of mitochondria from healthy tissue to moderate heating as well. We have compared the effect of moderate heating on the respiration and NAD(P)H fluorescence in isolated rat heart and liver mitochondria incubated at various Ca^{2+} concentrations. The rise of temperature from 37 to 42 °C caused substantial increase in the inner membrane permeability in both liver and heart mitochondria, but state 3 respiration in heart mitochondria increased by 30% whereas it decreased by 13–23% in liver mitochondria [NAD(P)H fluorescence did not changed in both cases]. The response of liver and heart mitochondria was very different in the range of temperature from 42 to 47 °C. Complete uncoupling of oxidative phosphorylation and the inhibition of the respiration was observed at 47 °C in isolated heart mitochondria. Respiration was completely ceased in liver mitochondria, indicating that their respiratory chain is more susceptible to higher temperature. Increase of temperature to 47 °C was followed by NAD(P)H fluorescence decrease both in heart and liver mitochondria. Change of free Ca^{2+} concentration in incubation medium from 5 nM and 1 μM did not have significant effect on the observed changes in mitochondrial respiration and NAD(P)H fluorescence; however, Ca^{2+} overload (10 μM Ca^{2+}) drastically increased the deleterious temperature effects in both types of mitochondria.

PP-3

The yeast Ccr4–Not complex controls ubiquitination of the nascent-associated polypeptide complex

O. Panasenko, E. Landrieux, M. Feuermann, A. Finka, N. Paquet and M. Collart
Department of Microbiology and Molecular Medicine, University of Geneva, CMU, Geneva, Switzerland.
E-mail: olesyapan@yandex.ru

In this study, we determine that the *Saccharomyces cerevisiae* Ccr4–Not complex controls ubiquitination of the conserved heterodimeric EGD (enhancer of Gal4p DNA binding) complex, which consists of the Egd1p and Egd2p subunits in yeast and is named nascent polypeptide-associated complex (NAC) in mammals. We determine that subunits of the EGD and Ccr4–Not complexes interact, and that both Egd1p and Egd2p are ubiquitinated proteins whose ubiquitination status is regulated by glucose levels. We show that the appropriate ubiquitination of Egd1p requires the Not4p E3 ligase, an intact RING finger domain of Not4p, and the UBA domain of Egd2p. In turn, the appropriate ubiquitination of Egd2p requires Not4p and Egd1p. Our results suggest that the control of EGD ubiquitination depends on Not4p within the Ccr4–Not complex. We also identify the Ubc5p E2 enzyme as a partner for Not4p in EGD ubiquitination. Finally, the functional importance of the control of EGD ubiquitination by Not4p is supported by the UBA-dependent mis-localization of Egd2p in cells lacking Not4p. Our results demonstrate a new function of the Ccr4–Not complex *in vivo*, namely protein ubiquitination, and a target for this function.

PP-4

The level of Ca^{2+} in blood at the experimental crush syndrome and under influence of 'proline-rich peptide'

R. Gevorkian[1], L. Melkonyan[1], H. Hayrapetyan[1], A. Guevorkian[1] and **A. Galoyan**[2]
[1]Laboratory of Pathological Biochemistry, Institute of Biochemistry, Yerevan, Armenia, [2]Department of Neurohormones, Institute of Biochemistry, Yerevan, Armenia.
E-mail: kevork_neurochem@mail.ru

Trauma of skeletal muscle by long-lasting compression, is followed by acute hemodynamic shock, myoglobinuria, acute renal insufficiency, and lethal endotoxicity. There are numerous data indicating that the main intoxication of the organism occurs during decompression period, in which toxic metabolic products are released into the blood and myocardium. Clinical data show that death are most frequently depends on hyperkaliemia, starting from the decompression. Natural cytokine – PRP was obtained from both neurohypophysis and hypothalamic neurosecretory

granules by A. Galoyan. In the experiments 108 Wistar male rats of 160–200 g mass were used. CS was induced by a compression of femoral soft tissues using a special press. Common amount of calcium ions was defined using crezolphtalein spectrophotometer method. The results show the level of Ca^{2+} in blood after 2 h compression and 2, 4, 24 and 48 h decompression and under the influence of PRP. After 2 h compression the level of Ca^{2+} decreases by 21% , and during decompression period the concentration of Ca^{2+} increases in blood by 20%, 21%, 36%, 47%, accordingly after 2, 4, 24 and 48 h decompression. So, the decompression period after 2 h compression is characterized by the increasing level of Ca^{2+} in blood. Under the influence of PRP the level of Ca^{2+} decreases, especially after 24 and 48 h decompression, when the level decreases, accordingly, by 29.8% and 31.5% in comparison with the analogous groups, but without PRP.

PP-5
Drosophila dUTPase: nucleocytoplasmic shuttling and nuclear localization signal

V. Muha[1], Zs. Venkei[2], J. Szabad[2] and **B. G. Beáta**[1]
[1]Genom Metabolism and Repair, Institute of Enzymology, Biological Research Center-Hungarian Academy of Science, Budapest, Hungary, [2]STAOK, Institute of Medical Biology, Szeged, Hungary. E-mail: villoe@enzim.hu

Uracil-free DNA is considered to be essential for most organisms.

Fruit fly larvae present a very exceptional case, as the uracil-preventative dUTPase is restricted only to the imaginal discs, while larval tissues associated with intensive DNA synthesis do not contain it. Moreover, the gene of the major uracil-eliminating UNG is missing, possibly leading to sustained presence of uracil in DNA. Tissues containing uracil-DNA are pre-destinated to death during metamorphosis, whereas imaginal discs survive. Within this context, dUTPase gains importance beyond DNA repair as a metamorphosis regulator factor.

In this study the subcellular localization of the two dUTPase isoforms were investigated.

They were expressed separately as fluorescent protein fused constructs in S2 cells and microinjected into early *Drosophila* embryos.

The 23 kDa isoform, which contains a nuclear localization signal (NLS), is present mainly in the nucleus. On the contrary, the 21 kDa isoform, lacking the NLS segment, remains in the cytoplasm. The 21 kDa shows an unexpected localization shift during nuclear mitosis. In prophase, with nuclear envelope disintegrated, this isoform accumulates in the karyoplasm. As nuclei enter telophase, the 21 kDa isoform gets again excluded from the nuclei. These localization shifts are closely timed to the nuclear cleavage phases. Data suggest that nuclear localization of the dUTPase is under strict regulation involving factors beyond the Ran transport system.

PP-6
ATP decrease is an important cause instauration muscle fatigue

J. Maulén[1], J. Rovira[2], J. A. Cadefau[2], J. M. Irimia[2] and M. R. Cussó[2]
[1]Catholic University of Talca, Talca, Chile, [2]Department of Physiological Sciences (I) of Barcelona University, Barcelona, Spain. E-mail: mcusso@ub.edu

Muscle fatigue has been attributed to many metabolic causes, such as changes in pH, creatine-P, ATP, glycogen, and Pi. We studied the role of these factors during fatigue.

Short-term muscle fatigue and its restoration was analyzed in rabbit muscle. Fast-twitch tibialis anterior was electrostimulated at 10 Hz for 20 s, 1, 5, 15 and 30 min and then allowed to rest for 30 min except for 30 min. Muscles stimulated for 30 min were rested for 3 h.

Muscles were analyzed for ATP, creatine-P, glycogen, phosphorylated glucose and fructose, and lactate. The fatigue index was measured after rest periods.

The fatigue index decreased significantly after 15 and 30 min of electrostimulation and did not recover after 30 min of rest. After 3 h of rest, muscle strength was nearly restored. Although all metabolites were modified during fatigue, only ATP remained significantly low after 3 h of rest, which prevented restoration of muscle strength. The other metabolites were restored quickly.

ATP regulated the sarcolemma ionic channels. The chloride channels (ClC-1) regulate the excitability of skeletal muscle. They are inhibited by high ATP levels which decreases their sensitivity to positive voltage. When ATP decreases, the activity of ClC-1 channels increases, reducing muscle excitability and inducing muscle fatigue. Decrease of ATP protects muscle against sustained contraction suggesting that changes in ATP concentration could be decisive in the control of fatigue.

PP-7
Suppression of expression of muscle-associated proteins by PPARα in brown adipose tissue

T. Nakajima, N. Tanaka and **T. Aoyama**
Department of Metabolic Regulation, Shinshu University, Graduate School of Medicine, Matsumoto, Japan.
E-mail: aoyamato@sch.md.shinshu-u.ac.jp

Peroxisome proliferator-activated receptor alpha (PPARα) belongs to the steroid/nuclear receptor superfamily. Two-dimensional SDS-PAGE analysis of brown adipose tissue (BAT) unexpectedly revealed six spots that were present only in PPARα-null mice. Proteomic analysis indicated that these proteins were tropomyosin-1 α-chain, tropomyosin β-chain, myosin regulatory light chain 2, myosin light chain 3, and parvalbumin-α. Analyses of mRNA have revealed that PPARα suppressed the genes encoding these proteins in a synchronous manner in adult wild-type mice. Histological and physiological analyses of BAT showed in adult wild-type mice, a marked suppression of BAT growth concurrent with a prominent decrease in lipolytic and thermogenesis activities. These results suggest that in adult mice, PPARα functions to suppress the expression of the proteins that may be involved in the architecture of BAT, and thus may function in keeping BAT in a quiescent state.

PP-8
The modulation of carnitine and gamma-butyrobetaine content triggers the cardioprotective effect of mildronate

R. Vilskersts[1], E. Liepinsh[2], D. Zhurina[2], O. Pugovichs[2] and M. Dambrova[2]
[1]Faculty of Medicine, University of Latvia, Riga, Latvia, [2]Department of Medicinal Chemistry, Latvian Institute of Organic Synthesis, Riga, Latvia. E-mail: Reinis.vilskersts@biomed.lu.lv

Mildronate [3-(2,2,2-trimethylhydrazinium)propionate dihydrate] is inhibitor of gamma butyrobetaine hydroxylase, an enzyme which catalyses the synthesis of carnitine from gamma-butyrobetaine (GBB) in liver. It was found that mildronate ameliorates cardiac function during ischaemia by modulating myocardial energy metabolism. In this study we measured the changes in the

contents of carnitine and GBB in rat plasma, heart and brain tissues during the long-term (28 days) treatment by mildronate (i.p. 120 mg/kg/daily). We used a HPLC set-up with pre-column derivatization which allowed us to determine mildronate, carnitine and GBB in a single run. Obtained data show that mildronate caused the time-dependent significant decrease in carnitine concentration and increase of GBB concentration in rat tissues. We detected about fivefold increase of GBB contents in plasma and brain and sevenfold increase in rat heart. We also tested the cardioprotective action of mildronate in the experimental model of heart infarction in isolated rat heart. Obtained results indicate that the cardioprotective effect of mildronate develops in concert with the induced changes in GBB and carnitine concentrations in rat tissues. In conclusion, our study provides the experimental evidence that the administration of mildronate not only decreases the free carnitine concentration, but also brings about a significant increase of GBB concentration in rat tissues, which underlies the cardioprotective action of mildronate.

PP-9
Glucose metabolism in normal and diabetic rat retina

R. Salceda, R. C. Carvajal and V. Coffe
Neuroscience Department, Instituto de Fisiología Celular, UNAM Mexico, D.F. México. E-mail: rsalceda@ifc.unam.mx

Diabetes mellitus is accompanied by a number of pathological abnormalities including retinopathy. Hyperglycaemia is presumably accompanied by metabolic disturbances. In the present work, we studied the influence of different glucose concentrations on lactate levels and CO_2 production in retina from normal and streptozotocin-treated rats.

Incubation of normal retina in a medium containing 5.6 mM glucose caused a rapid increase in lactate production. The NAD/NADH ratio was six times higher in a glucose-free medium that with any glucose concentration tested. Increasing glucose concentrations from 5.6 to 30 mM caused six times increase in glucose accumulation and three times increase in CO_2 production. The contribution of the pentose phosphate pathway was 15% of that produced from mitochondrial oxidation. Not significant differences in glucose accumulation and CO_2 production were observed in diabetic retinas. However, glycogen levels were 2.4-fold higher and high lactate levels have been reported in diabetic retina (Salceda et al. 1998).

Our results indicate an active oxidative metabolism in retina. The low NAD/NADH ratios found at any glucose concentration tested suggested that the aerobic pathway should be rapidly saturated. We proposed that gluconeogenesis could be a mechanism for lactate removal during periods of high metabolic activity and under pathological conditions.

PP-10
Phosphoinositides are involved in phagosome formation and maturation in the ciliate tetrahymena

D. Deli[1], G. Leondaritis[2], A. Tiedtke[3] and **D. Galanopoulou**[1]
[1]Laboratory of Biochemistry, Department of Chemistry, University of Athens, Zografou, Athens, Greece, [2]Laboratory of Developmental Neurobiology and Neurochemistry, Institute for Biomedical Research of the Academy of Athens, Athens, Greece, [3]Institute for General Zoology and Genetics, University of Münster, Münster, Germany.

Phagocytosis is a conserved process utilized by various types of cells for particle or pathogen endocytosis. In mammalian cells and *Dictyostelium*, phagocytosis is initiated by the interaction of particles with specific membrane receptors. In the ciliate Tetrahymena, it occurs in the cytostome, where phagosomes are formed by intracellular vesicle fusion and not by membrane invagination. In this study, we aimed at elucidating the possible regulation of Tetrahymena phagocytosis by phosphoinositides (PI). Wortmannin, a potent inhibitor of D-3 PI synthesis in Tetrahymena, caused an arrest both in the maturation and defecation of iron-dextran and fluorescent *Escherichia coli* cells-containing phagosomes. Treatment of cells with U73122, which inhibits PI-PLC in Tetrahymena, caused an increase in PtdInsP2 levels and a delay in phagosome formation. An independent analysis of PtdInsP2 during phagocytosis revealed a fluctuation in PtdInsP2, with maximal levels during the initial phase of the process. In addition, study of a mutant Tetrahymena strain, blocked in the biogenesis of phagosomes, showed an increased content in PtdInsP2, although PI-PLC activity was twofold higher compared to the wild-type cells. These results suggest that both D-3 and D-4 PI are involved in distinct steps of phagocytosis in Tetrahymena. Ongoing studies with purified phagosomes of different maturation stages and *in vivo* visualization of PI redistribution during phagocytosis will clarify their exact targets.

PP-11
Contribution of cGMP signaling pathway(s) in regulation of Leydig cell steroidogenesis

T. S. Tatjana and **S. A. Silvana**
Faculty of Science, University of Novi Sad, Novi Sad, Serbia and Montenegro. E-mail: tanjak@ib.ns.ac.yu

cGMP is formatted in Leydig cells but the role of this second messenger in androgen (T + DHT) production have been incompletely characterized. Here, we show presence of transcripts for the all elements of cGMP signaling pathways, i.e. membrane-bound guanylyl cyclase, NO synthethase (NOS), soluble guanylyl cyclase, GMP-specific phosphodiesterase 5 (PDE 5), protein kinase G (PKG I), multidrug resistance protein 5 (MRP5) as well as cyclic nucleotide-gated channels (CNG; rode, olfactory and cone). We also characterized effect of activation and inhibition of different elements of cGMP signaling pathway(s) on androgen production in static culture of purified adult rat Leydig cells under basal conditions and in response to stimulation with hCG and different steroidogenic substrates. In all treatments which rise cGMP production stimulation of androgen production was occurred and this phenomenon was more prominent in basal than in receptor-controlled androgen production. Moreover, androgen production was decreased in the presence of specific PKG inhibitor, indicate that PKG-dependent phosphorylation take place in regulation of Leydig cell steroidogenesis. Immunoprecipitation study showed PKG-dependent phosphorylation of steroidogenic acute regulatory protein (StAR), suggesting that both cAMP and cGMP have important and specific roles in control of androgen-producing cell functions and thus their crosstalk could be of the importance for synchronization of cellular functions.

PP-12
Molecular physiology of Leydig cells stress response: genes related to steroidogenesis and no-cGMP signaling pathway

S. A. Andric and **T. S. Kostic**
Faculty of Science, University of Novi Sad, Novi Sad, Serbia and Montenegro. E-mail: silvana@ib.ns.ac.yu

The ability of stress to interfere with Leydig cells capacity and activity of steroidogenic enzymes has been published earlier. The

specific goal of this study is to investigate the impact of NO-cGMP-related signaling pathways on molecular physiology of Leydig cells of rats exposed to stress. Here, we analyze the effect of acute (2 h) and chronic (10 days, 2 h each day) immobilization stress on the transcription of genes related to steroidogenesis (steroidogenic acute regulatory protein-StAR, CYP11A1, 3βHSD, CYP17, 17βHSD) and NO-cGMP signaling pathways in adult rat Leydig cells. Transcription analysis showed that immobilization did not change level of mRNA for StAR, CYP11A1, 3βHSD, and CYP17, but there was evidence about decreased level of 17βHSD transcript. At the same time, it is clear that immobilization bidirectionally (gradual stimulation followed by inhibition) affected transcription for inducible NO synthase (iNOS), while transcription of neural NOS (nNOS) and endothelial NOS (eNOS) was not changed. Moreover, level of transcripts for phosphodiesterase 5 (PDE 5) and multidrug resistance protein 5 (MRP 5), is gradually decreased during stress, while there were no changes in the level of mRNA for other elements of NO-cGMP signaling pathway(s). Results of this study, together with those published, suggest that NO-cGMP signaling pathway(s) are involved in stress-impaired testicular steroidogenesis.

PP-13
Estrogenic effects of natural and synthetic compounds assessed in *Saccharomyces cerevisiae*

G. Hasenbrink[1], L. Wildt[2], J. Ludwig[1] and **H. Lichtenberg-Frate**[1]
[1]IZMB, Molecular Bioenergetics, University of Bonn, Kirschallee 1, Bonn, Germany, [2]Klinik für gynäkologische Endokrinologie und Sterilität, Universität Innsbruck, Innsbruck, Austria.
E-mail: h.lichtenberg@uni-bonn.de

The human estrogen receptors α and ß, differentially localized and expressed in various tissues and cell types mediate transcriptional activation of target genes. These encode a variety of physiologic reproductive and non-reproductive functions involved in energy metabolism, salt balance, immune system, development, and differentiation. Toward developing a screening assay for the use in applications where significant numbers of compounds need to be tested for (anti)estrogenic bioactivity hERα and hERß were expressed in a *Saccharomyces cerevisiae* strain devoid of three endogenous xenobiotic transporters (PDR5, SNQ2, YOR1). By utilizing receptor-mediated transactivation of the GFP as reporter 17 natural, comprising estrogens and phytoestrogens or synthetic compounds, gestagens, and antiestrogens were investigated. The assay deployed a simple and robust protocol for the rapid detection of estrogenic effects within a 96-well microplate format. Results were expressed as effective concentrations (EC_{50}) and correlated with other yeast-based and cell line assays. Tibolone and its metabolites exerted clear estrogenic effects, though considerably less potent than all other natural and synthetic compounds. For the blood serum of two volunteer's considerable higher total estrogenic bioactivity than single estradiol concentrations as determined by immunoassay were found. Visualization of a hERα/GFP fusion protein in yeast revealed a subcellular cytosolic localization.

Integration of Defence and Survival

PP-14
YAP4P phosphorylation during yeast stress response

J. Pereira, T. Nevitt and C. Rodrigues-Pousada
ITQB, UNL, Oeiras, Portugal. E-mail: jpereira@itqb.unl.pt

YAP4 belongs to the YAP family of eight bZIP transcription factors. *YAP4* has been described as a gene that confers resistance to cisplatin and several antimalarial drugs. Recently, we were able to associate *YAP4* with the yeast stress response, showing that its mRNA levels increase under osmotic and oxidative stress and that Yap4 is induced and phosphorylated under these conditions. By direct mutagenesis we show that Yap4 phosphorylation is not involved in protein subcellular localization as the non-phosphorylated mutants T192A- and S196A-Yap4 still give rise to a nuclear resident protein. By blocking Yap4 transit to the nucleus through mutation of its nuclear localization signal, we observed that Yap4 phosphorylation is abolished. These results suggest that Yap4 phosphorylation occurs in the nucleus and is most probably related to its activation and/or stability. To address this, Yap4 protein kinetics was analysed in the double mutant T192A-S196A-Yap4. We observe that the mutant protein is expressed but not phosphorylated during the time course applied, suggesting that phosphorylation of T192 and S196 residues of Yap4 is not related to its stability under hyperosmotic stress conditions. Band-shift analyses is being used to study the role of Yap4 phosphorylation in its *cis*-element binding as well as determine whether Yap4 can heterodimerize with Yap6, its closest family member, *in vivo*.

PP-15
Investigation of apoptotic gene expression levels in multidrug-resistant MCF-7 cell lines

Ö. **Darcansoy İşeri**, M. Demirel Kars and U. Gündüz
Department of Biological Sciences, Middle East Technical University, Ankara, Turkey. E-mail: dozlem@metu.edu.tr

Bcl-2 gene family is involved in cell survival/death control and function in regulating the apoptotic pathway mostly through protein–protein interactions between various homologous members of the family. Bcl-2 is a proto-oncogene that encodes transforming protein Bcl-2 which inhibits apoptosis. Bax, is a proapoptotic gene which forms heterodimers with Bcl-2 and the balance between two components determines the activity of the apoptotic system. Resistance to broad spectrum of chemotherapeutic agents during cancer chemotherapy is named as multidrug resistance (MDR) and it is a major impediment to the successful treatment of different cancer types by chemotherapy. Altered expression of genes for survival/death is one of the mechanisms of multidrug resistance.

In this study investigation of Bcl-2/Bax expression levels in paclitaxel, docetaxel, doxorubicin and vincristine-resistant MCF-7 breast carcinoma cell lines is aimed to understand mechanism of

resistance in these cells. Resistant sublines were developed by continuous drug application in dose increments. According to cytotoxicity analysis, developed cell lines were found to be resistant to anticancer drugs used. Bcl-2 and Bax gene expression analysis was performed by RT-PCR and, related protein levels were determined by Western blot and immunostaining analysis. The results suggest that differential expression levels of Bcl-2 and Bax genes may be one of the mechanisms of acquired resistance in MCF-7 cells.

PP-16
Differential expression of isoforms of spleen tyrosine kinase in tissues: effects of the microbial flora

F. Duta[1], M. Ulanova[1], D. Seidel[2], L. Puttagunta[3], S. Musat-Marcu[4], K. S. Harrod[5], A. D. Schreiber[6], U. Steinhoff[2] and A. D. Befus[1]

[1]Department of Medicine, University of Alberta, Edmonton, AB, Canada, [2]Department of Immunology, Max Planck Institute of Infection Biology, Berlin, Germany, [3]Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada, [4]HistoBest Inc., Edmonton, AB, Canada, [5]Department of Infectious Disease, Lovelace Respiratory Research Institute, Albuquerque, NM, USA, [6]University of Pennsylvania School of Medicine, Philadelphia, PA, USA. E-mail: florentina.duta@ualberta.ca

Syk is a non-receptor tyrosine kinase expressed in various hematopoietic cells and also in non-hematopoietic cells such as lung and breast epithelial cells, fibroblasts, and endothelial cells. The role of Syk in leukocyte activation through receptors such as those for IgE and IgG is well known, but in non-hematopoietic cells it appears to influence cell proliferation, tumor growth, and cell interaction.

Given the widespread distribution of Syk and its role in host defenses, we postulated that its expression is influenced by microbial exposure. Accordingly, we investigated Syk expression in tissues of germ-free and conventional mice by immunohistochemistry, Western blot and real-time RT-PCR. Interestingly, Syk is present in both germ-free and conventional mice and the microbial flora has no major influence on overall expression of Syk.

We also investigated the distribution of Syk isoforms, long Syk (L) and short spliced variant Syk (S), in tissues of germ-free and conventional mice. They were widely expressed in mouse tissues, although previously it was thought that Syk (S) was restricted to bone marrow. Interestingly, Syk (S) protein was significantly elevated in lung and spleen in germ-free mice.

Thus, Syk is widely distributed in various cells and tissues and is likely involved in several pathways of development, and normal and abnormal physiology.

Acknowledgment: Funded by CSACI/CAAIF/Merck Frosst, Alberta Heritage Foundation for Medical Research and NIH.

PP-17
Expression of the human *HSPA2* gene in cancer cell lines

W. Piglowski, A. Kwiecien, A. Mazurek, M. Jarzab, Z. Krawczyk and **D. Scieglinska**

Department of Tumor Biology, Maria Skodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland. E-mail: dorotas@io.gliwice.pl

Heat-shock proteins are a group of highly conserved chaperone proteins. The human Hsp70 family consists of at least eight members that differ from each other by expression pattern. Many types of cancer cells constitutively express elevated level of Hsp70i protein which in normal cells is induced only by stress conditions. The Hsp70i protein influences the phenotype of tumor cells rendering them more resistant to agents inducing cell death. Another member of Hsp70 family is the HSPA2 protein, which is a crucial chaperone abundantly expressed during spermatogenesis.

Here, we present the analysis of the *HSPA2* gene expression in various human cancer cell lines. The structure of the *HSPA2* mRNA synthesized in cancer cell lines was determined by RT-PCR. The level of the *HSPA2* transcript assayed by Q-PCR significantly differed between the studied cell lines. Western blot analysis revealed that in some cell lines amount of the HSPA2 protein does not correspond to mRNA content. Our results suggest that the HSPA2 expression is regulated at both, transcriptional and post-transcriptional level in cell-specific manner. Using specific anti-HSPA2 antibody we searched for intracellular localization of the HSPA2 protein in cancer cells at normal and stressful conditions. We found that during heat shock the HSPA2 protein shifts from cytoplasm to nucleus and nucleoli. It appears that cancer cells contain additional chaperone protein which function hitherto was not described.

PP-18
Small heat shock proteins interact with membranes and affect membrane physical state and function

I. Horvath[1], Z. Balogi[1], K. Giese[2], O. Chergy[1], A. Glatz[1], I. Vass[1], P. Goloubinoff[3], E. Vierling[2] and L. Vigh[1]

[1]Biol. Res. Center, Szeged, Hungary, [2]Univ. Arizona, Tucson, AZ, USA, [3]Univ. Lausanne, Lausanne, Switzerland. E-mail: hibi@brc.hu

The cellular pool of small heat shock proteins (sHsps) is divided into a cytoplasmic subfraction responsible for regular chaperone activity and a membraneous subfraction, involved in membrane stabilization. We have isolated a series of Synechocystis Hsp17 mutants characterized with regard to *in vivo* thermotolerance, *in vitro* chaperone activity and propensity to form oligomers. We defined particular features of these mutants responsible for interacting with membrane lipids, a potential determinant of their membrane association. While causing destabilization of the oligomeric state, three mutations of Hsp17 caused no significant alterations in the lipid and/or thylakoid-binding characteristics compared to wild-type Hsp17. However, with a mutation at the N-terminus (Q16R), a dramatic change in the association of Q16R to thylakoids and liposomes was observed. Parallel with elevated insertion affinity of Q16R (versus wild-type Hsp17) into lipid monolayers, a strikingly increased protection against UV-B stress *in vivo* was detected.

Specific lipid binding is also a feature of the *Escherichia coli* sHsps, IbpA and IbpB. The IbpA/B membrane lipid interaction depends on the head group composition and the extent of lipid unsaturation. IbpA/B strongly regulated membrane fluidity and permeability. A comparative study conduced with wild type, ibpAB-disrupted and replacement strains provided the first evidence for the active involvement of sHsps in the homeostatic control of membrane physical state.

PP-19
Yap0 super-mutant – a tool to study the functional role of the Yap family members

L. Nascimento, R. Menezes, T. Nevitt, C. Amaral and **C. Rodrigues-Pousada**

Genomics and Stress, Instituto de Tecnologia Química e Biológica, Oeiras, Portugal. E-mail: lilianan@itqb.unl.pt

Yeast are continuously exposed to rapid and drastic changes in their external milieu. They possess a very flexible and complex

programme of gene expression when exposed to a plethora of environmental insults. *Saccharomyces cerevisiae* contains a family of eight bZip proteins, designated by Yap, which modulate the transcriptional activation of specific genes involved in the response to several stress conditions such as oxidative, osmotic, arsenic and heat stress, among others. The existing data concerning the function of Yap proteins support both a degree of functional overlap as well as distinct physiological roles. Furthermore, data are beginning to emerge on the crosstalk between the members of this family. Recent data obtained by us strongly indicate that Yap8 and Yap1 are able to interact in response to arsenic stress. This is the first evidence of the formation of heterodimers between bZIP transcription factors in yeast. The generation of a strain deleted in all YAP genes is an invaluable tool in order to study the function of each member of the Yap family individually. Thus, the main challenge of the present study was the construction of the 'YAP0 SUPER-MUTANT' deleted in all YAP genes. The strategy used was a combination of PCR-based gene disruption using the Cre/loxP system, tetrad and phenotypic analysis. Experiments are being carried out in order to understand the complex role of these transcription factors.

Rhythmic Signals: the Setting of Biological Time

PP-20
The effect of plant hormones (GA3, IBA and ABA) on ARF1 and SAR1 expression in *Pisum sativum* var. *araka*

O. Ertekin and A. R. Memon
TÜBİTAK – Research Institute for Genetic Engineering and Biotechnology, Kocaeli, Turkey.
E-mail: ozlem.ertekin@gmbae.tubitak.gov.tr

Plant hormones play a very important role in plant development and growth. Small GTP-binding proteins, ARF1 and SAR1, which shuttle between GDP-bound soluble and GTP-bound membrane-attached forms, play a regulatory role in vesicular trafficking. In this study we investigated the effect of plant hormones on the expression of ARF1 and SAR1 in different plant parts of *Pisum sativum*. We observed a decrease in the expression level of SAR1 protein in the radicle and plumule fractions of 12 and 18 days dark grown plants compared to 4 and 6 days old plants. Whereas there was no significant change in ARF1 expression level. In order to see the influence of plant hormones on the level of ARF1 expression, plants were supplied with the hormones Giberellin (GA3), Auxin [Indole Butyric Acid (IBA)] and Abscisic Acid (ABA). A significant increase in ARF1 expression in the radicle and plumule fractions was observed when plants were supplied with the IBA and ABA, compared to that of the control and GA3-treated plants. In this study, we demonstrate that SAR1 protein may play an important role in secretory pathway at early stages of plant development and plant hormones could influence ARF and SAR regulation in the cell.

NF-κB Pathway in Normal Physiology and Disease

PP-21
Post-translational modifications change the direction of Ras-dependent downstream pathways

N. Narmania, T. Barbakadze, E. Zhuravliova and D. G. Mikeladze
Laboratory of Neurochemistry, Institute of Physiology, Tbilisi, GA, USA. E-mail: nnarmania@mail.ru

The Ras family of small GTP-binding proteins has been implicated as a molecular switch that directs diverse cellular responses, such as cell cycle progression, transformation, and cell death. Ras is regulated by a series of post-translational modifications, including farnesylation, palmitation, and nitrosylation, but the role of these modifications on the regulation of downstream effectors is not known. We investigated the effects of manumycin, an inhibitor of farnesyltransferase and L-NAME, an inhibitor of nitric oxide synthase on the activity of various transcription factors in mixed primary neuronal/glial cells. We have found that both manumycin and L-NAME inhibit the DNA-binding activity of NF-κB (50 kDa subunit). L-NAME also decreases the activity of STAT and manumycin restore this inhibitory effect of L-NAME. Both inhibitors raise the activity of c-Fos and only manumycin elevate the DNA-binding activity of Sp1. Furthermore, manumycin, as well as L-NAME decrease the activity of c-Jun, while in the presence of both inhibitors the DNA-binding potency of this transcription factors does not change. It is concluded that simultaneously (nitrosylated and farnesylated) modified Ras alter the systems regulating the upstream pathway of c-Jun and does not change the activity of the systems, controlling STAT, Sp1, NF-κB, CREB-1, ATF-2, and c-Fos.

Acknowledgment: This study was supported by INTAS 2001-0666 grants.

PP-22
Treatment with substance P and caerulein induces chemokine synthesis in pancreatic acinar cells

R. Ramnath and M. Bhatia
Department of Pharmacology, National University of Singapore, Yong Loo Lin School of Medicine, Singapore.
E-mail: phcrdr@nus.edu.sg

Chemokines play a key role in the pathogenesis of acute pancreatitis. We have earlier shown that pancreatic acinar cells produce the CC chemokine MCP-1 in response to caerulein hyperstimulation. In mice with pancreatitis, levels of substance P (SP) and expression of NK-1 receptors in pancreatic acinar cells are increased. In the present study, we investigated the effect of caerulein and SP on pancreatic acinar cells. We found that CC chemokine MCP-1, MIP-1alpha and CXC chemokine MIP-2 were produced when acinar cells were stimulated with caerulein. Furthermore, pancreatic acinar cells produced MCP-1, MIP-1alpha and MIP-2 when treated with SP alone. Moreover, acinar cells treated with both caerulein and SP caused a significant increase in the chemokine levels compared to caerulein and SP treatment alone. Also, acinar cells stimulated with combined

treatment of caerulein and SP caused a significant increase in NFkappaB compared to the treatment with caerulein or SP alone. These results suggest that both SP and caerulein are acting through NFkappaB pathway to induce chemokine synthesis. To further confirm this, acinar cells were treated with NEMO-binding domain (NBD), a selective inhibitor of NFkappaB activation. Treatment with NBD significantly attenuated the stimulation in chemokine synthesis caused by treatment with both caerulein and substance P. This study shows that caerulein and substance P induce chemokine synthesis through NFkappaB pathway.

PP-23
ERK and JNK activation differentially regulates phosphatidic acid-induced matrix metalloproteinase-9 expression

S. H. Baek, J. G. Lee and C. H. Lee
Department of Biochemistry & Molecular Biology, College of Medicine, Yeungnam University, Daegu, South Korea.
E-mail: sbaek@med.yu.ac.kr

Phosphatidic acid (PA) is implicated in pathophysiological processes associated with cellular signaling events and inflammation, which include regulating the expression of numerous genes. The present study examined whether the temporal control of ERK and JNK could differentially regulate the expression of NF-κB-dependent gene, matrix metalloproteinase-9 (MMP-9). PA induced the expression of MMP-9 in a dose-dependent manner, but mRNA showed a biphasic increase by PA treatment. PA induced phosphorylation of ERK1/2 and JNKs. Inhibition of ERK1/2 with U0126 suppressed PA-induced MMP-9 expression, whereas inhibition of JNKs with SP600125 enhanced cell migration, with strong increase of MMP-9 expression. PA activated NF-κB pathway as measured by increased IκBα degradation, promoter activity, and NF-κB-DNA binding. The expression of MMP-9 and the cell migration was inhibited when NF-κB activation was downregulated by SN-50, NF-κB inhibitor. In addition, tumor necrosis factor-α antibody strongly suppressed PA-induced MMP-9 expression, suggesting the involvement of tumor necrosis factor-α pathway. Overall, these observations demonstrate that activation of ERK1/2 and JNKs play a different role in the activation of NF-κB and the subsequent regulation of MMP-9.

PP-24
The serum interleukin 6 and C-reactive protein levels in the patients after trauma

C. Karakaya[1], T. Noyan[1], N. Sayilir[1] and S. Ekin[2]
[1]Department of Biochemistry, School of Medicine, Yuzuncu Yil University, [2]Faculty of Arts and Sciences, Chemistry Department, Biochemistry Division, Yuzuncu Yil University.
E-mail: karakayac2003@yahoo.com

Aim: To observe the changes that will occur in the serum cytokine and acute phase response developing based on bone fracture trauma.
Materials and methods: 21 patients diagnosed with femur and tibia bone fracture has been measured serum IL-6 and CRP levels during the 6, 24 and 48 h.
Results: After trauma IL-6 serum level was measured at the highest rank at the 24th hour and found out that the rank at the 48th hour decreased less than at the 6th hour. Statistically the level of IL-6 at the 24th hour occurred a meaningful increase than at the 6th hour ($P < 0.01$), and a decrease at the 48th hour ($P < 0.01$). On the other hand, serum CRP level reached to the highest level after trauma at the 48th hour.
Conclusion: Statistically the 24 and 48th hour CRP serum level showed a meaningful increase compared to the 6th hour ($P < 0.01$). These results make the measured IL-6 level after trauma at the 24th hour helpful to estimating the tissue defeats occurring based on trauma.

PP-25
Cytokine levels in the seminal plasma of fertile and infertile men

N. Sayilir[1], M. Tarakcioglu[2] and C. Karakaya[1]
[1]Department of Biochemistry, School of Medicine, Yuzuncu Yil University, [2]Department of Biochemistry and Clinical Biochemistry, School of Medicine, Gaziantep University.
E-mail: karakayac2003@yahoo.com

Aim: Cytokines are peptides used for the controlling of intracellular activities and the in the cellular communication. They are released from various specialized cells of urogenital systems of men. These molecules are considered to have some effects on sperm functions and fertility. In this study, examining the levels of IL-6 and TNF-α in the seminal plasma of men who were infertile due to various reasons, and correlations between various sperm parameters and urogenital infections have become the chief focus of our concern.
Methods and materials: A total of 29 infertile men constituting three groups were studied for our clinical trials: the group with infections ($n = 10$), the group with varicocele ($n = 12$) and oligozoospermi group ($n = 7$); a control group with offspring was also included to our clinical studies ($n = 11$).Within the course of our study we have determined routine sperm parameters, the levels of seminal plasma IL-6 and TNF-α as serum FSH, LH, PRL and total testosteron levels. The levels of IL-6 and TNF-α in the seminal plasma and plasma hormones were measured with chemilluminescence method.
Results: Compared to the other infertile and control group, the infected infertile group was found to have higher IL-6 and TNF-α levels ($P < 0.05$). Statistically, no correlation has been found between plasma hormone and cytokine levels; the case was also true between IL-6, TNF-α and sperm parameters.
Conclusion: Consequently our findings have provided ample evidence in that IL-6 and TNF-α levels in the seminal plasma are higher only in the infected group among the infertile groups in a statistically significant way and there is no correlation between these parameters and FSH, LH, PRL as well the total testosterone levels; so these parameters cannot be used as a distinctive marker in the diagnosis of infertility, but could be used in distinctive diagnosis of urogenital infections in men.

PP-26
The inhibition of NF-κB activation is protective in the LPS-induced brain inflammation model

E. Liepinsh[1], L. Zvejniece[2], R. Vilskersts[2], R. Muceniece[2] and M. Dambrova[1]
[1]Medicinal Chemistry, Latvian Institute of Organic Synthesis, Riga, Latvia, [2]Faculty of Medicine, Latvian University, Riga, Latvia. E-mail: ledgars@latnet.lv

In the recent years the nuclear factor kappa B (NF-κB) has attracted considerable interest due to its key role in responses to injury and inflammation, and regulation of a multitude of genes of which several are shown to become activated during the inflammation. We have shown earlier that the guanidine compound ME10092 [1-(3,4-dimethoxy-2-chlorobenzylideneamino)-guanidine] possesses a strong cardioprotective effect in an experimental heart infarction model in the rat. We have also found

that the compound possesses a certain antioxidative profile, as well as inhibition of activation of NF-κB in the rat cardiomyocytes in simulated ischemia and reperfusion *in vitro*. In the present study, we tested the activity of ME10092 in the lipopolysacharide (LPS)-induced brain inflammation model in mice *in vivo*. By electron paramagnetic resonance (EPR) we showed that ME10092 in a dose-dependent manner (1–100 pmol/mouse) inhibited the LPS-induced increase in nitric oxide (NO) contents in mice brain tissues. The immunohistochemical analysis of brain tissue slices indicated that ME10092 treatment also suppressed the expression of inducible nitric oxide synthase (iNOS) *in vivo*. In cell nuclear extracts, we found that ME10092 inhibited the LPS-induced nuclear translocation of the NF-κB. We conclude that the inhibition of NF-κB activation by ME10092 mediates the suppression of the brain inflammation in the LPS-induced experimental brain inflammation model *in vivo*.

PP-27
Transglutaminase 2 inhibition promotes sensitivity to the chemotherapy in cancer cells via NF-κB inhibition

D.-S. Kim, J.-M. Kim, K.-S. Park, S.-S. Park and S.-Y. Kim
Molecular Oncology Branch, National Cancer Center, Goyang, Korea. E-mail: kimsooyoul@gmail.com

Although TGase 2 expression is often observed in the apoptotic process, there is lack of evidence that TGase 2 itself is responsible for triggering the apoptosis. However, overexpression of TGase 2 is able to make cells sensitive to the apoptotic stimuli as a sensitizer. Recently, an evidence of TGase 2 expression associated with antiapoptosis has been reported in drug-resistant and metastatic breast cancer cells that present upregulated TGase 2 expression. Furthermore, TGase 2 inhibition in chemo-resistant breast cancer cells promotes sensitivity of chemotherapy. TGase 2 inhibition together with chemotherapeutic agent showed that efficiently increase of cell death. However, antiapoptotic mechanisms of TGase 2 remain to be elucidated. Recently, we have found that TGase 2 is able to activate a survival factor NF-κB in several cell types independently to the I-κB kinase signaling. TGase 2 induces the polymerization of I-κB rather than stimulating I-κB kinase. This polymerization of I-κB results in direct activation of NF-κB in breast cancer cell lines. Consequently chemotherapeutic resistance appears to be acquired in cancer cells due to TGase 2-mediated NF-κB activation. We also found that TGase inhibition reverses NF-κB activation concomitantly with drug resistance in breast cancer cells. Taken together, developing TGase 2 inhibitors will benefit on cancer therapy as chemotherapeutic sensitizers.

PP-28
Tracking NF-κB activation upon genotoxic stress: a non-classical mechanism

S. Çöl Arslan and C. Scheidereit
Max Delbrück Center for Molecular Medicine. E-mail: arslan@mdc-berlin.de

The NF-κB family of transcription factors play multiple roles in immune system, development and regulation of apoptosis. In the basal state, NF-κB dimers are bound to the inhibitor IκB molecules and kept in the cytoplasm. Upon receptor stimulation, the kinase complex consisting of IKKα, IKKβ and IKKγ/NEMO gets activated. The activated complex phosphorylates IκB and leads to its proteosomal degradation. The released NF-κB dimers then translocate to the nucleus and regulate transcription.
In addition to well-described molecules like LPS, TNFα or IL-1, genotoxic stress also activates NF-κB. The mechanism of this activation has been proposed as sequential sumoylation, ATM phosphorylation and ubiquitination of NEMO, which then induces NF-κB activation. This mechanism is of great interest, for unlike other stimuli mentioned above, it uses a nucleus-to-cytoplasm-to-nucleus signaling. In our study, we further investigated this process to find the key molecules required for sequential modification of NEMO and if ubiquitinated NEMO is actually sufficient for IKK activation without further input. How these modifications affect the association of NEMO with the IKK complex is also being investigated.
Understanding the exact nature of NEMO modifications upon genotoxic stress will help us to solve the complex puzzle of how the IKK complex is regulated in various conditions.

PP-29
Flagellin is a potent inducer of nuclear factor-κB-dependent proinflammatory signaling in cardiomyocytes

J. Rolli[1], S. Levrand[2], B. Waeber[2], F. Feihl[2] and L. Liaudet[1]
[1]Service of Adult Critical Care Medicine, University Hospital, Lausanne, Switzerland, [2]Division of Clinical Pathophysiology, University Hospital, Lausanne, Switzerland. E-mail: joelle.rolli@chuv.ch

Introduction: Flagellin (FLAG), a 55 kDa monomer obtained from the flagella of gram-negative bacteria, induces inflammatory responses *in vitro*, mediated by Toll-like receptor 5 (TLR5). Gram-negative sepsis is associated with myocardial failure, which is related to myocardial cytotoxicity and inflammation triggered by putative circulating mediators. Whether FLAG may exert such a cytotoxic role during gram-negative sepsis has not been evaluated. Thus, the aim of the present study was to explore a potential role of FLAG as an inducer of cardiomyocyte inflammation *in vitro* and *in vivo*.
Methods: *In vitro*, H9C2 rat cardiomyocytes were stimulated with recombinant *Salmonella* FLAG (1–100 ng/ml, 10–24 h). *In vivo*, BALB/c mice were injected (tail vein) with 1–5 μg FLAG. Proinflammatory effects of FLAG were evaluated by its ability to activate NFκB (monitored by degradation and phosphorylation of IκB, nuclear p65 translocation, NFκB DNA binding and NFκB-luciferase gene reporter), and to induce transcription and/or expression of the inflammatory cytokines TNFα and MIP-2.
Results: FLAG-activated NFκB in a concentration-dependent manner in cardiomyocytes both *in vitro* and *in vivo*, and also upregulated the transcription and expression of TNFα and MIP-2.
Conclusion: Flagellin is a potent mediator of proinflammatory signaling in cardiomyocytes and may represent a previously unrecognized mediator of myocardial failure during gram-negative sepsis.

PP-30
Regulation of antiviral response at the level of TBK1-NAP1 interaction

G. Ryzhakov and **F. Randow**
MRC Laboratory of Molecular Biology, University of Cambridge, Cambridge, UK. E-mail: gr@mrc-lmb.cam.ac.uk

TANK-binding kinase 1 (TBK1) is essential mediator of antiviral immunity. TBK1-deficient cells are unable to produce interferons and other IRF3-dependent cytokines in response to virus infection or TLR agonists. On the other hand, TBK1-mediated activation of IRFs and NF-κB may lead to the overinflammation problems such as lupus erythematosus. They are two known adaptors of this kinase: NAP1 and TANK. NAP1 is essential for TBK1-dependent NF-κB and IRF3 activation, though its precise function is unknown. Thus, it is interesting to know how the

protein binds and activates TBK1. We used a recently developed approach called LUMIER to study the architecture of the TBK1-containing complex. First, we confirmed that NAP1 specifically interacts with TBK1 but not with related kinases – IKK-alpha and IKKbeta. Then, using deletion mutagenesis we narrowed down the regions within TBK1 and NAP1 that interact with each other. Ectopically expressed TBK1-binding domain of NAP1 selectively inhibits IRF3 but not NF-kB activation induced by various stimuli. Thus, targeting this spot in the pathway may have an important therapeutic application.

Signalling and Cancer: Nuclear Receptor Connection

PP-31
DNA topo I is a cofactor for c-jun in the regulation of EGFR expression and cancer proliferation

A. Mialon[1,2], M. Sankinen[1], H. Söderström[1], T. T. Junttila[2,3,4], T. Holmström[1], R. Koivusalo[4], A. C. Papageorgiou[1], R. S. Johnson[5], S. Hietanen[4,6], K. Elenius[2,4] and J. Westermarck[1]

[1]Centre for Biotechnology, University of Turku and Åbo Akademi University, Turku, Finland, [2]Department of Medical Biochemistry and Molecular Biology, University of Turku, Turku, Finland, [3]Turku Graduate School of Biomedical Sciences, University of Turku, Turku, Finland, [4]Medicity Research Laboratory, University of Turku, Turku, Finland, [5]Molecular Biology Section, Division of Biological Sciences, School of Medicine, University of California, San Diego, La Jolla, California, U.S.A, [6]Department of Obstetrics and Gynaecology, Turku University Hospital, Turku, Finland.
E-mail: amialon@btk.fi

DNA topoisomerase I (Topo I) is a molecular target for the anticancer agent topotecan in the treatment of small cell lung cancer and ovarian carcinomas. However, the molecular mechanisms by which topotecan treatment inhibits cancer cell proliferation are unclear. We describe here the identification of Topo I as a novel endogenous interaction partner for transcription factor c-Jun. Reciprocal coimmunoprecipitation analysis showed that Topo I and c-Jun interact in transformed human cells in a manner that is dependent on JNK activity. c-Jun target gene epidermal growth factor receptor (EGFR) was identified as a novel gene whose expression was specifically inhibited by topotecan. Moreover, Topo I overexpression supported c-Jun-mediated reporter gene activation and both genetic and chemical inhibition of c-Jun converted cells resistant to topotecan-elicited EGFR downregulation. Topotecan-elicited suppression of proliferation was rescued by exogenously expressed EGFR. Furthermore, we demonstrate the cooperation of the JNK-c-Jun pathway, Topo I, and EGFR in the positive regulation of HT-1080 cell proliferation. Together, these results have identified transcriptional coactivator Topo I as a first endogenous cofactor for c-Jun in the regulation of cell proliferation. In addition, the results of the present study strongly suggest that inhibition of EGFR expression is a novel mechanism by which topotecan inhibits cell proliferation in cancer therapy.

PP-32
Structural investigations of insect ecdysteroid nuclear receptor with natural DNA response element

M. Jakób[1], R. Koodziejczyk[1], M. Orowski[1], S. Krzywda[2], A. Kowalska[1], M. Jaskólski[2] and A. Ożyhar[1]

[1]Biochemistry Department, Wrocaw University of Technology, Wrocaw, Poland, [2]Department of Crystallography, Adam Mickiewicz University, Poznań, Poland.
E-mail: michal.jakob@pwr.wroc.pl

Ecdysteroid receptor acts as a dimeric ligand-inducible transcription factor composed of ecdysone receptor (EcR) and ultraspiracle (Usp), members of nuclear receptor superfamily. Its key role is to regulate insect metamorphosis by inducing moulting process in response to 20-hydroxyecdysone hormone. The heterodimer of EcR-Usp mediates transcription through a highly degenerated pseudo-palindromic natural DNA response element *hsp27*. In order to be able to use the receptor as artificial building block in gene therapy and to rationally design inhibitors of dimerisation we started crystallization and crystallography analysis of the receptor. Until now most of the structures of nuclear receptors were determined with artificial highly symmetric DNA response elements, therefore we have purified and co-crystallised EcR and Usp DNA binding domains from *D. melanogaster* with the 20 bp natural response element *hsp27*. Crystals obtained by vapour diffusion method diffracted synchrotron radiation to 1.95 A. Our research show that both proteins use similar dimerisation surfaces, and rely on the deformed DNA geometry to establish protein-protein contacts. We observe that in comparison to structure with artificial DNA response element the main fold is preserved, however the pattern of interactions differs which emphasizes the previously suggested plasticity of ecdysteroid receptor.

Acknowledgment: Work is supported by a State Committee for Scientific Research grant 3T09A04038.

PP-33
Molecular beacon for determining the *hsp27* response element – ecdysteroid receptor interaction

T. Krusiński, P. Dobryszycki, A. Kowalska and A. Ożyhar
Biochemistry Department, Wroclaw University of Technology, Wroclaw, Poland. E-mail: tomasz.krusinski@pwr.wroc.pl

The ecdysteroids are crucial during moulting and metamorphosis processes among the insects. They act via a receptor, which belongs to the nuclear receptors' superfamily. Functional ecdysone receptor consists of two proteins: the ecdysone receptor (EcR) and the ultraspiracle (Usp). The EcR-Usp complex regulates the transcription through an $hsp27_{pal}$ (natural 20-hydroxyecdysone response element – an imperfect palindrome from the promoter region of the *Drosophila melanogaster hsp27* gene). Usp acts as an anchor defining complex orientation on the DNA. This work is one of the first example of using molecular beacon for quantitative examining a protein – DNA interaction. In this method the protein-dependent association of two fluorescent-labelled DNA fragments each containing about half of a sequence defining a protein-binding site is crucial. This methodology was used to estimate the sequence-specific interaction of $hsp27_{pal}$ with the DNA binding domain from Usp protein (UspDBD). The dissociation constant, K_d, of the UspDBD-$hsp27_{pal}$ complex was determined to be 1.42 ± 0.28 nM, whereas K_d for the deletion mutant of UspDBD with truncated A-box – UspDBDΔA-$hsp27_{pal}$ equals 9.42 ± 1.72 nM. Results obtained with molecular beacons are in agreement with those obtained with fluorescence anisotropy measurements as well as with EMSA.

PP-34
Oestrogen receptor-alpha activates transcription of the mammary gland Na^+/I^- symporter (mgnis) gene

E. Yaman Çankaya[1], H. Alotaibi[1], E. Demirpençe[2] and U. H. Tazebay[1]
[1]*Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey,* [2]*Department of Biochemistry, Faculty of Medicine, Hacettepe University, Ankara, Turkey.*
E-mail: eyaman@fen.bilkent.edu.tr

Sodium Iodide Symporter (NIS) function in mammary gland (mg) epithelial cells is essential for the accumulation of I- in mother's milk which is the newborn's first source of I- for thyroid hormone synthesis. Furthermore, increased mgNIS expression has previously been shown in a large number of human breast cancers, and the potential uses of radioiodide and other radioactive substrates of mgNIS in breast cancer diagnosis and therapy is currently studied by various groups. We investigated possible roles of oestrogen receptor-(ERalpha) and 17-β-estradiol (E2) in regulation of mgNIS expression in mammary cancer cell models such as MCF-7 and MDA-MB-231. We are showing that in a previously ERalpha negative (ERalpha-) mammary gland cell line, MDA-MB-231, both transient and stable expression of ERalpha activates expression of mgNIS in the absence of its ligands. Furthermore, E2 treatment increases all-trans-retinoic acid (tRA) dependent mgNIS mRNA accumulation in MCF-7 cells, an ERalpha + human mammary cell line. We obtained evidences implicating that the effect of ERalpha on mgNIS gene activation is carried out through a novel oestrogen responsive element (ERE) sequence located in close proximity of mgNIS TATA box in the promoter region. Our results indicate that ERα and E2 contribute to the regulation of mammary gland NIS gene (mgNIS) expression, and E2 and tRA-activated factors functionally interact in mgNIS regulation in mammary cancer cell models.

PP-35
ATRA's inhibitory effect on prostate cancer cell growth involves harp expression

O. Theodorakopoulou, M. Hatziapostolou and E. Papadimitriou
Department of Pharmacy, Laboratory of Molecular Pharmacology, University of Patras, Patras, Greece. E-mail: otheodor@upatras.gr

It is becoming increasingly recognized that all-trans retinoic acid (ATRA) plays a role in cancer cell growth arrest through regulation of the expression of several genes. Heparin Affin Regulatory Peptide (HARP) is an 18 kDa secreted polypeptide growth factor with high affinity to heparin. HARP is mitogenic for endothelial cells, stimulates angiogenesis *in vitro* and *in vivo* and plays a key role in the progression of several types of tumours of diverse origin. In the present study we found that exogenous ATRA significantly decreased human prostate cancer LNCaP cell proliferation. Heparin affin regulatory peptide (HARP) seems to be involved in the inhibitory effect of ATRA, because the latter had no effect on stably transfected LNCaP cells that did not express HARP. Moreover, ATRA significantly decreased HARP mRNA and protein amounts in a concentration- and time-dependent manner. These data suggest that ATRA affects prostate cancer LNCaP cell growth through an effect on the expression of HARP and further studies are in progress to elucidate mechanisms involved.

PP-36
Gene expression analysis of hedgehog signalling pathway genes in breast cancer

O. Akilli-Ozturk[1], B. Gur[1], B. Bozkurt[2], **S. Seckin**[3] and I. G. Yulug[1]
[1]*Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey,* [2]*General Surgery, Ankara Numune Research and Teaching Hospital, Ankara, Turkey,* [3]*Department of Pathology, Ankara Numune Research and Teaching Hospital, Ankara, Turkey. E-mail: akilli@fen.bilkent.edu.tr*

The Hedgehog (HH) signal pathway has been investigated in many cancers and shown to have important effects, but not effectively studied in breast cancer. Signal pathways with a role in development are known to interact with each other and disturbance in one pathway can influence the regulation of others. It is therefore important to study these signal pathways in cancer. We have been analysing the gene expression profiles of Bcl2, a downstream target of HH pathway, and Shh, Smo, Ihh, Ptc1, Gli1, Gli2 and Gli3, genes involved in HH pathway, in breast carcinoma cell lines, primary breast tumour and normal tissue sample pools by real-time quantitative RT-PCR. We have analysed the HH pathway genes in 10 primary breast tumour samples and three matched normal sample pools. Observed overexpression of Gli1 and Gli3 in 70% of the tumour samples make them potential indicators of an active HH signalling in breast cancer. All the other genes that were analysed displayed low expression levels in the tumour samples when compared to normals. Ptch expression was stable or low while the Gli3 expression was high in 100% of grade III tumours. Since grade III tumours displays poor prognosis, this result may show the importance of components of the HH pathway in breast cancer progression. This is the first study to show the expression profiling of the HH pathway genes in breast cancer, which will help us to understand the initiation and development mechanisms of this cancer.

PP-37
Regulatory role of FAK/PI-3k/actin signalling in cancer cells

G. Kalergi[1], D. Mavroudis[2], V. Georgoulias[2] and **C. Stournaras**[1]
[1]*Department Biochemistry, University of Crete Medical School, Heraklion, Greece,* [2]*Department Clinical Oncology, University of Crete Medical School, Heraklion, Greece.*
E-mail: cstourn@med.uoc.gr

Recent findings in malignant MCF7 human breast epithelial- and LNCaP human prostate cancer-cells suggested that actin cytoskeleton reorganization regulated by activation of FAK and PI-3 kinase may regulate their phenotypic and metastatic profile. Here we report that incubation of human A375 melanoma cells with the opioid casomorphin induces activation of the same signalling cascade FAK/PI-3K/Rac1, leading to potent actin reorganization and inhibition of cell motility. To further assess the clinical impact of these findings, cytospins of peripheral blood mononuclear cells prepared from 45 breast cancer patients were investigated for the expression and/or activation of cytokeratin (CK), FAK, PI-3 kinase and actin organization. Immunofluorescence analysis revealed that 28 out of 45 samples were tested CK-positive, indicating the existence of circulating micrometastatic occult tumour cells (OTC). Interestingly, expression of phosphorylated-FAK (p-FAK) was documented in all 28-CK-positive samples, implying a sound correlation in the expression of both molecules in OTC. In 15 out of 17 CK- and p-FAK positive-tested samples, phosphorylation of PI-3 kinase was as well documented. Finally, actin morphology in OTC's was comparable to that observed in MCF7 and A375 malignant cells. Our findings suggest a

regulatory role of FAK/PI-3K/actin signalling in micrometastatic cells that may regulate migration mechanisms, supporting the presumption of their malignant and metastatic nature.

PP-38
Analysis of molecules differentially interacting with the highly homologous ER-α corepressors safb1 and safb2

D. Tsianou, A. Lyberopoulou, R. Kohen, S. Bonanou and E. Georgatsou
Medical School, University of Thessaly, Larissa, Greece. E-mail: dtsianou@med.uth.gr

Scaffold attachment factor-B1 (SAFB1) is a nuclear matrix protein that is implicated in a multitude of cellular processes. It has been reported to be a corepressor of oestrogen receptor- α(ER-α) transcriptional activity and it has been implicated in chromatin organization, transcriptional regulation, RNA processing, as well as stress response. SAFB2, a protein highly homologous to SAFB1 and also an ER-alpha corepressor, shares with it numerous highly conserved domains. Their genes are localized head to head on the same chromosome and their expression is regulated by a common promoter. Although indirect evidence suggests that SAFB1 and SAFB2 might have unique properties, any functional differences especially regarding their corepressor activity are still obscure. In this study, we have examined the interaction of SAFB2 with SAFB1 molecular partners fished out by the yeast two hybrid system. Among the clones tested only one clearly distinguishes between the two proteins in the yeast system and it was chosen for further examination of its structural and functional relation to SAFB1 and SAFB2.

PP-39
Clinicopathological study of survivin expression in colorectal cancer

F. Shimamoto[1], **H. Tuncel**[2], S. Sai[1], E. Aoki[1], S. Tanaka[3], S. Oka[3], R. Takahashi[3], I. Kaneko[3], H. Tatuka[4] and T. Takada[5]
[1]Faculty of Human Culture and Science, Department of Pathology, Prefectural University of Hiroshima, Hiroshima, Japan, [2]Cerrahpasa Medical Faculty, Department of Biophysics, Istanbul University, Istanbul, Turkey, [3]Department of Endoscopy, Hiroshima University, Hiroshima, Japan, [4]Department of Molecular Radiobiology, Division of Genome Biology, Graduate school of Biomedical sciences, Hiroshima, [5]Department of Oral Maxillofacial Pathobiology, Graduate School of Biomedical Sciences, Dental School, Hiroshima University, Hiroshima, Japan. E-mail: simamoto@pu-hiroshima.ac.jp

Survivin is a bifunctional protein that suppresses apoptosis and regulates cell division. It is expressed in various human cancers, but not in most normal adult human tissues. There are few comparative studies of survivin expression between the cytoplasm and nucleus of individual cells. The aims of the present study were to investigate survivin expression in colorectal carcinoma and to elucidate the relationships among the survivin, clinicopathological features and tumour progression. Immunohistochemical analyses of 144 cases of advanced colorectal cancer revealed 17 N+C+ cases with survivin (+) staining in both the cytoplasm (C) and the nucleus (N), 92 N+C– cases with survivin expression on only the nucleus, 12 N–C+ cases with survivin expression on only one side of the cytoplasm, and 21 N–C– cases that were (–) for survivin in both the cytoplasm and the nucleus. The occurrence of metastasis was higher in the N–C+ group than in the N+C– group, and the frequency of metastasis and number of cases with stage D were lower in the N+ group than in the N- group. Furthermore, the number of cases with stage D was higher in the C+ group than in the C- group. The N+ cases were associated with a better prognosis, while the C+ cases were not. These findings suggest that the biological behaviour of colorectal cancer may differ according to the localization of survivin within the cancer cells.

PP-40
The JAK/STAT pathway constitutively activated in cervical cancer cell lines is inhibited by piceatannol

A. Valle-Mendiola, J. Mendoza-Rincon, B. Weiss-Steider and **I. Soto-Cruz**
Lab. de Oncología, Unidad de Investigación en Diferenciación Celular y Cáncer, FES Zaragoza, UNAM. Apartado México D.F., Mexico. E-mail: sotocruz@servidor.unam.mx

The Jak-STAT pathway is one of the important signalling pathways downstream of cytokine receptors. Following binding of IL-2 to its cognate receptor, receptor-associated Jaks are activated. STAT proteins are then in turn activated by tyrosine phosphorylation by Jak kinases, allowing their dimerization and subsequent translocation into the nucleus, where they modulate expression of target genes. We have found that the JAK/STAT pathway is constitutively activated in transformed cervical cells, and we have demonstrated that stimulation with 10 U/ml of IL-2 prompted a significant increment of JAK3 and STAT5 phosphorylation, indicating that, in these cells, IL-2 triggers the activation of STAT5 as an important upstream factor. It has been shown that piceatannol is able to inhibit the JAK/STAT pathway, therefore, we analysed the effect of piceatannol in the phosphorylation of JAK3, and STAT-3 and -5. The cells were stimulated with 10 U/ml of IL-2 and 100 μM of piceatannol for different periods of time. IL-2 induced phosphorylation of JAK3, STAT3 and STAT5 in both cell lines, but the treatment with piceatannol prevents the phosphorylation of these proteins and also prevents translocation into the nucleus of the phosphorylated species of STATs, indicating that JAK3 is a target for this inhibitor. The basal activation of the Jak/STAT pathway involved in IL-2R signal transduction in CALO and INBL cells suggest that this pathway may play a role in the pathogenesis of cervical cancer.

PP-41
The effect of simvastatin on signalling pathways involved in pathogenesis of pancreatic cancer

H. Gbelcova[1], T. Ruml[1], Z. Knejzlik[1], M. Lenicek[2], J. Zelenka[2] and **L. Vitek**[2]
[1]Institute of Chemical Technology, Prague, Czech Republic, [2]Institute of Clinical Biochemistry and Laboratory Diagnostics, Prague, Czech Republic. E-mail: vitek@cesnet.cz

Introduction: Inhibitors of HMG-CoA reductase are widely used for treatment of hypercholesterolemia. However, inhibition of this enzyme results also in depletion of intermediate biosynthetic products contributing importantly to the cell proliferation. In the present study, we investigated the effects of simvastatin on the signalling pathways involved in the pathogenesis of pancreatic cancer.
Methods: The effect on simvastatin (17 μM) on phosphorylation of Akt protein kinase (ELISA) was tested on CAPAN-2 human pancreatic cancer cells. In a second study, the impact of simvastatin on localization of farnesylated Ras proteins was also investigated. RNA from He-La cell line was isolated and K-ras

and N-ras oncogenes were isolated using RT-PCR and inserted into pEGFP-CI vector enabling expression of these gene products in N-terminal fusion with GFP in COS-1 cells. Expression was assessed by fluorescent microscopy.
Results: Simvastatin decreased Akt protein phosphorylation by 42%; addition of mevalonate led to complete elimination of this effect. Simvastatin also caused accumulation of N- and K-Ras in cytoplasm of treated cells, while these proteins remained predominantly on the cytoplasmic membrane in unexposed cells.
Conclusions: Simvastatin effectively inhibits Akt protein phosphorylation in pancreatic cancer cells as well as blocks translocation of ras oncogenes to cytoplasmic membrane. These effects seem to importantly contribute to antiproliferative effects of statins.

PP-42
Cytochrome C release after nur77 mitochondrial translocation is abrogated in thymic lymphoma cells

I. Stasik, A. Rapak, E. Ziolo and L. Strzadala
Laboratory of Tumour Molecular Immunobiology, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland. E-mail: izabela@iitd.pan.wroc.pl

Nuclear orphan receptor Nur77 is an essential mediator of apoptosis in T cells and numerous cancer cell lines. It can act by two alternative mechanisms: regulation of target genes expression or translocation from the nucleus to the mitochondria with subsequent release of cytochrome C. Thymic lymphoma VIII/d cell line, derived from TCR transgenic mice, is resistant to Nur77-mediated apoptosis, despite of unaffected expression and DNA-binding activity of Nur77. We also observed mitochondrial translocation of this nuclear receptor. However, we found abrogation of cytochrome C release in these cells. HA1004 (an inhibitor of serine-threonine kinases) and FK506 (an inhibitor of calcineurin) were shown to restore the sensitivity of examined lymphoma to apoptosis induction. Here we show that apoptosis enhancement by these agents correlated with increased cytochrome C appearance in the cytosol. In conclusion, we show that despite of DNA-binding and successful translocation to the mitochondria in VIII/d thymic lymphoma cells, Nur77 is not able to trigger apoptosis. The failure seems to be located at the level of cytochrome C release and can be modulated by HA1004 or FK506 treatment.
This work was supported by grant 2/P05A/10929 from the Polish State Committee for Scientific Research.

PP-43
Investigation of the effects of anastrozole and quercetin on breast cancer *in vitro*

A. Demiroglu Zergeroglu[1], E. Dulger[1], E. Kilic[2], O. G. Yildiz[3] and R. Saraymen[2]
[1]*Department of Biology, Gebze Institute of Technology, Gebze, Kocaeli, Turkey,* [2]*Department of Biochemistry, Medical Faculty, Erciyes University, Kayseri, Turkey,* [3]*Department of Radiation Oncology, Medical Faculty, Erciyes University, Kayseri, Turkey. E-mail: ademiroglu@gyte.edu.tr*

Breast cancer is the second leading cause of cancer deaths in western women. Chemotherapy has been used at different stages of the disease. The flavonoid, quercetin has a strong growth inhibitory effect on several human cancer cell lines. The development of the third-generation aromatase inhibitors therefore represented a welcome potential alternative to others anti-cancerogens. Anastrozole is the aromatase inhibitor of choice. The drug is approximately used when using substantial amounts of aromatizing steroids. In this work, two different cell lines MCF-7 and T47-D were used. These cell lines showed different sensitivity to the anastrozole, quercetin alone or in combination as time and dose-dependent manner. The results showed that combination of quercetin and anastrozole suppressed cell proliferation in T47-D but not in MCF-7. Suppression effect on T47D was observed after 48–72 h.

PP-44
Effects of serum nitrite/nitrate and VEGF-A levels on survival of lung cancer patients

M. Colakogullari[1], E. Ulukaya[1], A. Yilmaztepe[1], G. Ocakoglu[2], M. Yilmaz[1], M. Karadag[3] and A. Tokullugil[1]
[1]*Department of Biochemistry, Uludag University Faculty of Medicine, Bursa, Turkey,* [2]*Department of Biostatistics, Uludag University Faculty of Medicine, Bursa, Turkey,* [3]*Department of Chest Diseases and Tuberculosis, Uludag University Faculty of Medicine, Bursa, Turkey. E-mail: mcolak@uludag.edu.tr*

Objective: As nitric oxide (NO) was proposed to be both an upstream and downstream regulator of vascular endothelial growth factor (VEGF), relationship between NO and VEGF remains unclear.
Methods: Blood samples of 31 patients with primary lung carcinoma before chemotherapy ($n = 31$) and healthy controls ($n = 15$) were collected. Serum nitrite/nitrate were measured by Griess reaction. Serum VEGF-A analysis was performed by ELISA kit. Effects of serum nitrite/nitrate and VEGF-A levels on survival were evaluated.
Results: Serum nitrite/nitrate and VEGF-A levels of lung cancer patients and control group were 93.7 ± 48.9 and 63.7 ± 32.2 μM ($P = 0.018$), and 620 ± 491 pg/ml and 255 ± 157 pg/ml ($P = 0.001$), respectively. Cut-off value of pre-treatment serum nitrite/nitrate of the cancer patients was determined as 67.2 μM (ROC analysis, area under curve = 0.859, $P = 0.002$). High nitrate/nitrite (> 67.2 μM) concentration had statistically significant effect of on overall survival (Cox analysis, $P = 0.026$ and Odds ratio = 1.009) Overall survival of the lung cancer patients with higher serum nitrate concentrations is significantly less than the lung cancer patients with lower serum nitrate concentration (Kaplan-Meier survival functions test log rank significance = 0.0007) and risk of death is 8.070 times higher ($P = 0.005$).
Conclusion: Our data suggests that having high serum nitrite/nitrate concentration is a strong indicator of poor prognosis for late stage lung cancer patients.

PP-45
Relationship between a single nucleotide polymorphism in the matrix metalloproteinase-1 promoter and ovarian cancer

O. F. Bayrak[1], F. Sahin[1], I. Pirim[2], M. E. Yalvac[1] and C. D. İzbırak[1]
[1]*Genetics and Bioengineering, Yeditepe University, Istanbul, Turkey,* [2]*Medical Biology, Ataturk University, Erzurum, Turkey. E-mail: ofbayrak@yeditepe.edu.tr*

Matrix metalloproteinase-1 (MMP-1) is an enzyme, which is degrading extracellular matrix. Thus, MMP-1 is known to have a contribution to tumour initiation and development due to alteration of the cellular microenvironment that facilitates tumour formation and angiogenesis. MMP-1 is thought to play a critical role in tumour invasion and metastasis. Human MMP-1 has two differently glycosylated proenzymes. Human MMP-1 gene is

expressed in a various physiological processes such as embryonic development, and wound healing and number of pathological processes, such as malignant tumours. The expression of MMP-1 is partly regulated by the upstream promoter sequences of the gene. The polymorphic sites due to insertion of 1G base have been found to be located in a core recognition sequence of the binding sites for transcription factors that consequently modifies the level of MMP-1 expression. In this study, we aimed to elucidate whether SNP in the MMP-1 promoter enhances ovarian cancer susceptibility. Total genomic DNA was isolated from the blood samples of 66 patients and 72 healthy controls. Then, a primer set was designed and used for detection of SNP in the promoter region of MMP-1 gene by sitedirect-mutagenesis method getting an appropriate cutting region of ALU I restriction enzyme. The results of the present study showed that 81 and 72% of the patients and controls have 2G/2G or 1G/2G genotype, respectively. Therefore, the data suggested that MMP-1 SNP might enhance ovarian cancer susceptibility.

Cell Surface Receptors and Downstream Targets

PP-46
The role of HGF/C-met signalling pathway on the non-small cell lung cancer

M. Gumustekin[1], B. Sis[2], G. Bulut[3], A. Kargı[2], I. Oztop[4], N. Olgun[4] and N. Atabey[3]
[1]*Department of Pharmacology, Dokuz Eylul University School of Medicine, Izmir, Turkey,* [2]*Department of Pathology, Dokuz Eylul University School of Medicine, Izmir, Turkey,* [3]*Department of Medical Biology and Genetics, Dokuz Eylul University School of Medicine, Izmir , Turkey,* [4]*Institute of Oncology, Dokuz Eylul University, Izmir, Turkey. E-mail: gumustek@deu.edu.tr*

Aim: It is thought that HGF/c-Met signalling pathway has a role in the invasion and metastasis processes of lung cancer. The aim of this study is to determine the role of HGF/c-Met pathway in NSCLC.
Methods: The expression of HGF and c-Met were determined immunohistochemically in tissue samples of 63 NSCLC patients. DNAs obtained from sections of the same tissues were amplified by PCR using exon-14-specific primers that encode tyrosine kinase domain of the c-Met receptor.
Results: C-Met and HGF expressions were determined in 81% and 48% of NSCLC tissues, respectively, consequent to the immunohistochemical analyses. A correlation between the overexpression of HGF/c-Met and tumour size, tumour stage, lymph node metastasis and relapse rate was not observed. C-Met was found to be overexpressed in patients with distant metastasis. The sequence analyses of exon 14 have been completed for only 31 PCR products until now. Mutation was not detected in these analyses. The sequence analyses of the remaining PCR products still continue.
Conclusion: These result show that HGF/c-Met pathway may play a role in NSCLC development and/or progression. Our data support the opinion that c-Met overexpression may be independent of HGF. The completed sequence analyses suggest that there may not be a relation between HGF/c-Met overexpression and the mutations in exon 14. All the sequence analyses must be completed for a definite result.

PP-47
A G-protein based biological sensor to reveal signal transduction mechanism in living cells

M. Akgoz[1] and **N. Gautam**[2]
[1]*Department of Chemistry, Art and Science Faculty, Kafkas University, Kars, Turkey,* [2]*Department of Anaesthesiology, School of Medicine, Washington University, Saint Louis, MO, USA. E-mail: makgoz@yahoo.com*

A biological sensor was developed to study signal transduction mechanisms. This sensor uses the phenomenon of translocation of the G-protein beta-gamma subunit upon receptor activation in living cells in real time. After activation of the receptor, the G-protein beta-gamma-YFP subunit on the membrane translocates to the golgi apparatus in less than 1 min. On deactivation of the receptor with antagonist, it translocates back to the membrane. This can be observed under the fluorescent microscope. The translocation process takes place in seconds and can be repeated several times. This sensor was used to elucidate the receptor stimulated G protein activation mechanism. Rapid and efficient screening of commercial drugs for receptors will also be possible with this biological sensor.

PP-48
Towards understanding the structure and function of g protein-coupled receptors: a multidisciplinary approach

A. Shukla, C. Reinhart and H. Michel
Max Planck Institute of Biophysics. E-mail: arun.shukla@mpibp-frankfurt.mpg.de

GPCRs are integral membrane proteins with seven transmembrane helices that regulate many cell signalling pathways via activation of G proteins. GPCR malfunction leads to several human diseases and majority of commonly prescribed medicines act on these receptors. However, structure based drug designing on GPCRs has not been possible due to lack of high resolution structure. Therefore, my efforts focus on expression, purification and structural studies of selected GPCRs. Three different GPCRs namely, bradykinin receptor, neuromedin receptor and angiotensin receptor, have been purified in milligram amounts and being subjected to crystallization trials to obtain structural information. *In vitro* and *in vivo* reconstitution of GPCR signalling complexes (GPCR heterodimer, GPCR-arrestin and GPCR-G protein) is also being pursued, which may provide insights into signalling mechanism. During heterodimerization studies, it was observed that coexpression of angiotensin receptor drives the bradykinin receptor to cell surface. This is the first report where cell surface trafficking of a peptide GPCR is driven by another distantly related GPCR. In addition, we also use solid state NMR spectroscopy to understand the conformational changes in ligands upon binding to GPCRs and we are very close to obtain the high resolution structure of active conformation (bound to receptor) of bradykinin. This should pave the way towards structure based designing of potent and specific drugs acting on GPCRs.

PP-49
Structural basis of cell adhesion signalling of syndecan-4 proteoglycan as a cell surface receptor

B-K. Koo[1], I. Han[2], E. Mortier[3], P. Zimmermann[3], J. R. Whiteford[4], J. R. Couchman[4], E-S. Oh[2] and W. Lee[1]
[1]Department of Biochemistry and Protein Network Research Center, College of Science, Yonsei University, Seoul, Korea, [2]Department of Life Sciences, Division of Molecular Life Sciences and Center for Cell Signalling Research, Ewha Womans University, Seoul, Korea, [3]Laboratory for Glycobiology, University of Leuven & Flanders Interuniversity Institute for Biotechnology, Leuven, Belgium, [4]Division of Biomedical Sciences, Faculty of Medicine, Imperial College of Science, Technology and Medicine, London, UK. E-mail: wlee@spin.yonsei.ac.kr

The syndecan transmembrane proteoglycans are involved in the organization of the actin cytoskeleton and they have important roles as cell surface receptors during cell-matrix interactions. Syndecan-4 can regulate cell-matrix interactions and it is enriched in focal adhesions. We have shown that the syndecan-4 cytoplasmic domain (4L) forms oligomeric complexes that bind to and stimulate PKCα activity in the presence of PtdIns(4,5)P2, emphasizing the importance of multimerization in the regulation of PKCα activation. Oligomerization of the cytoplasmic domain of syndecan-4 is regulated either positively by PtdIns(4,5)P2, or negatively through phosphorylation of serine 183 (Ser183). Phosphorylation results in reduced PKCα activity by preventing PtdIns(4,5)P2-dependent oligomerization of the syndecan-4 cytoplasmic domain. Data from NMR and gel filtration chromatography shows that the phosphorylated cytoplasmic domain (p-4L) exists as a dimer. Solution structure showed that the overall conformation of p-4L is a compact intertwined dimer with unusual symmetric clamp shape and its molecular surface is highly positively charged. A marked effect of phosphorylation is a dramatic conformational change in the C2 region, which ablates an interaction site with the PDZ protein syntenin. The detailed molecular interactions of syndecan-4 with PDZ domain are also discussed based on NMR experimental data.

PP-50
Tumour growth is impaired in Semaphorin4D knockout animals

J. R. Sierra[1], S. Corso[1], A. Casazza[1], H. Kikutami[2], L. Tamagnone[1] and S. Giordano[1]
[1]Division of Molecular Oncology, Institute for Cancer Research and Treatment, University of Turin School of Medicine, Candiolo (Turin), Italy, [2]Department of Molecular Immunology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan. E-mail: jose.sierra@ircc.it

The secreted and membrane bound protein Semaphorin4D (Sema4D) is endowed with angiogenic properties. Sema 4D binds to its receptor, PlexinB1, and this interaction lead to the activation of Met, the tyrosine kinase receptor for the Hepatocyte Growth Factor. Met activation induces cell proliferation, migration, prevention of apoptosis, and differentiation. To investigate the *in vivo* angiogenic function of this Sema4D and its role in tumour progression, we use Sema4D KO mice (kindly provided by Dr Kikutami) that were injected with syngeneic tumourigenic cells. KO animals showed an impairment of tumour growth and a significant decrease of the number of lung metastases, compared with Wt mice. Analysing the status of vessels inside the tumours, KO animals displayed a five-time fold decrease in the total vessel area, maintaining a similar vessel number. Tumour vessels in KO animals seemed to be less well organized. Further analyses would identify the host cell population producing Sema4D and reveal how it activates endothelial cells and stimulates the invasive/metastatic properties of tumour cells. Our preliminary data suggest that Sema4D plays an important role in the tumourigenic/metastatic process and that it is a likely candidate for an anti-neoplastic target therapy.

PP-51
CXCR4 expression in Ishikawa endometrial cell lines

L. Kubarek and P. P. Jagodzinski
Department of Biochemistry and Molecular Biology, University of Medical Sciences, Poznan, Poland. E-mail: lukasz_quba@wp.pl

CXCL12 chemokine binds to CXCR4 receptor that belongs to the G-protein-coupled-receptors family. This activates a variety of intracellular signal transduction pathways and effector molecules, which regulate cell survival, adhesion, migration, proliferation and angiogenesis. The increased expression of CXCR4 in breast cancer cells may correlate with tumour progression and metastasis to bone marrow, lymph nodes, lungs and other organs. We determined the transcript and protein level of CXCL12 and CXCR4 in oestrogen receptor positive (ER+) and negative (ER-) Ishikawa endometrial cancer cell lines. Total RNA was isolated according to the method of Chomczyński and Sacchi, treated with DNase I and reverse-transcribed into cDNA. Quantitative analyses of CXCL12 and CXCR4 transcripts were performed by real-time PCR SYBR Green I system. The quantity of transcripts was normalized with polymerase II transcript level. The protein level of CXCR4 was determined using Western blot and flow cytometry analysis. We observed approximately higher level of CXCR4 and CXCL12 expression in ER- compared with ER+ endometrial cancer cell line. Oestrogen might regulate CXCR4 and CXCL12 expression, which can be associated with progression and metastasis of ER+ endometrial tumour cells.

PP-52
Proper activity of PTP-PEST in mast cell signalling is based on the expression level of adaptor LAT2

P. Heneberg, L. Dráberová, G. M. Shaik and P. Dráber
Institute of Molecular Genetics AS CR, Prague, Czech Republic & 3rd Medical Faculty, Charles University, Prague, Czech Republic. E-mail: petrhen@biomed.cas.cz

Changes in activity of the protein tyrosine phosphatase PTP-PEST during mast cell activation through the high affinity IgE receptor type I (FcεRI) was studied. After antigen-mediated aggregation of the IgE-FcεRI complexes, the enzymatic activity of PTP-PEST was rapidly enhanced with the peak at about 2 min after triggering. In cells with down-regulated expression of a linker for activation of T-cells family member 2 (LAT2, formerly NTAL) by RNA interference, PTP-PEST was not activated by the FcεRI-aggregation, probably due to the observed absence of an increase of actin polymerisation after receptor triggering. On the other hand, in cells with upregulated expression of LAT2 after transfection of LAT2 cDNA under cytomegalovirus promoter, the activity of PTP-PEST was increased in both resting and activated cells. Enhanced activity of PTP-PEST in LAT2 overexpressors led to markedly decreased tyrosine phosphorylation of transmembrane adaptor protein PAG and decreased association of Csk with PAG. This led to enhanced activity of Lyn kinase and extremely hyperactive SHP-2 in both resting and activated cells. Consequently FcεRI was less phosphorylated, causing the inhibition of phosphorylation of Syk kinase and

adaptor LAT and decreased activity of PLCγ and subsequent activation events. The combined data suggests that PTP-PEST is an important regulator of mast cell signalling via FcεRI and its activity is tightly regulated by adaptor protein LAT2.

PP-53
Isomerization of the adenosine A_{2a} receptor-[^{3}h] zm241385 complexes

A. Uustare[1], A. Terasmaa[2], A. Vonk[1] and **A. Rinken**[1]
[1]Institute of Organic and Bioorganic Chemistry, Tartu University, Tartu, Estonia, [2]Laboratory of Clinical and Translational Studies, National Institute of Alcohol Abuse and Alcoholism, NIH, Bethesda, MD, USA. E-mail: deffi@ut.ee

[^{3}H] ZM241385, a specific radiolabelled antagonist for A_{2A} adenosine receptors, bound to a homogenous population of binding sites in rat striatal membranes with affinity K_d = 0.14 nM and density B_{max} = 1620 fmol/mg protein. Similar binding properties have been also obtained for transfected CHO cell membranes (K_d = 0.23 nM and B_{max} = 360 fmol/mg protein), but in this case the pretreatment with adenosine deaminase (ADA) was required to remove internal adenosine. The binding of [^{3}H] ZM241385 was fast and reversible, achieving equilibrium within 20 min at all radioligand concentrations. The analysis of the obtained kinetic and saturation data indicated that the [^{3}H] ZM241385 binding might have at least two subsequent steps, where a fast equilibrium is followed by a slow conformational isomerization. The potency of ZM241385 to inhibit CGS21680-induced cAMP accumulation in CHO cells (K_i = 6.6 nM) was considerably lower than its apparent affinity in binding experiments, but in good agreement with the estimated equilibrium constant for the first step of the binding reaction (K_A = 8.5 nM) determined in kinetic experiments. Obtained data indicated that isomerization step of the radioligand binding to the receptors has high impact in interpretation of experimental data and has to be taken into account in prediction of potencies of drugs.

PP-54
Searching for possible interactions between CB1 and GABAB receptors in rat brain hippocampal membranes

R. Çınar[1], K. Mackie[2], T. F. Freund[3] and M. Szűcs[1]
[1]Institute of Biochemistry, Biological Research Center, Hungarian Academy of Science, Szeged, Hungary, [2]Department of Anaesthesiology and Physiology and Biophysics, University of Washington School of Medicine, Seattle, USA, [3]Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary. E-mail: szucsm@nucleus.szbk.u-szeged.hu

GABAB receptors are unique among G-protein coupled receptors in their requirement for heterodimerization between two subunits, R1 and R2 for functional expression. Recent studies have revealed that hetero-oligomerization between very different receptors can also occur and this may profoundly change the binding and signalling properties of the receptors. First we performed a thorough characterization of the GABAB and CB1 cannabinoid receptors by using ligand-stimulated [35S] GTPγS binding assays in rat hippocampal membranes. Win55,212-2 (a CB1 agonist) displayed high potency (ED50 = 23.26 ± 1.2 nM) and efficacy (148 ± 2.2%) in stimulating [35S] GTPγS binding. This effect was completely blocked with SR141716 (a CB1 antagonist), proving that the CB1 receptors are fully functional. The GABAB agonists baclofen and SKF 97541 also elevated [35S] GTPγS binding by 149 and 186%, respectively. Interestingly, nanomolar concentrations of the GABAB antagonist phaclofen slightly but significantly lowered the maximal stimulation of [35S] GTPγS binding compared to that obtained with Win55,212-2 alone. These results can be interpreted to show an interaction, possibly hetero-oligomer formation between CB1 and GABAB receptors with altered functionality.

PP-55
Extracellular RNA in culture of transformed and primary human cells

E. Morozkin, P. Laktionov, E. Rykova and V. Vlassov
Cellular Biology Group, Institute of chemical biology and fundamental medicine, Novosibirsk, Russia.
E-mail: morozkin@niboch.nsc.ru

In order to investigate cell free and cell surface associated RNA in culture of human cells we developed original glass fibre filters (GFF) and buffers for isolation of single and double stranded RNA and ribooligonucleotides from biological fluids. Developed GFF-based procedure provides 70% recovery of ribooligonucleotides and 95% recovery of polymeric RNA from cells and human plasma. Cell free and cell surface bound RNA were isolated from He-La and HUVEC cells using developed procedure, followed by concentration detection by fluorescence-based assay using SYBR Green II. Isolated cell surface bound RNA was 5-[^{32}P]-labelled and analysed by PAGE, which revealed the presence of the individual RNA molecules. One of the major low molecular weight RNA fragments was isolated and sequenced by chemical method for RNA sequencing. The nucleotide sequence of ribooligonucleotide (5-AC GGG UGG GGU CCG CGC AGU CCG CCC GGA GG) corresponds to 5-end of 28S rRNA. To investigate the different rRNA secretion out of cells, the primers specific for different regions of rRNA were developed and RT-PCR technique was applied. 18S rRNA fragment was found only on the cell surface, whereas, fragments of 28S and 5.8S rRNA was found both on the cell surface and in culture medium. The data obtained suggest the existence of sequence-specific interaction of RNA with cell surface.

PP-56
The role of actin cytoskeleton in calcium response: C2C12 as a model of excitable cells

D. Suplat, P. Pomorski and J. Baranska
Department of Cellular and Molecular Neurobiology, Laboratory of Signals Transduction. E-mail: d.suplat@nencki.gov.pl

Skeletal muscle satellite cells are precursors of mammalian skeletal muscles. Differentiation of those precursors *in vivo* is regulated by extracellular growth factors that transmit signals into the cells. Extracellular ATP acting trough P2X and P2Y purinergic receptors is also involved in this process. The effects of actin cytoskeleton disruption by cytochalasin D on calcium signals evoked by ATP, thapsigargin and caffeine were investigated in C2C12 myoblasts and myotubes. In myoblasts the high and rapid Ca^{2+} response is generated mainly by P2X ion-gated receptors. In myotubes, ATP generates weak Ca^{2+} response acting through P2Y metabotropic receptors only. Cytoskeleton disruption strictly decreases general calcium response in myoblasts. Otherwise, the calcium response evoked only by P2Y receptors seems not to depend on cytochalasin D treatment. Thapsigargin, an irreversible inhibitor of the SERCA ATPase, promotes the leak of Ca^{2+} from the ER. Caffeine acts through ryanodine receptors releases Ca^{2+} from internal stores. Both do not provoke any second messenger formation. Ca^{2+} mobilization is slightly decreased after cytoskeletal disruption both in myoblasts and myotubes. Those experiments show differences in the role of actin

cytoskeleton in calcium responses between C2C12 excitable cells and glioma C6, a popular model of non-excitable cells. The role of actin cytoskeleton in signal transduction is different and depends on type of the cell and its development stage.

PP-57
IL-1β counteracts TGFβ signal in chondrocytes by downregulating TBRII through NFκB pathway

C. Baugé[1], S. Leclercq[2], J. M. Elissade[3], J. P. Pujol[1] and K. Boumediene[1]
[1]Laboratory of Connective Tissue Biochemistry, Faculté de Médecine, Caen cedex, France, [2]Department of Orthopaedic Surgery, Clinique Saint Martin, Caen, France, [3]Department of Anatomy, Faculty of medicine, Caen cedex, France. E-mail: catherine.bauge@unicaen.fr

Interleukin-1β (IL1β) and Transforming Growth Factor-β (TGFβ) play a key-role in osteoarthritis (OA). In this present study, we attempt to determine the influence of IL1β on TGFβ responsiveness in human articular chondrocytes (HAC). HAC were treated with IL1b and TGFβ-induced gene expression was analysed through PAI1 and p3TPLux induction. R-Smads phosphorylation and TGFβ receptors (TbRI, TbRII) and Smads expression were defined by Western-Blot and real-time RT-PCR. Transduction pathways (NO, MAPK, NFκB) were investigated using specific inhibitors. Transfections of TbRII promoter or expression vectors were performed to delineate DNA sequences and to define transcriptional factors involved in IL1β effect. IL1β pre-treatment inhibits TGFβ-induced gene expression and Smad2/3 phosphorylation, causes a dramatic decrease of TbRII expression, and up-regulates Smad7. Interestingly, TbRII overexpression counteracts the loss of TGFβ-responsiveness induced by IL1b. TbRII downregulation is prevented by cycloheximide and is attenuated by inhibition of NFκB pathway. This regulation implicates TbRII core promoter that contains a putative binding site for p65 and p65 overexpression decreases TbRII expression. In conclusion, IL1b impairs TGFβ signalling through TbRII downregulation, which is probably, mediated by NFκB, and secondarily though Smad7 upregulation. These findings may account for the reduced responsiveness of articular chondrocytes to TGFβ during the late stages of OA.

PP-58
Inactivation of DNA methylotransferases affects expression of several genes involved in TCR signalling

A. Kozlowska and **P. P. Jagodzinski**
Biochemistry and Molecular Biology University of Medical Sciences, Poznan, Poland. E-mail: kozlowa@am.poznan.pl

DNA methylation occurs on cytosine in CpG dinucleotide of promoter and first exon of genes. This process is carried out by DNA methyltransferases (DNMTs) and serves as an epigenetic regulation of gene expression. DNMT1 is responsible for maintenance of methylation pattern, whereas other DNMT3A and DNMT3B methylate CpG sites de novo. DNMTs are involved in T cell lineage development, activation and Th1/Th2 helper T cell polarization. We examined the effect of DNMT1 depletion on expression of genes involved in T cell receptor (TCR) pathway in Jurkat T cells. Using lentiviral vector expressing short hairpin RNA, we stably depleted DNMT1. Quantitative western blot showed 90% reduction of DNMT1 protein in Jurkat T cell line. Total RNA was isolated, treated with DNase I and reverse-transcribed into cDNA. Quantitative analyses of FYN, PKC-θ, CD4, LCK, ITK, ZAP-70, LAT, SLP-76, CD45 and CD3ε transcripts were performed by real-time PCR system. The quantity of transcripts was normalized with β-actin(transcript level. We observed an increase of mRNA level of FYN, PKC-θ, CD4 and LCK in Jurkat T cells with stable depletion of DNMT1. Our studies indicate that DNA methylation may have a role in regulation of expression of several genes involved in TCR signalling. This finding also suggests that level of DNA methylation can be responsible for improper function of CD4+ T cells in patients with autoimmune disease.

Acknowledgment: This work was supported by grant KBN No. 2PO5B-019-27.

PP-59
Adenosine receptors in growth arrested glioma cells

P. Krzeminski and J. Baranska
Nencki Institute of Experimental Biology, Department of Molecular and Cellular Neurobiology, Laboratory of Signal Transduction. E-mail: pkrzemin@nencki

Adenosine is final product of ATP and ADP metabolism that can act on specific P1 receptors located mainly on plasma membrane. Signal transduction through the group of four already known P1 receptors is tightly regulated not only by interaction of downstream proteins but also by the mechanisms of receptors desensitization and elimination of adenosine by specific nucleotide transporter. Adenosine levels as well as ADP and ATP differs among tissues and is elevated in many tumours. Stimulation of nucleotide receptors can have various effects on cell faith what depends on concentration, tissue model and growth conditions. Recent result from our laboratory showed that level of ADP sensitive receptor $P2Y_1$ is strongly decreased in growth arrest induced by serum deprivation. As an ADP can be metabolised to the adenosine by ectonucleases we decided to examine expression profile and role in proliferation of P1 receptors of two glioma cell lines c6 and T98g in serum deprivation induced growth arrest. Data from our experiments suggest that cells growing in normal, full medium have only A2B and A3 receptors. During serum deprivation of glioma C6 cells the level of A2b remains unchanged, while A3 level is gradually increasing what coexist with growth arrest.

PP-60
Human interferon gamma: significance of lysine 88 for its biological activity

S. Petrov[1], G. Nacheva[1], M. Boyanova[1], A. Berzal-Herranz[2], A. Karshikoff[3] and **I. Ivanov**[1]
[1]Gene Regulation Department, Institute of Molecular Biology, Bulgarian Academy of Sciences, Sofia, Bulgaria, [2]Molecular Biology Department, Instituto de Parasitologia y Biomedicina 'Lopez-Neyra', Granada, Spain, [3]Department of Biosciences at Novum, Karolinska Institute, Huddinge, Sweden. E-mail: stefart@abv.bg; genoveva@obzor.bio21.bas.bg; mayab@obzor.bio21.bas.bg; Aberzalh@ipb.csic.es

Interferons accomplish their multiple biological activities by activating the STAT transcription factors, which are translocated to the nucleus through specific nuclear localization sequence (NLS) located in their ligands. Two putative NLS have been pointed out in the human interferon gamma (hIFNγ) spread over residues 83–89 and 124–132. To investigate the significance of the putative upstream NLS for the biological activity of hIFNγ we have prepared a new construct of the hIFNγ gene in which a Gln codon was substituted for the Lys88 codon. The mutated gene was cloned and expressed in *E. coli* LE392. This mutation

led to a 1000-fold decrease in both hIFNγ antiviral and antiproliferative activities. When co-incubated with the wild type hIFNγ (standard), the mutant hIFNγ competed for the cell receptors that led to a 30% inhibition of the standard activity. This indicates that the mutation does not interfere with the interaction of the protein to its cell receptor but affects the intracellular transduction in which Lys88 seems to play an important role. To study the role of the C-terminal NLS, 21 C-terminal codons have been deleted from the mutant hIFNγ gene and this led to a 10 000-fold decrease in biological activity and 55% inhibition of the standard activity in the competition assay. These data confirm our hypothesis that the lack of the C-terminus stabilizes the hIFNγ/receptor complex.

Acknowledgment: Supported by NSF, grant K-1405.

PP-61
Production of recombinant Go alpha protein using the pqe80 expression vector system

P. Mega Tiber, C. Nacar, O. Orun and B. Kan
Department of Biophysics, School of Medicine, Marmara University, Istanbul, Turkey. E-mail: pinarmet@yahoo.com

Heterotrimeric G Proteins, which couple cell-surface receptors to intracellular enzymes, channel proteins and other effector systems are composed of an alpha, a beta and a gamma subunit. G protein-mediated signalling is involved in diverse physiological functions. Go protein, a member of the Gi/Go family, is the most abundant type of heterotrimeric G protein in brain and the central nervous system, which plays key roles in pain perception, motor control and Ca^{2+} channel regulation. We have previously subcloned Goalpha into the pGEX-4T2 system and over-expressed Goalpha as a GST-fusion protein; however most of the protein produced was in the form of inclusion bodies. In this study, Go alpha sequence was amplified with PCR from pT7/NdeI/Go alpha template and subcloned into the pQE80 expression vector system (Qiagen) using *Hind*III and *Eco*RI restriction enzymes, in accordance with the classical protocol of Lee et al. The construct was then transformed into the TOP10 *E. Coli* cell line. After transformation, colonies were scanned for the insert sequence. Different Isopropyl-β-D-Throgalactopyranoside (IPTG) concentrations and incubation temperatures were used to induce over-expression of Go alpha protein. Attempts to increase the amount of soluble protein and optimize purification are in progress.

Acknowledgments: Go alpha cDNA was kindly provided by Dr Joel Moss from National Institutes of Health. This work was supported by the Marmara University Research Fund.

PP-62
Juvenile hormone binding protein from *G. mellonella* binds to membrane protein in the specific manner

M. Zalewska, A. Ożyhar and M. Kochman
Department of Biochemistry, Wrocaw University of Technology, Wrocaw, Poland. E-mail: marta.b.zalewska@pwr.wroc.pl

Juvenile hormone (JH) is essential for multiple physiological processes: it controls larval development, metamorphosis and adult reproduction. In the insect haemolymph, JH is in 99.9% in the bound state with juvenile hormone binding protein (JHBP). This protein protects JH molecule from non-specific hydrolases and serves as a carrier to supply the hormone to the target tissues, preventing its non-specific binding to hydrophobic surfaces. However, mechanism describing the way in which JH enters the target cells has not yet been elucidated. In this report we present the studies on JHBP binding to membrane proteins. Membranes isolated from VIIth instar larvae fat body of the *G. mellonella* were incubated with increasing concentrations of radioiodinated JHBP. We found that the interaction between JHBP and membranes occurs with saturation kinetics and is specific and reversible. Specificity of binding was determinated by competitive binding experiments in the presence of 100-fold excess of unlabeled JHBP or in the presence of non-specific protein (serum albumin). The above results indicate the existence of a membrane protein, which recognizes JHBP molecule and perhaps may take part in the transfer of JH to the target cells. Currently, investigations are being performed to identify the JHBP binding protein in the cell membrane proteins.

Acknowledgment: This work was supported by the Polish Ministry of Education and Science.

PP-63
A role of neutrophils in allogeneic immune response

E. V. Maryukhnich, E. S. Zvezdova, L. A. Pobezinsky and D. B. Kazansky
Laboratory of Regulatory Mechanisms in Immunity, Carcinogenesis Institute – Russian N. N. Blokhin Cancer Research Center, Kashirskoye Shaussae, Moscow, Russian Federation. E-mail: marjuchnich@yandex.ru

Recently we have shown that accumulation of mature, non-apoptotic neutrophil-like cells (Gr-1 + CD31–CD80 + CII+) occurs in mouse spleen after intraperitonal injection of allogeneic tumour cells. They reach its peak on 6th day after immunization, which precedes the CD8+ T cell expansion and the acquirement of effector functions by them. Depletion of CD8 and CD4 cell *in vivo* revealed dependence of neutrophil response on CD8 T lymphocytes. Migration of bone marrow neutrophils toward the gradient of factors released by splenocytes of immunized mice may point that CD8 T cells attract granulocytes to the spleen, where they can be the source of costimulatory signals. Indeed, while the *in vitro* incubation of splenocytes with allogeneic tumour cells in MLTC didn't lead to their activation, adding of immune splenocytes containing neutrophil-like cells induced their proliferation. Also we have found the expression IL-12 mRNA in spleen neutrophils. Expression B7.1 molecules on neutrophils were detected by flow cytometry, but not RT-PCR. This suggests that neutrophils can express B7 related protein, how the human neutrophils do that under some pathological conditions. Thus, we have shown that neutrophils play role in response to allogeneic tumour cells. Expression of costimulatory molecules suggests that neutrophils can acquire properties of professional antigen-presenting cells (APC) and their potential to polarize the immune responses to tumour antigens.

PP-64
Screening for new proteins interacting with endoglin, by the phage display technique

E. M. Garrido, F. J. Blanco, A. Fernandez-L, L. M. Botella and **C. Bernabeu**
Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, Madrid, Spain. E-mail: evagarrido@cib.csic.es

Endoglin, mainly expressed at the surface of the endothelial cells, is one of the components of the transforming growth factor-beta receptor complex and is involved in angiogenesis and cardiovascular development. Its mutation is responsible for Hereditary Haemorrhagic Telangiectasia, an autosomal dominant vascular disorder characterized by arteriovenous malformations and telangiectases. Due to the relatively high expression of endoglin at the surface of

endothelial cells, respect to other TGF-beta receptor components, we postulate that endoglin might have other ligands unrelated to the TGF-beta system. In order to identify new binding molecules, a phage display screening was performed using the extracellular domain of endoglin as bait. After three rounds of bioplanning a total of forty phage plaques were selected. DNA was extracted, PCR-amplified and sequenced to identify the proteins encoded by the phages. Twelve of the sequences corresponded to middle size transcripts of known proteins. Among them, we have focused our interest in the Smad interacting protein 1 (SIP-1), which is a transcription repressor of the transforming growth factor-beta signalling pathway, and in the Faciogenital dysplasia protein 3 (Fgd3), which is a Rho-GEF (Rho GTP exchanging factor) specific for Cdc42. We are performing experiments to verify the *in vitro* and *in vivo* interaction with these proteins and to elucidate the physiological role of these putative endoglin partners.

PP-65
Mutation and functional analysis in hereditary haemorrhagic telangiectasia (HHT) patients

A. Fernandez-L[1], F. Sanz-Rodriguez[2], E. M. Garrido[1], F. J. Blanco[1], C. Bernabeu[1] and **L. M. Botella**[1]
[1]Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas. Madrid, Spain, [2]Departamento de Biología y Genética, Facultad de Biología, Universidad Autonoma de Madrid, Madrid, Spain. E-mail: africa@cib.csic.es

Hereditary Haemorrhagic Telangiectasia (HHT) is an autosomic dominant vascular disease clinically characterized by spontaneous and recurrent epistaxis, telangiectases, and arteriovenous malformations in internal organs. Mutations in Endoglin and ALK1 genes are responsible for HHT1 and HHT2, respectively. Both genes are mainly expressed by endothelial cells and code for components of the transforming growth factor β (TGF-β) receptor complex. Blood outgrowth endothelial cells were obtained from patient's peripheral blood, characterized by specific endothelial markers and functionally studied compared to endothelial cells from healthy donors. HHT1 and HHT2 showed low levels of endoglin expression. In the case of HHT1 it is due to endoglin haploinsufficency, and in HHT2 it is probably due to endoglin regulation by ALK1. Moreover, HHT cells showed impaired ALK1 and ALK5/TGF-β signalling. Endoglin is able to interact with proteins at the F-actin polymerization sites. Accordingly, endoglin deficiency in HHT cells is associated with an altered actin cytoskeleton as well as areas of F-actin fiber depolymerization. Endoglin role in cytoskeleton organization was confirmed by siRNA silencing leading to altered fibres and by partial recovery of fibre organization after endoglin overexpression in HHT cells. A disorganized cytoskeleton, in addition to TGF-β signalling alterations, would lead to cellular fragility, which could explain the clinical traits of the disease.

PP-66
Oncomining the receptome for the genes that are differentially expressed in HCC

M. E. Avci, O. Konu and T. Yagci
Molecular Biology and Genetics, Bilkent University, Ankara, Turkey. E-mail: aender@bilkent.edu.tr

The entire repertoire of genes that encode plasma membrane receptors is recently defined as the 'receptome' (http://Receptome.Stanford.edu). On the other hand, oncogenomic analyses through DNA microarray studies have generated a wealth of data uncovering the complex gene expression patterns of cancer. Such data are available in another data-mining platform, namely ONCOMINE (www.oncomine.org). The aim of our study is to retrieve membrane receptors and their cognate ligands that are over-expressed in hepatocellular carcinoma (HCC) and to exploit these proteins as diagnostic markers and therapeutic targets. Receptor proteins were selected from analyses performed in aforementioned databases. We have restricted our initial studies to a subgroup of receptors and ligands functioning in axon guidance. In ONCOMINE (2.0) the genes having statistically significant up- or down-regulation with respect to their adjusted *P*-values were selected for further functional studies. Out of 119 target genes containing receptors and ligands of Netrin, Ephrin, Roundabout and Plexin families, nine were up-regulated, while 12 were down-regulated significantly. RNA interference was used as a second filter for the selection of target molecules. As a first attempt, we investigated the expression of slit-robo genes in HCC cell lines and tumours. Our first results allowed us to hypothesize that the members of this receptor family are differentially expressed in HCC, according to differentiation status of HCCs.

PP-67
T-cadherin mediates low-density lipoprotein–initiated mitogenic signalling

D. Kipmen-Korgun[1], K. Osibow[2], C. Zoratti[2], J. Greilberger[3], G. M. Kostner[2], G. Juergens[3] and W. F. Graier[2]
[1]Department of Biochemistry, Akdeniz University, Antalya, Turkey, [2]Institute of Molecular Biology & Biochemistry, Center of Molecular Medicine, Medical University of Graz, Graz, Austria, [3]Institute of Medical Chemistry and Pregl Laboratory, Center of Integrative Physiology, Medical University of Graz, Graz, Austria. E-mail: dijlekipmen@akdeniz.edu.tr

T-cadherin is a unique cell adhesion molecule that is anchored to the cell membrane through its GPI-moiety. T-cadherin was found to be an atypical LDL binding site that is expressed in various types of cells. The expression of T-cadherin was reduced in numerous types of cancers while it was upregulated in tumour-penetrating blood vessels and atherosclerotic lesions. However, our knowledge on the physiological role and the signal transduction pathways associated with this protein is limited. This study was aimed to investigate whether or not T-cadherin has a role in LDL-initiated signal transduction. Therefore, T-cadherin was overexpressed in the human umbilical vein derived endothelial cell line EA.hy926 and the human embryonic kidney cell line HEK293 and the LDL-initiated signal transduction and its consequences were elucidated. Our data revealed that T-cadherin serves as a receptor specifically for LDL. Following LDL binding to T-cadherin, a mitogenic signal transduction was initiated that involved activation of PLC and IP3 formation, which yielded intracellular Ca^{2+} mobilization. Downstream to these phenomena, activation of tyrosine kinase(s), Erk1/2 kinase and the translocation of NFkB towards the nucleus were found. Finally overexpression of T-cadherin resulted in accelerated cell proliferation in a LDL dependent manner. Our data suggest that T-cadherin serves as a signalling receptor for LDL that facilitates a LDL-dependent mitogenic signal in the vasculature.

PP-68
CREB mediates arterial smooth muscle cell migration via the regulation of osteopontin gene transcription

S. Jalvy, M. A. Renault, L. Lam Shang Leen, I. Belloc, A. P. Gadeau and C. Desgranges
INSERM U441, Atherosclerose, Pessac, France. E-mail: sandra.jalvy@etud.u-bordeaux2.fr

The cAMP responsive element-binding factor (CREB) is activated in arterial smooth muscle cells (SMC) by several growth factors

involved in arterial wall remodelling, such as PDGF-BB. The extracellular nucleotide UTP, which induces SMC migration via osteopontin (OPN) production, also increases CREB activation. The aim of this study is to identify the mechanisms of CREB activation and its consequences on SMC migration and OPN regulation. Stimulation of cultured SMC by UTP and PDGF-BB highly induces CREB activation via ERK1/2 and p38, and via p38 and PKA respectively. The role of CREB in SMC migration was determined using the Transwell approach and a dominant negative form of CREB (A-CREB). A-CREB expression inhibits both UTP- and PDGF-induced migration suggesting that the migratory process is dependent on CREB activation. Moreover, using A-CREB, we demonstrate that OPN expression, which is necessary for UTP and PDGF migration, is also CREB-dependent. Gel shift and ChIP assays reveal that CREB binds three sites on the OPN promoter: a CRE site (–1410) and two AP-1 sites (–1872 and –76) by forming a multicomplex CREB/c-Fos. Using gene reporter assays with mutated constructions, we show the two AP-1 sites are involved in UTP-induced OPN expression, while CRE and AP-1 –76 sites are involved in PDGF induction. So we demonstrate that CREB is involved in SMC migration and OPN expression induced by agonists of either G-protein coupled receptor (UTP) or tyrosine kinase receptor (PDGF).

PP-69
Stress hormones and cytokine release from PBMC: mode of action

L. Jansky, T. Matejovska and A. Stara
Faculty of Biology, University of South Bohemia, C. Budejovice, Czech Republic. E-mail: Janskyl@seznam.cz

Cytokines are released from lymphocytes by exocytosis. Various substances, e.g. lipopolysaccharide (LPS) stimulate cytokine release by stimulating protein synthesis. While the pathway leading to increased protein synthesis after administration of LPS is well described, the detailed mode of action of various cytokine releasing substances (CRS) on cytokine exocytosis has not been not described in detail. The time course of action of different concentrations of different CRS (noradrenaline, IL-1 beta) on release of different cytokines from human PBMC was studied. Noradrenaline (NA) in physiological doses increases the release of IL-1 beta, TNF alfa and L-6, while higher concentrations of NA inhibit release of these cytokines. The release of INF gamma is not influenced. The effect of NA is not mediated by specific adrenergic receptors, because the response is delayed and small. A typical dose-response curve cannot be established. IL-1 beta stimulates the release of TNF alfa. Its effect, being immediate and long lasting, is mediated by specific receptors. Release of IL-6 is not influenced. The data indicate selectivity of IL-1 beta action on different groups of lymphocytes. Data suggest that the mode of action of different humoural substances on cytokine release from PBMC is mediated not only by activation of specific receptors and by mechanisms stimulating protein synthesis, but also by mechanisms facilitating exocytosis, probably due to modulation of cell membrane properties.

PP-70
Cell surface hsp90 interacts with the extracellular domain of her2 and contributes to receptor activation

K. Sidera, M. Gaitanou, R. Matsas and E. Patsavoudi
Department of Biochemistry, Hellenic Pasteur Institute, Athens, Greece. E-mail: sidera@pasteur.gr

HSP90 is a molecular chaperone that controls the folding assembly intracellular disposition and proteolytic turnover of many proteins most of which are involved in signal transduction processes. HER2 is a 185-kDa receptor-like glycoprotein and a member of the ErbB family of receptor tyrosine kinases that play a central role in cellular proliferation differentiation and migration. The role of HSP90 in the regulation of HER2 has been attributed to stabilization of the receptor at the cell surface via interaction with its cytoplasmic kinase domain such that disruption of the HER2/HSP90 association leads to receptor degradation. We have previously demonstrated the cell surface localization of HSP90 and its involvement in cell migration processes during development of the nervous system. In the present work we show using GST-pull down assays that surface HSP90 interacts with the extracellular domain of HER2.Furthermore we explore the effect of a function blocking monoclonal antibody against HSP90, mab4C5 on (a) phosphorylated and total levels of HER2, (b) cell invasion and (c) actin rearrangement and lamellipodia formation using MDAMB453 breast cancer cells under ligand stimulation conditions with Heregulin (HRG). Our data suggests that surface HSP90 is involved in HER2 activation and signalling by HRG contributing thus to the ligand-receptor interaction which in turn will activate the cytoplasmic signal transduction cascades leading to cytoskeletal rearrangement essential for cell motility.

PP-71
Association of a variant in exon 31 of the sulfonylurea receptor 1 (sur1) gene with type 2 diabetes mellitus and obesity in Turks

O. Evliyaoglu[1], E. Sogut[2], N. Uzuncan[1], B. Karaca[1], A. Başkesen[1] and N. Çelik[3]
[1]Department of Biochemistry, Social Security İzmir Educational Hospital, Izmir, Turkey, [2]Department of Biochemistry, Ataturk Training and Research Hospital, Izmir, Turkey, [3]Department of Biochemistry, Social Security Tepecik Educational Hospital, Izmir, Turkey. E-mail: oevliyaoglu@hotmail.com

Diabetes mellitus is a metabolic disease caused by absence of insulin or by insulin resistance that results in inappropriate insulin action. We investigated the relationship between polimorfizm of exon 31 of SUR1 gene with obese and non-obese Type II Diabetes Mellitus (DM). SUR1 gene codes the SUR1 protein that takes part in secretion of insulin. Study is planned with 17 healthy persons, 20 non-obese Type II DM, 25 obese Type II DM. We determined the serum glucose, cholesterol, trigiseride levels, and blood (%) HbA1c. DNA is extracted from peripheral blood. Single Nucleotide Polymorphisms (SNP) is determined by Restriction Fragment Length Polymorphism (RFLP). We observed a significant association between A allele and Type II DM ($P < 0.05$) and this was stronger in obese Type II DM patients while there were no association with the non-obese Type II DM patients. The patients with hypertrigliseridemia showed the same significant association with A allele frequency. This study reports that SNP's of exon 31 of SUR1 gene can be used as a risk factor in Tip II DM, and in determining the other risk factors, the genes that participate in obesity must be considered more carefully.

PP-72
N-terminal conformational changes of dopamine transporter determined by FRET analysis

O. Orun[1], S. G. F. Rasmussen[2], J. H. Cha[3], A. H. Newman[3], J. A. Javitch[4] and U. Gether[2]
[1]*Marmara University School of Medicine, Department of Biophysics, Istanbul, Turkey,* [2]*Molecular Neuropharmacology Group, Department of Pharmacology, The Panum Institute, University of Copenhagen, Denmark,* [3]*Medicinal Chemistry Section, NIDA-IRP, Baltimore, MD, USA,* [4]*Center for Molecular Recognition, Columbia University College of Physicians and Surgeons New York, NY, USA. E-mail: oyaorun@yahoo.com*

Dopamine transporter (DAT) is a member of monoamine transporters family. DAT is the major target for psychostimulants like cocaine and amphetamine (AMPH).The main effect of AMPH is to induce DA efflux. It has recently been shown that phosphorylation of serines in N-terminal (N-T) tail of DAT regulates the AMPH induced DA-efflux through an unknown mechanism. To address this question we are establishing techniques to characterize conformational changes of the N-T tail of DAT. By applying fluorescence resonance energy transfer (FRET) between YFP fused in the N-terminal tail and a rhodamine labelled cocaine analogue (JHC1-64) bound in the transmembrane domain of DAT the movement of the tail relative to the fixed rhodamine position could be monitored. To mimic the phosphorylated and dephosphorylated state of the N-T serines we have mutated Ser7 and Ser12 to aspartate and alanines, respectively. These mutations have been introduced in DAT constructs, one with YFP fused to the N-T end and the one introduced in position 55 of the N-T tail. FRET measurements between YFP in the YFPp1-DAT construct and the bound JHC1-64 did not result in measurable energy transfer suggesting a long distance for FRET to occur. StoD and StoA mutations did not result in measurable energy transfer either. However, in the YFPp55-DAT construct we found significant FRET. We are currently testing the effect of StoD or StoA mutations in YFPp55-DAT construct by FRET as an indication of movement of the N-T tail.

PP-73
Regulation of transcobalamin receptor expression in cobalamin (vitamin b12) deficiency

S. Kalra[1], R. Ahuja[1], E. Mutti[2], D. Veber[2], S. Seetharam[1], G. Scalabrino[2] and **B. Seetharam**[1]
[1]*Department of Medicine, Med. Coll. of Wisconsin, Milwaukee, WI, USA,* [2]*Institute of General Pathology and Center of Excellence on Neurodegenerative Diseases, University of Milan, Milan, Italy. E-mail: giuseppe.scalabrino@unimi.it*

Total gastrectomy (TG) causes cobalamin (Cbl)-deficiency followed by increases in tumour necrosis factor (TNF)-α and homocysteine (HCY) levels in the spinal cord of the rat. In order to understand how these events may influence Cbl transport, we have measured by immunoblotting membrane transcobalamin-receptor (TC-R) levels using both animal and cell models. TC-R protein levels were elevated (2- to 3-fold) in the total membranes of kidney, liver and intestine of rats made Cbl-deficient by either TG (maintained for 2, 4, and 8 months) or feeding Cbl-deficient diet for 12 months. However, elevation of TC-R levels in the spinal cord was delayed and occurred after 8 months of TG or 12 months of feeding Cbl-deficient diet. Postoperative Cbl-replacement treatment normalized the TC-R levels. Treatment of human intestinal epithelial Caco-2 cells with TNF-α or addition of HCY during culture resulted in 8- to 10-fold upregulation of TC-R levels. These data indicate that in Cbl deficiency (however induced): (a) TC-R is upregulated in most tissues, (b) increases in TNF-α and HCY levels may be responsible for TC-R upregulation and (c) TC-R upregulation may facilitate increased import of Cbl in cells under stress of Cbl-deprivation.

PP-74
The role of protein kinase C in migration of neuroblastoma cells

H. Stensman and C. Larsson
Laboratory Medicine, Molecular Medicine, Lund University, Malmö, Sweden. E-mail: helena.stensman@med.lu.se

The capacity of cancer cells to migrate is crucial for its malignancy. Here we demonstrate that stimulation with platelet-derived growth factor (PDGF) induces an increased migration of SK-N-BE(2)C neuroblastoma cells. Treatment with the general PKC inhibitor GF109203X or the inhibitor of the classical isoforms Gö6976 completely inhibits migration while an inhibitor of PKCβ isoforms, LY333531, partially suppresses PDGF-induced migration. Experiments using PD98059 and LY294002, specific inhibitors of the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K), respectively, show that treatment with PD98059 does not inhibit PDGF-induced migration while LY294002 has some inhibitory effect. 12-O-tetradecanoylphorbol-13-acetate (TPA) is an activator of PKC and here we show that TPA induces an increased migration and this is inhibited by GF109203X and Gö6976. Neither a MAPK inhibitor nor a PI3K inhibitor could inhibit TPA-induced migration. Thus, activation of a classical PKC isoform is sufficient to drive migration of neuroblastoma cells and crucial for PDGF-induced migration. PDGF partially signal via the PI3K pathway while the MAPK pathway is not necessary for either PDGF- or TPA-induced migration.

PP-75
Characterization of [^{35}s] GTPγs binding stimulated by endomorphin-2 and morphiceptin analogues

A. Janecka[1], J. Fichna[1], M. Piestrzeniewicz[1], J. Costentin[2] and J-C. do-Rego[2]
[1]*Laboratory of Biomolecular Chemistry, Medical University of Lodz, Lodz, Poland,* [2]*Laboratoire de Neuropsychopharmacologie Expérimentale, CNRS-FRE 2735, IFRMP 23, Université de Rouen, Rouen, France. E-mail: ajanecka@zdn.am.lodz.pl*

Endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2) and morphiceptin (Tyr-Pro-Phe-Pro-NH_2) are two structurally related endogenous opioid peptides with high affinity and selectivity for the μ-opioid receptor. The aim of the present study was to examine the properties of endomorphin-2, morphiceptin, and their analogues, modified in position 3 or 4 by introducing 3-(1-naphthyl)-D-alanine (D-1-Nal) or 3-(2-naphthyl)-D-alanine (D-2-Nal), using a functional [^{35}S] GTPγS binding assay. Endomorphin-2, morphiceptin, and their analogues were synthesized by a standard solid-phase procedure using techniques for Fmoc-protected amino acids. The [^{35}S] GTPγS binding assays were performed on rat thalamus membrane preparations. Endomorphin-2 and morphiceptin stimulated [^{35}S] GTPγS binding in a naloxone-reversible, dose-dependent, and saturable manner. Two novel analogues, [D-1-Nal^3] endomorphin-2 and [D-1-Nal^3] morphiceptin, were μ-receptor agonists. [D-2-Nal^3] endomorphin-2, [D-1-Nal^4] endomorphin-2, [D-2-Nal^4] endomorphin-2, and [D-2-Nal^3] morphiceptin had antagonist properties at the μ-opioid receptor. The

most potent μ-receptor antagonist was [D-2-Nal^3] endomorphin-2.

Conclusion: The size and topographical location of the aromatic ring of the position 3 and 4 amino acid residues seem to be critical for the stimulation of the [^{35}S] GTPγS binding and the activation of the downstream effector systems.

PP-76
The effect of phospholipase C in angiotensin II-induced p42/p44 MAPK phosphorylation in cultured vascular smooth muscle cells

A. Cetin and A. Yesilkaya
Department of Biochemistry, Faculty of Medicine, Akdeniz University, Antalya, Turkey. E-mail: arzudolgun@akdeniz.edu.tr

Angiotensin II (Ang II) is the active component of the renin-angiotensin system, which has an important role in atherosclerosis, hypertension, pathogenesis of cardiovascular diseases and regulating blood pressure. It was shown that, in vascular smooth muscle cells (VSMC), by stimulating Gq protein through AT1 receptors, Ang II activates highly complex intracellular signalling pathways, which were known as ERK1-2 or p42/p44 Mitogen Activated Protein Kinase (MAPK). These immediate signal transduction processes are, G protein mediated activation of phospholipase C (PLC), leading to phosphatidylinositol hydrolysis, formation of inositol trisphosphate (IP3) and diacylglycerol accumulation (DAG), increase in cytosolic free calcium concentration (Ca^{2+}), activation of protein kinase C (PKC), and vascular constructions/MAPK activations. This study was aimed to investigate whether or not PLC activation has a role in MAPK phosphorylation after stimulation with Ang II in VSMC cultured. Phosphorylation was shown using western-blot techniques with specific phospho-antibodies against MAPK proteins. In cultured rat vascular smooth muscle cells, Ang II induced a rapid increase in MAPK activity through the Ang II type 1 receptor. The Ang II-induced MAPK activation was inhibited by the phospholipase C inhibitor, U73122. Our results showed that Ang II-induced MAPK activation might be PLC depended.

PP-77
Immunohistochemical assay of enolase expression as plasminogen receptor on surface of rat and human muscle cells

J. Pietkiewicz, E. Seweryn, J. Saczko and T. Banas
Department of Medical Biochemistry, Medical University, Wroclaw, Poland. E-mail: egdn@bioch.am.wroc.pl

Enolase is a glycolytic enzyme in the cell cytosol of all organisms metabolizing glucose on Embden-Meyerhoff-Parnas pathway. This protein has been also identified on the surface of many eukaryotic and prokaryotic cells, where it plays a role as effective plasminogen receptor [1, 2]. Many reports are available demonstrating the direct correlation between increased expression of enolase and progression of tumours such as neuroendocrine tumours, neuroblastoma and lung cancer [3]. Such high enolase expression as well as its surface localization indicates that the enzyme may serve some other functions except for its involvement in glycolysis. It may bind certain extracellular ligands such as plasminogen, which would enable proteolysis of ECM, facilitating tumour growth. In the present report we demonstrated localization of enolase protein on the surface and in the cytosol of normal and transformed rat muscle cells and human sarcoma cells by electron microscope technique and immunohistochemical analysis. We compared interaction of cell surface enolase-like receptor with plasminogen on sarcoma and normal rat muscle cells in different conditions.

References

1. Ge J, Calt MD, Gregory RL. Infect Immun 2004; 72: 6748–6752.
2. Merkulova T, Lucas M, Jabet N, Rouzeau JD, Gros F, Lazar M, Keller. A. Biochem J 1997; 323: 791–800.
3. Pancholi V. Cell Mol Life Sci 2001; 58: 902–920.

PP-78
Establishment of genetically engineered neural cells that express doxycycline-inducible TrkC

D. Klopotowska, L. Strzadala, E. Ziolo and J. Matuszyk
Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland.
E-mail: matuszyk@iitd.pan.wroc.pl

TrkC is a high affinity receptor for neurotrophin-3 (NT-3). The goal of this study was to construct genetically engineered neural cells that express TrkC under the control of a doxycycline (DOX)-inducible promoter. MBG18 is a neural cell line derived from brain of mouse embryos (BBRC 309:91), stably transfected to express the reverse tetracycline-responsive transactivator (rtTA) under the control of the EF1alpha promoter. The aim was to engineer the cells (MBG18 and PC12 Tet-On) to express the target gene (firefly luciferase, TrkC) at high level in response to DOX and at low level in the absence of DOX. We studied the expression of luciferase gene driven by either tetracycline-response element (TRE) or modified TRE (TRE-tight). We found that although DOX-induced expression of luciferase target gene linked to TRE-tight promoter was much higher than for TRE promoter, uninduced expression in the absence of DOX was also higher for TRE-tight promoter. However, leaky expression of target gene was almost completely eliminated by tetracycline-responsive transcriptional silencer (tTS). In conclusion, application of tTS in combination with TRE-tight-driven target gene leads to low leaky and high DOX-induced expression of target gene of interest. Finally, we demonstrate that NT-3 treatment led to activation of signalling pathways in cells showing DOX-induced expression of TrkC receptor.

Acknowledgment: This work was supported by grant PBZ-MIN-005/P04/2002.

PP-79
P10, a HARP derived peptide that exhibits anti tumour biological actions

Z. Diamantopoulou[1], O. Bermek[2], Ch. Birmbas[1], J. Courty[2] and P. Katsoris[1]
[1]*Department of Biology, University of Patras, Patras, Greece,*
[2]*Biology, University Paris, Paris, France.*
E-mail: katsopan@upatras.gr

HARP (Heparin Affin Regulator Peptide) is an 18 kDa growth factor (1) detected in various tissues and cell lines (2). HARP displays several biological actions, such as induction of cellular proliferation, migration and angiogenesis, indicating its possible involvement in carcinogenesis. Recently, we have identified and characterized several HARP's proteolysis fragments with either similar or opposite to HARP's biological activities (3). In the present work, we investigated the biological activity of P10, a synthetic peptide corresponding to HARP residues 122–131. Our results suggest that P10 inhibits the *in vitro* proliferation, adhesion, migration and anchorage independent cell growth of human prostatic tumour cell lines PC3 and DU145. Using Western blot analysis, we showed that SRC, AKT, ERK1/2 kinases and PTEN

phosphatase are activated following a treatment with P10. In addition, studies of the mechanism of action indicated that P10 interfered with the binding of HARP receptors ALK and RPTPb/z. Furthermore, we have shown that P10 inhibited *in vivo* angiogenesis on the chicken embryo CAM assay. Taken together these results indicated that P10 could be constitutes an interesting tool for tumour therapy strategy.

References

1. Rauvala H. Embo J 1989; 8, 2933–2941.
2. Wellstein A et al. J Biol Chem 1992; 267: 2582–2587.
3. Polykratis A et al. Int J Biochem Cell Biol 2004; 36: 1954–1966.

PP-80
Farnesyl phosphates are endogenous ligands for lysophosphatidic acid receptors

K. Liliom[1], A. Baksa[1], T. Tsukahara[2], R. Tsukahara[2], M. Zelman-Femiak[3], E. Swiezewska[3] and G. Tigyi[2]
[1]*Institute of Enzymology, Hungarian Academy of Sciences, Budapest, Hungary,* [2]*Department of Physiology, University of Tennessee, Memphis, TN, USA,* [3]*Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland. E-mail: attila.baksa@enzim.hu*

The polyisoprenol derivatives oligoprenyl phosphates are key metabolic intermediates for the biosynthesis of steroids, the side chain of ubiqinones, dolichols and for the posttranslational modifications of proteins. Lysophosphatidic acid (LPA) is an important lipid regulator of fundamental cellular processes like proliferation, apoptosis, differentiation and motility. Fatty alcohol phosphates, in which molecules the phosphate moiety is directly attached to a hydrocarbon chain, represent the minimal pharmacophores of LPA receptors as we have shown recently. Here we have investigated whether farnesyl phosphates, which are polyunsaturated fatty alcohol phosphates, can interact with the cell surface and nuclear receptors for LPA. Both farnesyl phosphate and farnesyl diphosphate potently and specifically antagonized the LPA-elicited intracellular Ca^{2+}-mobilization mediated through the LPA3 receptor, while causing only modest inhibition at LPA2 and had no measurable effect at LPA1. The transcription factor peroxisome proliferator-activated receptor gamma (PPARγ) is activated by LPA and its mimetics including fatty alcohol phosphates. We found that both farnesyl phosphate and diphosphate compete with the binding of the synthetic PPARγ agonist (^{3}H) rosiglitazone and weakly activate the PPARγ-mediated gene transcription. These results indicate new roles for the oligoprenyl phosphates as endogenous modulators of LPA receptors.

PP-81
Diverse effects of vascular endothelial growth factor on pulmonary endothelial barrier

T. Mirzapoiazova, I. Kolosova, P. Usatyuk, V. Natarajan and **D. Verin**
Department of Medicine, The University of Chicago, Chicago, USA. E-mail: averin@medicine.bsd.uchicago.edu

Increased endothelial permeability is involved in the pathogenesis of many cardiovascular and pulmonary diseases. VEGF is considered to be a major permeability-increasing cytokine. At the same time, VEGF is known to have beneficial effect on endothelial cells (EC), increasing their survival. The mechanisms, by which VEGF may control endothelial barrier function is not completely understood. The purpose of our work was to evaluate effects of VEGF on barrier function of cultured human pulmonary artery EC (HPAEC). We found that 10 ng/ml VEGF significantly improved barrier properties of HPAEC, as indicated by transendothelial resistance measurement. In contrast, challenge with 100 ng/ml VEGF decreased endothelial barrier and caused disruption of adherence junctions. VEGF at both concentrations increased cellular migration; however, 10 ng/ml VEGF had significantly stronger effect. VEGF caused dose-dependent increase in intracellular Ca^{2+} concentration, however phosphorylation of myosin light chain was detectably elevated after treatment with 100 ng/ml only. In contrast, 10 ng/ml VEGF caused significant increase in intracellular cAMP and Y576-specific phosphorylation of focal adhesion kinase. Our data suggest that depending on its concentration, VEGF may cause diverse effects on pulmonary endothelial permeability via different signalling pathways.

PP-82
Thyroid-stimulating hormone promotes the growth of human melanoma cells

J. A. Ellerhorst, M. K. Johnson, C. P. Cooke and A. H. Diwan
Department of Experimental Therapeutics, M.D. Anderson Cancer Center, Houston, TX, USA. E-mail: jaellerh@mdanderson.org

We have reported a high prevalence of hypothyroidism in the cutaneous melanoma population, suggesting that the pathologic hormonal environment of hypothyroidism promotes melanoma growth. The objective of this study was to test the hypothesis that thyroid-stimulating hormone (TSH), which is elevated in the circulation of hypothyroid individuals, stimulates the growth of melanoma cells. TSH receptors were detected by immunostaining in all benign nevi, dysplastic nevi, and melanomas examined. There was a trend toward increased staining intensity in melanomas when compared to benign nevi, suggesting that melanomas may express high levels of the receptor. Melanoma cells and cultured melanocytes both responded to physiologically relevant concentrations of TSH by alterations in cAMP levels. In the presence of TSH, melanoma cells activated the MAPK and PI3K pathways as evidenced by phosphorylation of ERK and Akt. These pathways were not activated in melanocytes. Furthermore, melanoma cells, but not melanocytes, demonstrated a proliferate response to TSH. We conclude that melanoma cells have phenotypic features similar to thyrocytes: they carry TSH receptors, respond to TSH through cAMP, activate growth related signal pathways, and proliferate. These findings support the hypothesis that hypothyroidism promotes melanoma growth through TSH. Clinical studies are warranted to examine the association of hypothyroidism and elevated TSH levels with outcomes of melanoma patients.

PP-83
Activation of mitogen activated protein kinases by purinergic receptors in endothelial cells

M. Montiel and E. Jiménez
University of Malaga. E-mail: ejg@uma.es

The effect of extracellular nucleotides on mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) in human umbilical vein endothelial cells (HUVEC) has been investigated. ATP, 2-meSATP, UTP and UDP cause a rapid and transitory increase in the phosphorylation of MAPK/ERK, but a negligible response was seen for P2X receptors agonist α, β-meATP. MAPK/ERK activation by ATP was prevented in

cells pre-treated with pertussis toxin (PTX), PD98059, a MEK inhibitor, and wortmannin and LY294002, two selective phosphoinositide 3-kinase (PI3K) inhibitors, but not by U73122, an inhibitor of phospholipase C (PLC) or a calcium-free medium. Furthermore, an inhibition of ATP-dependent MAPK/ERK phosphorylation was observed in HUVEC pre-treated with high doses of GF109203X, a non-selective protein kinase C (PKC) inhibitor, or myristoylated PKC-ζ pseudosubstrate, a specific inhibitor of PKC-ζ. We also found that ATP stimulates both the phosphorylation of 3-phosphoinositide-dependent protein kinase-1 (PDK1) and its translocation to plasma membrane. These observations suggest that the effect mediated by ATP on MAPK/ERK activation in HUVEC occur via P2Y receptors through down-stream mechanisms dependent of PI3K.

PP-84
Investigation of the PKC and Raf-1 interaction

L. Sunesson and C. Larsson
Molecular Medicine, Laboratory Medicine, Malmoe, Sweden.
E-mail: lovisa.sunesson@med.lu.se

Neuroblastoma is one of the most common solid tumours in childhood and the malignancy has a rather poor prognosis. Most neuroblastomas are undifferentiated tumours, consisting of neuroblasts lacking neuronal processes. The protein kinase C (PKC) family of protein kinases has been implicated to play roles in many different cellular processes. For example, it has been shown to be involved in the process of neurite outgrowth. We have previously shown that introduction of the regulatory domain of the novel PKC isoform ε (PKCεRD) in neuroblastoma cells induce neurite outgrowth. However, by which mechanism PKCε accomplish this is still unknown. One way of studying this effect has been to investigate different possible interaction partners to PKCε. Raf-1 is another important kinase involved in many different signal transduction pathways and it has been shown to interact with PKC, especially with PKCε. In our study, we have investigated the structures in PKC that enable this interaction. By using co-immunoprecipitation techniques, we have shown that both full-length classical and novel PKC isoforms bind Raf-1, as well as the regulatory domains of the different isoforms. Furthermore, our data suggests that PKCε binds Raf-1 both via its regulatory as well as its catalytic domain. Additional studies on the substructures of the regulatory domain of PKCε indicate that C1a and C2 bind Raf-1 better than the C1b domain.

PP-85
Isolated native, oxidized LDL and HDL influence platelet binding characteristics of fibrinogen and glycoprotein IIb/IIIa

D. Özsavcı[1], H. Avcı[1], A. Şener[1], E. Enç[1], B. Vanizor Kural[2], G. Yanıkkaya Demirel[3] and K. T. Yardımcı[1]
[1]*Biochemistry Department, Marmara University Faculty of Pharmacy, İstanbul, Turkey,* [2]*Biochemistry Department, Karadeniz Technical University Faculty of Medicine, Trabzon, Turkey,* [3]*Centro Laboratory, İstanbul, Turkey.*
E-mail: deryaozsavci@hotmail.com

Background: LDL and oxidized LDL (ox-LDL) enhance platelet function via binding to its receptors on platelets, which are different from the 'classical' receptors of the nucleated cells. The present study was designed to observe the effects of isolated LDL, ox-LDL and HDL to platelet binding properties of fibrinogen and Glycoprotein (Gp) IIb/IIIa.

Methods: Washed platelets (WP) were prepared from of nine healthy volunteers. Human LDL and HDL were separated by density gradient ultracentrifugation and LDL was oxidized with CuSO4 for 24h at 37°C. ADP (10 μM) induced WP were treated with increasing concentrations of LDL/ox-LDL (7.5–400 μg/ml) but HDL was added to final three concentrations. Expressions of GPIIb/IIIa (CD41a) and antifibrinogen were measured by flow cytometry. Results were converted to specific antibody binding capacities per platelet (plt).

Results: Following ADP activation, levels of antifibrinogen/plt and CD41a/plt increased significantly ($P < 0.001$). After treatment with LDL/ox-LDL (7.5–400 μg/ml), levels of CD41a/plt decreased significantly ($P < 0.001$) whereas levels of antifibrinogen/plt increased significantly in dose dependent manner ($P < 0.001$), however, addition of HDL inhibited the increase in antifibrinogen ($P < 0.001$).

Conclusion: These data showed that while LDL and ox-LDL enhanced fibrinogen binding to platelets, they also abolished the binding of antiGpIIb/IIIa to platelets dose dependently. HDL reversed this effect of LDL/ox-LDL on only fibrinogen binding. We concluded that GpIIb/IIIa might be a receptor for LDL and ox-LDL, and it seems that binding site of these lipoproteins on GpIIb/IIIa differs from fibrinogen domain.

PP-86
Signal transduction through the AtoS-AtoC/az two component system towards poly (3-OH-butyrate) biosynthesis in *E. coli*

M. C. Theodorou[1], E. C. Theodorou[1], C. A. Panagiotidis[2] and D. A. Kyriakidis[1]
[1]*Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece,* [2]*Department of Pharmaceutical Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece. E-mail: marina_theodor@hotmail.com*

The AtoS-AtoC/Az two-component system activates the atoDAEB operon expression upon acetoacetate induction for *E. coli* growth in short-chain fatty acids. It also enhances the poly (3-OH-butyrate) (cPHB) biosynthesis, upon acetoacetate induction as well as in the presence of spermidine. The response regulator of the system is the antizyme (Az) of ornithine decarboxylase and is the product of *atoC* gene. It belongs to the NtrC-NifA family of sigma54-RNA polymerase transcriptional activators. AtoC contains two putative phosphorylation sites, i.e. a conserved aspartic acid among the response regulators and a histidine residue in an H box consensus sequence, normally common to histidine kinases. We report here, that only phosphorylation-competent AtoC can lead to enhanced production of cPHB in *E. coli*, when overexpressed with AtoS. Specifically, upon acetoacetate induction, the mutation of Asp reduces cPHB accumulation, compared with cells expressing wild-type AtoC. The mutation of His residue has an even more pronounced effect. The relative effects of these mutations on cPHB accumulation are consistent with their effects on atoDAEB operon expression, i.e. the mutation of Asp has a more potent phenotype than the substitution of His, in the presence of spermidine. Introduction of both AtoC mutations render the system unresponsive to acetoacetate as well as polyamine, resulting in total abrogation of the AtoS-AtoC/Az overexpression effect phenotype to cPHB levels in *E. coli*.

PP-87
Reactivity of antibodies against *K. pneumoniae* enolase and some cell wall omp of *K. pneumoniae* and *P. aeruginosa* strains

I. S. Bednarz, I. Ceremuga, J. Pietkiewicz and T. Banas
Department of Medical Biochemistry, Medical University, Wroclaw, Poland. E-mail: ibednarz@bioch.am.wroc.pl

The glycolytic enzyme alpha-enolase, despite its common catalytic function in cytosol compartment of the cell, constitutes a receptor for plasminogen at human, mammals and fungi bacterial cell surface [1]. Pericellularly promotions of plasminogen activation in Gram-positive *Streptococcus pneumoniae* bacterial strain plays a critical role in fibrinolysis and degradation of extracellular matrix and appears one of important factors of the cell transmigration and host tissue colonization [2]. Enolase-like proteins were identified also in the cell wall outer membranes of some Gram-negative bacteria [3]. In our previous studies we obtained homogenic enolase from cytosolic fraction of *Klebsiella pneumoniae* cells. The aim of present report was to obtain rabbit polyclonal antibodies specific against *Klebsiella pneumoniae* enolase. In SDS-polyacrylamide gel electrophoresis and immunoblotting assay we demonstrated interaction of these antibodies with purified enolase-like protein from cell wall outer membrane fraction of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* cells. Our results provided evidence that some similarity of epitopes between cell wall outer membrane proteins from *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and glycolytic enzyme – enolase existed in cytosol of *K. pneumoniae* cells.

References:

1. Pancholi V. Cell Mol Life Sci 2001; 58: 901–920.
2. Bergman S et al. Thromb Hoemost 2005; 94(2): 304–311.
3. Witkowska D et al. FEMS Immunol Med Microbiol 2005, 45(1): 53–62.

PP-88
Structural analysis of the RCS signalling pathway in pathogenic enterobacteria

V. Rogov, N. Rogova, K. Sengupta, F. Loehr, F. Bernhard and V. Doetsch
Institute of Biophysical Chemistry, University of Frankfurt, Frankfurt, Germany. E-mail: rogov@bpc.uni-frankfurt.de

Bacteria as well as lower eukaryotes use phosphorylation cascades in order to respond to changing environmental conditions. A key mechanism of signalling pathways is the communication of membrane integrated sensor kinases with cytoplasmatic effector proteins by complex reversible phosphorylation reactions involving multistep phosphorelay systems. The Rcs regulatory network is a global signalling system that controls a variety of operons involved in capsule synthesis, virulence, motility or cell division. The membrane bound sensor unit is formed by a heterodimer of the hybrid kinases RcsC and RcsD while the cytoplasmatic effectors RcsA and RcsB form a heterodimer upon DNA-binding. The arrangement of enzymatic domains with histidine kinase, phospho-receiver, phospho-transfer and DNA-binding activities is characteristic for the Rcs system and essential for the modulation of signal transfer. We present the structural evaluation of three central functional domains of this signalling system by heteronuclear high resolution NMR spectroscopy. We further describe the so far unique structural fold of a newly identified domain integrated in the Rcs sensor kinases as well as in kinases of other bacterial signalling systems. Phospho-transfer mechanisms, the complex formation between sensor and effector proteins and the phosphorylation dependent DNA binding activity of Rcs effector proteins has been analysed by multiple approaches and will be presented.

PP-89
The RGD-independent signalling pathway in response to fibronectin-tissue transglutaminase complex

D. Telci[1], H. Basaga[1], E. Edwards Verderio[2], X. Li[3], T. Tezil[1], M. Baccarini[4] and M. Griffin[3]
[1]Biological Sciences and Bioengineering Program, Faculty of Engineering and Natural Sciences, Sabanci University, Orhanlı Tuzla, Istanbul, Turkey, [2]School of Biomedical and Natural Sciences, The Nottingham Trent University, Clifton, Nottingham, UK, [3]School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham, UK, [4]Department of Microbiology and Genetics, Max F. Perutz Laboratories, University Departments at the Vienna Biocenter, Vienna, Austria.
E-mail: teziltugsan@su.sabanciuniv.edu

Tissue transglutaminase (TG2) may act as a cell surface adhesion mediator by its association with fibronectin (FN). Formation of a FN-TG2 complex provides pro-survival and distinct adhesive characteristics. Here we show the RGD (Arg-Gly-Asp) independent signalling pathways in cell adhesion. To investigate the novel RGD-independent mechanism, we inhibited the typical integrin-mediated adhesion by using RGD peptides. The rescue of cell adhesion does not depend on the binding of FN-TG2 complex to $\alpha_4\beta_1$ integrins. However, RGD-independent cell adhesion is inhibited by heparitinase digestion suggesting that FN-TG2 complex interacts with a heparan sulfate proteoglycan receptor. The cooperative effect of syndecan-2 with -4 during FN-TG2 mediated RGD-independent cell adhesion was further investigated using syndecan-4 null fibroblasts and siRNA technology. We previously showed that RGD-independent cell adhesion to FN-TG2 was linked to the activation of focal adhesion kinase. Here we show that RGD-independent cell adhesion pathway by FN-TG2 is not functional in c-Raf-1 null fibroblasts. Moreover, the results showing the activation of ERK and JNK suggest that the MAPK pathway is involved during this process. This study indicates that binding of TG2 to FN represents a novel cell adhesion signalling mechanism through the MAPK survival pathways, which can either act in synergy or as an alterative to integrin RGD-dependent cell adhesion at sites of tissue injury.

PP-90
Regulation of M2 muscarinic receptor expression in k562 cells by carbachol

H. Cabadak, B. Aydın and **B. Kan**
Biophysics, Marmara University, Istanbul, Turkey.
E-mail: hcabadak@yahoo.com

Muscarinic receptors belong to the G protein coupled receptor family. Multiple subtypes of muscarinic receptors are expressed in different human cells. These receptors mediate a variety of cellular responses, including inhibition of adenylate cyclase, PI hydrolysis and regulation of K channels. mAChR subtypes M1, M3, M5 lead to activation of phospholipase C and hydrolysis of inositol 4,5 biphosphate. M2 and M4 receptors inhibit adenylyl cyclase activity via PTX-sensitive Gi protein and cause only a modest stimulation of PI hydrolysis when overexpressed. Agonists change muscarinic receptor expression in a number of cell lines. Our previous studies have demonstrated that K562 cells express m2, m3 and m4 muscarinic acetylcholine receptors (mAChR). In this study, we were interested in investigating the

effect of agonist stimulation on the levels of muscarinic receptor m2 protein expression in K562 cells. We have therefore used Western blotting procedures to monitor the changes in m2 receptor protein level in K562 cells treated with carbachol. K562 cells grown RPMI 1640 medium supplemented with 10% FCS at 37°C for 1, 3, 5, 24 and 48 h. Proteins were separated by polyacrylamide gel electrophoresis followed by Western blot analysis which demonstrated that M2 protein level decreased upon carbachol treatment.

Acknowledgment: This work was supported by grant from L'ORĒAL Turkey (UNESCO).

Keywords: Muscarinic receptors, carbachol, K562 cell.

PP-91
Structural and functional analysis of cell-free expressed integral membrane proteins

D. Schwarz, C. Klammt, F. Loehr, A. Shypitsyna, S. Sobhanifar, B. Schneider, A. Koglin, V. Doetsch and F. Bernhard
Institute of Biophysical Chemistry, University of Frankfurt, Frankfurt, Germany. E-mail: fbern@bpc.uni-frankfurt.de

Membrane proteins are involved in many human diseases but extreme difficulties upon production of sufficient amounts have excluded them so far almost completely from structural analysis. Recent advances in the high-level cell-free production of integral membrane proteins enable now production rates of several milligram of protein per 1 ml of reaction and labelled samples suitable for analysis by NMR spectroscopy can be generated in as fast as 24 h. The synthesized membrane proteins can furthermore be inserted in the desired detergent micelles directly upon translation. We have produced a variety of structurally and functionally diverse membrane proteins from prokaryotic and eukaryotic origins including G-protein coupled receptors and multi-drug transporters. Highly effective detergents for the solubilization of cell-free produced membrane proteins have been selected and we could establish modified protocols for the production of functionally folded membrane proteins. The ligand binding activity, oligomeric complex formation and functional reconstitution of the endothelin B receptor and of the vasopressin receptor were analysed by multiple approaches. We further demonstrate that rationally designed combinatorial labelling schemes in combination with cell-free expression result in the rapid assignment of even larger alpha-helical membrane proteins and we present the structural evaluation of the multifunctional drug transporter TehA containing five transmembrane segments.

PP-92
SHP-1 regulates pc3 cells migration through the modulation of SRC activity and its interaction to focal complexes

F. J. Rodríguez-Ubreva, A. E. Martinez-Cariaga, M. C. Almaraz-Pro, M. P. López-Ruiz and B. Colás-Escudero
Department of Biochemistry, University of Alcalá, Alcalá de Henares, Madrid, Spain. E-mail: pilar.lopezruiz@uah.es

Prostatic tumour cell migration to bone is a significant event contributing to morbidity and mortality associated with prostate cancer. This process is partly controlled by tyrosine kinases and tyrosine phosphatases. Recently, we have demonstrated that the tyrosine phosphatase SHP-1 is expressed in normal human prostate, but not in poorly differentiated prostate cancer. Thus, we analysed the role of SHP-1 in the regulation of cellular adhesion and migration on collagen type I, the major bone extracellular matrix (ECM) component, where prostate cancer preferentially metastasizes. Our results show that, in PC3 cells, SHP-1 associates, in a molecular complex, with focal adhesion kinase (FAK) and Src, both of which are implicated in cell adhesion and migration processes, this association being regulated by collagen type I. PC3 cell adhesion on this ECM component induces the release of Src from the complex, coinciding with Src dephosphorylation at the Tyr-416 but not Tyr-527 residue. Moreover, RNAi-mediated gene silencing of SHP-1 in PC3 cells induces Src phosphorylation at both Tyr-416 and Tyr-527. In addition, SHP-1 knock down decreases cell migration on collagen type I. These results suggest that the tyrosine phosphatase SHP-1 plays a crucial role in prostate cancer progression, regulating PC3 cell migration via modulation of Src activity and its association to focal complexes.

Acknowledgment: Source of Funding: FIS (PI020964), C.S. Castilla la Mancha (04077-00) & Fundación MMA.

PP-93
The extracellular linker of transmembrane neuregulins regulates their sorting and juxtacrine function

J. C. Montero, R. Rodríguez-Barrueco, L. Yuste, P. P. Juanes, J. da Silva Borges, L. Ferreira, A. Esparis-Ogando and A. Pandiella
Centro de Investigación del Cáncer (CSIC), Salamanca. E-mail: ruthroba@hotmail.com

Membrane-anchored polypeptide factors play important roles in animal physiology, and their disregulation has been linked to diseases such as cancer. Membrane-anchored factors may undergo proteolytic cleavage at their ectodomains to generate soluble forms of these factors. Whether this shedding event is necessary for their action is still a matter of debate. During studies aimed at solving this question in the case of pro-Neuregulin (proNRG), we found that a small region locatedin the extracellular juxtamembrane domain participates in the sorting of proNRGα2c to the plasma membrane. Deletion of this region, termed the linker, caused intracellular entrapment of the mutant proNRG Linker form. This mutant accumulated at the cis-Golgi, and at this location it was biologically inactive, as indicated by its failure to stimulate ErbB receptors and cell proliferation. In contrast, more subtle mutations of the linker that allow correct sorting to the plasma membrane but prevent cleavage, demonstrated that cell surface-exposed proNRG forms were biologically active. These results indicate that structural information present in the linker is required for efficient cleavage and sorting of proNRG to the plasma membrane, and opens the possibility to the existence of a Golgi checkpoint that may control proper trafficking of membrane-bound growth factors.

PP-94
The carboxyl-terminal tail of the mu opioid receptor – docking site for RGS4 protein binding

L. J. Leontiadis[1], H. E. Hamm[2] and Z. Georgoussi[1]
[1]*Laboratory of Cellular Signalling and Molecular Pharmacology, Institute of Biology, N.C.S.R. 'Demokritos', Athens, Greece,*
[2]*Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN, USA.*
E-mail: lleontas@bio.demokritos.gr

Opioid receptors modulate a variety of physiological responses in the nervous system and belong to the superfamily of G protein coupled receptors (GPCRs). Opioid receptor signalling mechanisms have demonstrated that the third intracellular loop and the carboxyl tail (CT) are critical in mediating the signal through the G proteins and are also known to mediate protein-protein

interactions by recruiting novel cytoplasmic proteins at specific modular domains located in them (Georgoussi et al., 1997; Mazarakou and Georgoussi 2005). Regulator of G protein signalling (RGS) proteins are molecules that serve as GTPase activating proteins and effector antagonists acting upon members of G proteins. Recent observations reveal that these proteins can directly interact with GPCRs and serve as scaffolds regulating their function. In order to map opioid receptor subdomains important for interaction and begin to identify components of a putative signal transduction complex mediated by these intracellular domains, we focused on the CT of the μ-opioid receptor (μ-CT) and generated glutathione-S-transferase (GST) fusion proteins to be used as probes to screen for new interacting proteins. We were able to demonstrate for the first time that RGS4 protein (a) binds directly and selectively with the μ-CT, (b) forms stable heterotrimeric complexes with the μ-CT and active Gα subunits, (c) modulates DAMGO-mediated adenylyl-cyclase inhibition in HEK293 cells by acting as effector antagonist.

PP-95
Ribosomal protein l10 interacts with the SH3 domain and regulates GDNF-induced neurite growth

S. Park
Department of Applied Chemistry, Dongduk Women's University, Seoul, Korea. E-mail: sypark21@dongduk.ac.kr

The 24.5 kDa ribosomal protein L10 (RP-L10), which was encoded by QM gene, was known to interact with the SH3 domain of Yes kinase. Herein, we demonstrate that RP-L10 interacts with the SH3 domain of Src and activates the binding of the Nck1 adaptor protein with skeletal proteins such as the Wiskott-Aldrich Syndrome Protein (WASP) and WASP interacting protein (WIP) in neuroblastoma cell line, SH-SY-5y. The RP-L10 was associated with the SH3 domains of Src and Yes. It is shown that two different regions of RP-L10 are associated with the Src-SH3. The effect of ectopic RP-L10 expression on neuronal cell scaffolding was explored in cells transiently transfected with QM. SH-SY-5y human neuroblastoma cells transfected with QM were considerably more susceptible to neurite outgrowth induced by glial cell line-derived neurotrophic factor (GDNF). However, RP-L10 did not directly interact with actin assembly. Taken together, these results suggest that the RP-L10 may positively regulate the GDNF/Ret-mediated signalling of neurite outgrowth in the neuroblastoma cell line, SH-SY-5y.

PP-96
Functional relationships among egg raft-associated molecules UPIB, UPIII and Src in *xenopus* fertilization

A. K. M. Mahbub Hasan[1], K. Sato[2], K. Sakakibara[1], T. Iwasaki[2], Z. Ou[2] and Y. Fukami[1]
[1]*The Graduate School of Science and Technology, Kobe University, Kobe, Japan,* [2]*Research Center for Environmental Genomics, Kobe University, Kobe, Japan. E-mail: ripplemahbub@yahoo.com*

In *Xenopus* fertilization, egg rafts play important roles in sperm-egg interaction and subsequent activation of the raft-associated Src tyrosine kinase (xSrc) (Sato et al., 2002, 2003). Recently, we have shown the importance of Xenopus uroplakin III (xUPIII), a raft-associated single transmembrane protein in fertilization and its interaction with Xenopus uroplakin Ib (xUPIb), a raft-associated tetraspanin member (Sakakibara et al., 2005; Hasan et al., 2005). Mizote et al. in 1999 showed that cathepsin B, which has sperm protease-like activity can activate *Xenopus* eggs, although the molecular target(s) of that enzyme was not identified. We showed that xUPIII is one of the targets of sperm protease/cathepsin B, and after the cathepsin B treatment xUPIII was tyrosine-phosphorylated (Hasan et al., 2005). To analyse the relationships among xUPIb, xUPIII and xSrc, cultured HEK293 cells have been used. During co-expression of xUPIb and xUPIII, both of them co-localize to the plasma membrane and associate with rafts. Co-expression of xUPIb and xUPIII inhibit the phosphorylation at Tyr-415 of xSrc, a hallmark of xSrc activation. Treatment of the triple-expressed cells with H_2O_2 enhances the phosphorylation at Tyr-415 of xSrc and promotes xSrc to interact with and phosphorylate xUPIII. Our results imply the importance of egg membrane-associated molecules, xUPIb and xUPIII, in the regulation of xSrc activity, whose up-regulation is required for the egg activation.

PP-97
Effect of change in surface expression of the αvβ3 integrin receptor of suspended BHK 21 cells on plaque character of food and mouth disease virus (FMDV)

M. Akay[1], N. Ünal[2], G. Aynagöz[2] and F. Özyörük[2]
[1]*Biology, Hacettepe University, Ankara, Turkey,* [2]*Foot and Mouth Disease Institute. E-mail: sukranyilmaz@gmail.com*

In this study, it is aimed to reveal the effects of variations on αvβ3 integrin receptor expression at subsequent passages of BHK-21 suspension cell cultures on the character of FMD virus. Monolayer BHK-21 cell culture was adapted to suspension cell culture and the cells were passaged for 40 subsequent serial passages. The expression of αvβ3 integrin receptor was determined with Western Blotting technique in the intervals of subsequent cell culture passages before FMD virus was inoculated. Changes in plaque characteristics of FMD virus was detected with plaque assay in the samples from subsequent BHK-21 suspension cell culture passages. The progressive reduction of spreading on surfaces and abnormal expression of the αvβ3 integrin were found to be correlated with the number of passages in suspension culture. It is reported that changes occur on the surface properties of cells and significant reduction is observed in the integrin receptor expression, where these changes negatively effect plaque characteristics. Effect of change in surface expression of the αvβ3 integrin receptor of suspended BHK 21 cells on plaque character of foot and mouth disease virus (FMDV).

PP-98
Met acts on Mdm2 through mTOR to signal cell survival *in vivo*

A. Moumen, A. Tasmadjian, R. Dono and **F. Maina**
IBDML, CNRS UMR 6216, Marseille, France.
E-mail: maina@ibdm.univ-mrs.fr

Coordination of cell death and survival is crucial during embryogenesis and adulthood and alterations to this balance can result in degeneration or cancer. Growth factor receptors such as Met can activate phosphatidyl-inositol-3' kinase (PI3K), a major intracellular mediator of growth and survival. PI3K can antagonise p53-triggered cell death but the underlying mechanisms are not fully understood. Using genetic and pharmacological approaches, we demonstrated that PI3K acts through mTOR to regulate p53 activity both *in vitro* and *in vivo*. mTOR inhibits p53 by promoting translation of Mdm2, the negative regulator of p53. Increased Mdm2 protein levels require the mTOR effector p70s6k/S6 ribosomal protein. Unexpectedly, although it is required for Mdm2 nuclear translocation, Akt is dispensable for

Met-triggered mTOR activation and Mdm2 up-regulation. Inhibition of mTOR is sufficient to block cell survival induced by HGF/Met *in vitro*. Moreover, blocking mTOR by rapamycin *in vivo* down-regulates Mdm2 protein levels and induces p53-dependent apoptosis. Our studies identify a novel mechanism for cell survival, involving translational regulation of Mdm2 by mTOR, thus reinforcing mTOR as a potential drug target in cancer.

PP-99
Interaction of RSK2 with the ETS-domain transcription factor ELK-1

I. Aksan Kurnaz
Genetics and Bioengineering, Yeditepe University, Istanbul, Turkey. E-mail: iakurnaz@yeditepe.edu.tr

Immediate early gene activation upon mitogenic activation occurs through the serum response element (SRE). Activation of the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway exerts its effects through increased binding of the Ternary Complex Factor (TCF), such as Elk-1, to the SRE and transcriptional activation, and activation of a histone kinase, such as the MAPK-activated protein kinase (MAPKAP-K) ribosomal S6 kinase (RSK2). RSK2 is a serine/threonine kinase activated by ERK in response to growth factors, and mutations in the RSK2 gene lead to the Coffin-Lowry syndrome (CLS), an X-linked disorder characterized by psychomotor retardation, facial abnormalities, and progressive skeletal deformations. In this study we have investigated a direct role for Elk-1 in recruiting RSK2 to the SRE element. We show that RSK2 can directly interact with Elk-1 both in GST pulldowns and immunoprecipitation experiments, and Elk-1 deletion constructs show that that this interaction is independent of ERK binding or activation of RSK or Elk-1 proteins. Preliminary results also provide a link between the SRE element and histone modifications by RSK2 and show that this interaction facilitates Elk-1-dependent transcription. Current studies attempt at generating RSK2 deletion mutants in order to pinpoint the exact region of interaction between RSK2 and Elk-1.

PP-100
Molecular approach on breast cancer cell biology and bone metastasis

N. Karamanos, **I. Kanakis**, A. Roussidis, O. Kousidou and A. Theocharis
Laboratory of Biochemistry, Department of Chemistry, University of Patras, Patras, Greece. E-mail: N.K.Karamanos@upatras.gr

Matrix effectors involved in invasion and metastasis of breast cancer cell include growth and mobility factors, adhesion molecules, and proteolytic enzymes. Malignant cells induce a modified stroma through the expression of soluble factors that promote the altered expression of extracellular matrix macromolecules including metalloproteinases (MMPs) and proteoglycans (PGs). Modified stroma favours the growth and metastasis of malignant cells. Bone also provides a fertile microenvironment for distant metastasis of breast cancer cells. In order to examine whether gene expression of PGs and MMPs is related with breast cancer and invasive potential, we performed *in vitro* studies on a panel of breast cancer cell lines. The obtained results showed that breast cancer is associated with significant changes in gene expression of secreted and cell surface PGs as well as MMPs and their inhibitors. Studies to elucidate whether the modified gene expression of these molecules is associated with certain molecular targets and their respective signalling pathways, using specific tyrosine kinase inhibitors, such as STI571 and genistein, as well as the specific P450 aromatase inhibitor letrozole, showed that both tyrosine kinase pathways and oestrogen receptors are important modulators of gene expression. Furthermore, zoledronic acid, a third generation bisphosphonate, seems to inhibit breast cancer-induced osteolysis through suppression of MMPs gene expression.

PP-101
Megalin mediates albumin uptake in astrocytes through caveola-mediated endocytosis

A. F. Bento-Abreu, A. Velasco, A. Tabernero and J. M. Medina
Departamento de Bioquímica y Biología Molecular, Universidad de Salamanca, Salamanca, Spain. E-mail: aabreu@usal.es

We have previously shown that the uptake and transcytosis of albumin in astrocytes promote the synthesis of the neurotrophic factor oleic acid. Although the mechanism by which albumin induces oleic acid synthesis is well know, the mechanism of albumin uptake in astrocytes remains unknown. Here we show that albumin interacts with megalin, an endocytic receptor widely described in renal cells, in the surface of the plasma membrane and that this interaction is blocked by antibodies against megalin. In addition, we found that the silencing of megalin expression by using specific siRNAs also inhibits albumin uptake by astrocytes. Moreover, megalin colocalizes with caveolin-2 in the presence of albumin and the silencing of caveolin-2 expression reduces albumin uptake, indicating a caveolae-mediated endocytosis of albumin. Nevertheless, megalin has been reported to be internalized via clathrin-mediated endocytosis in the renal tubule cells. Consequently, the involvement of clathrin in albumin uptake by astrocytes is also being investigated. In brief, we describe for the first time the mechanism of albumin uptake in astrocytes, which is the initial step to promote the synthesis of the neurotrophic factor oleic acid.

PP-102
The role of megalin in the uptake of albumin-oleic acid complex by neurons

J. C. Valle, A. Tabernero and J. M. Medina
Departamento de Bioquímica y Biología Molecular, Universidad de Salamanca, Salamanca, Spain. E-mail: jcvcse@usal.es

In previous works, we have reported that albumin uptake and transcytosis in astrocytes induces the synthesis and release of the neurotrophic factor oleic acid, which promotes neuronal differentiation, but little is know about the mechanism by which the albumin-oleic acid complex is internalized in neurons. Recent studies performed in our group indicate that megalin is the receptor for albumin in astrocytes. In the present study, we try to address the possible role of megalin in the uptake of albumin-oleic acid complex by neurons. Megalin is, in some tissues, coexpressed with cubilin, and these two proteins are multiligand endocytic receptors with significant physiological functions, such as the reabsorption of albumin in the proximal tubule and also the modulation of cell signalling. In this work we show, by RT-PCR and immunocytochemistry, that megalin and cubilin are present in neurons sharing a similar cellular localization. In addition, we found that clathrin and caveolin-1 are expressed in these cells. Immunoprecipitation studies are been carried out to determine if megalin is internalized via clathrin or caveola-mediated endocytosis in neurons.

PP-103
Caveolin and c-met interaction in hepatocellular carcinoma cells

M. Cokakli[1], E. Erdal[1], O. Sagol[2], S. Karademir[3] and N. Atabey[1]
[1]*Department of Medical Biology and Genetics, Faculty of Medicine, Dokuz Eylul University, Inciralti-IZMIR, Turkey,* [2]*Pathology Department, Faculty of Medicine, Dokuz Eylul University, Inciralti-IZMIR, Turkey,* [3]*Department of General Surgery, Faculty of Medicine, Dokuz Eylul University, Inciralti-IZMIR, Turkey.*

Caveolin, which is the main protein component of caveolae, interacts with several signalling molecules such as Ras, Epidermal Growth Factor Receptor (EGFR), and Platelet Derived Growth Factor Receptor (PDGFR). It is also possible that it interacts with c-Met, which is Hepatocyte Growth Factor (HGF) receptor and a tyrosine kinase receptor. In this study, we investigated the role of caveolin cross talk in HGF/c-Met stimulated biological responses. In the first step, c-Met and caveolin expression levels were analysed in Hepatocellular Carcinoma (HCC) cell lines, including SNU-475, SNU-398, SNU-449, HepG2, Hep3B, SK-Hep1, Huh7, Mahlavu ve PLC/PRF-5. Among these cell lines, in SNU-398, c-Met and caveolin 1 and 2 and in HepG2, caveolin 1 and 2 expressions were found to be negative. Within the rest, expression of these genes were found to be variable. Interaction between c-Met and caveolin at protein level was also analysed by immunoprecipitation and immunohistochemistry. The effect of cholesterol depletion on basal and HGF induced cell proliferation, adhesion, motility and invasion were also analysed. Identification of role of caveolin in HGF/c-Met signalling will be helpful in understanding the cross talks leading to HGF stimulated biological responses such as invasion, metastasis, proliferation and their contribution level to carcinogenesis.

PP-104
Cell-extracellular matrix interactions modulate HGF induced adhesion, proliferation, motility and invasion of HCC cells

D. Ciglidag, M. Cokakli and N. Atabey
Faculty of Medicine, Department of Medical Biology and Genetics, Dokuz Eylul University, Izmir, Turkey.
E-mail: dilayciglidag@hotmail.com

Liver carcinogenesis is associated with changes in coordinated actions of several molecules like growth factors (GF), ECM proteins and proteoglycans, and their ligands. There is a close collaboration between GF's and ECM in cell adhesion, proliferation, motility and invasion. These processes are regarded as important steps in development of metastasis and may be important for liver carcinogenesis. HGF is a pleiotrophic growth factor that stimulates cell proliferation, motility and morphogenesis of a wide variety of cells via activation of its receptor, c-Met. In this study, we examined the effects of HGF and/or ECM components on HGF induced activities such as adhesion, proliferation, motility and invasion of HCC cells. We showed that fibronectin and heparin modulate responses of HCC cells to HGF and change their gene expression patterns. Heparin modestly decreased both basal and HGF induced adhesion, proliferation and motility of SK-Hep1 cells. Fibronectin but not collagen IV increased both basal and HGF induced cell motility. HGF stimulation induced a significant increase in integrin alpha6 and decrease in integrin alpha v and beta4 expression levels. In the presence of fibronectin and/or HGF, a significant increase in integrin alpha3 and alpha5 expression levels was observed. These data support the role of HGF and/or ECM components in HCC cell behaviour and may help to identify the mechanism of HGF signalling in HCC cells involving cooperation between c-Met and integrins and their ligands.

PP-104a
Expression profiling of candidate TOR signalling components in zebrafish

E. Dalkic, C. Kuscu, C. Sucularli and **O. Konu**
Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey. E-mail: ertugrul@bilkent.edu.tr

Robo2 is an axon guidance receptor, having a role also in the cell migration outside the nervous system. In our previous studies, we have identified alternatively spliced isoforms of robo2 gene in zebrafish (Dalkic et al. unpublished). Bioinformatics analysis using VxInsight showed a possible involvement of robo2 along with some other genes known to take part in embryogenesis in Tor signalling network. Tor signalling is crucial in regulation of cellular and organismal growth. Accordingly we have analysed the expression profiles of a selected set of candidate genes in the presence and absence of rapamycin, an inhibitor of Tor. Preliminary analyses by real-time rt-pcr have indicated that robo2 expression tend to increase in the presence of 20 μM rapamycin, whereas mitfa, ckit and sox9 expression levels decrease at 48 h post fertilization during zebrafish embryogenesis. In addition, we determined semi-quantitatively the expression levels of genes known to participate in pi3K/akt/tor signalling, such as gsk3b, pten, akt2, pi3K, in multiple adult tissues. Our findings also suggested that a decrease occurred in pi3K expression in the presence of rapamycin. This study represent the first report on the expression profiles of a selected set of genes likely to be involved in Tor signalling in zebrafish.

PP-105
Cortictropin-releasing factor (crf) and urocortins affect adrenal catecholamines in a CRF receptor type-specific manner

E. Dermitzaki[1], C. Tsatsanis[1], M. Venihaki[1], V. Minas[2], A. Androulidaki[1], A. Gravanis[2] and N. Margioris[1]
[1]*Department of Clinical Chemistry – Biochemistry, School of Medicine, University of Crete, Heraklion, Crete, Greece,* [2]*Department of Pharmacology, School of Medicine, University of Crete, Heraklion, Crete, Greece. E-mail: andym@med.uoc.gr*

Corticotropin-Releasing Factor (CRF) affects adrenal catecholamine production. Aim of the present work was to evaluate as a whole, the hypothesized adrenal CRF network including CRF, Urocortin 1 (UCN1), UCN2, UCN3 and their receptors CRF1 and CRF2 on catecholamine secretion and synthesis. Histochemical analysis of whole rat adrenal sections revealed a differential expression of the CRF system in the rat. More specifically, CRF and UCN2 were evenly expressed throughout the gland; UCN1 was mainly expressed in the medulla, zonae fasciculate and glomerulosa and weakly in zona reticularis; the CRF1 and CRF2 receptors were mainly expressed in the medulla, weakly in zonae fasciculata and glomerulosa and barely in zona reticularis. *In situ* hybridization and confocal laser microscopy supplemented these data. Exposure of dispersed rat adrenal chromaffin cells to UCN2 and UCN3 (specific CRF2 agonists) suppressed catecholamine secretion while Cortagine (specific CRF1 agonist) induced it; the effects were dose- and time-dependent peaking at 30 min. CRF1 and CRF2 mediated effects on catecholamine secretion were blocked by antalarmin (CRF1 antagonist) or anti-savaugine-30 (CRF2 antagonist) respectively. CRF and UCN1 had more complex effects. Changes of the subplasmaliminal actin filament mesh preceded the effects of CRF peptides on catecholamine secretion. At 48 h, activation of either CRF1 or CRF2 induced catecholamine synthesis via enhancement of tyrosine hydroxylase expression.

Signalling Through Ion-Channels

PP-106
Electroresponsive properties of dorsal raphe nucleus neurons in wild type and 5-HTT-/- knockout mice

B. M. Macri[1], P. Bonnavion[2] and T. Jacquin[2]
[1]*Department of Animal Physiology and Biophysics, Faculty of Biology, University of Bucharest, Bucharest, Romania,* [2]*UMR 677, INSERM-UPMC, NeuroPsychoPharmacologie, Faculté de Médecine Pitié-Salpêtrière, Paris, France.*
E-mail: beatrice_macri@yahoo.com

Lower potency of 5-HT1A receptor agonists to inhibit serotonergic neuron activity in dorsal raphe nucleus (DRN) is observed in 5-HT transporter knockout (5-HTT-/-) mice in comparison to wild-type mice. As 5-HTT-/- mice is a model of whole-life treatment with selective serotonin reuptake inhibitors, the goal of our study was to elucidate the underlying mechanisms using patch clamp techniques in DRN slices of adult mice. Stimulation of 5-HT1A receptors, via G-coupled receptors elicits an inward rectifying potassium current. Three distinct types of DRN neurons were recorded in brain stem slices from adult females and males of wild-type and 5-HTT-/- genotype, namely with a linear (type I) or rectifying (type II) I/V relationship, or a time dependent inward rectification (type III). Other electrophysiological characteristics of these neurons were analysed. Action potential duration in type III neurons is shorter than in type I and type II neurons. After hyperpolarization tau constant is shorter in type III than in type II neurons. For type I and type II, but not for type III neurons, a decrease of spike discharge frequency and an increase of inward current evoked by hyperpolarizing steps were recorded. Type I- and II-neurons are inhibited by 5-HT1A agonists being serotonergic neurons, while type III-neurons are not. These biophysical characteristics could explain various mechanisms underlying 5-HT1A receptor agonists effects. No differences were induced by the 5-HTT mutation.

PP-107
The influence of potassium channel openers on skeletal muscle mitochondria

D. Malinska, J. Skalska and A. Szewczyk
Department of Cellular Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland.
E-mail: d.malinska@nencki.gov.pl

ATP-regulated potassium channels (K_{ATP}) were first identified in plasma membrane. More recently it was shown that they are also present in the inner mitochondrial membrane in various tissues, like cardiac or brain tissue. Opening of the mitochondrial potassium channels affects mitochondrial respiration, membrane potential and matrix volume. An evidence exists that mitochondrial K_{ATP} channel (mitoK_{ATP}) is involved in cytoprotection, as the openers of the channel can mimic the effects of ischemia preconditioning, however the mechanism is not fully established. One of the proposed explanations is that opening of the mitoK_{ATP} modulates the mitochondrial production of reactive oxygen species (ROS), which can act as signalling molecules in cytoprotective pathways. Pharmacological modulators of K_{ATP} channel activity are a very popular research tools in investigation of the physiological role of the channel. BMS-191095 is one of the openers selective towards mitoK_{ATP}. Most of the data concerning mitoK_{ATP} comes from the study performed on cardiac tissue. In our experiments we used BMS-191095 to investigate the involvement of the channel in the physiology of skeletal muscle mitochondria. We have shown that BMS-191095 affects respiration rate and membrane potential of mitochondria isolated from rat skeletal muscle. We have also checked its influence on the production of reactive oxygen species in the skeletal muscle cell lines.

PP-108
The role of hydrogen bonding and water in ion channel gating

A. M. Kariev, V. S. Znamenskiy and **M. E. Green**
Department of Chemistry, City College of the City University of New York, New York, NY, USA.
E-mail: green@sci.ccny.cuny.edu

Great effort has gone into understanding gating of ion channels. New data on ion channel structure and function rule out certain models in which a voltage sensor physically moves in an extracellular direction; the maximum movement perpendicular to the membrane now appears <2Å. The model we are proposing has the sensor essentially stationary. There are three steps in the model: (a) a proton tunnelling event that occurs when depolarisation crosses a threshold; (b) a proton cascade along the sensor follows, and constitutes the gating current and (c) side chain opening in response to the change in charge occasioned by the proton cascade. We will show how this model fits the available data in more cases than any other model. We emphasize *ab initio* calculations on the gating regions of the KcsA channel, as well as results of *ab initio* calculations demonstrating the possibility of proton transfer between guanidinium moieties (side chains of arginines), as in the S4 voltage sensor of $Na+$ and $K+$ voltage-gated channels. The calculations show a key role for water and for hydrogen bonding. The KcsA closed state shows a plug of four water molecules 'below' the Q119 guanidiniums of the four domains, holding the domains together and blocking the channel. Adding charges (one proton per domain) breaks the plug, the side chains separate, and the channel opens. If the proton cascade in voltage gated channels leaves the gating region similarly charged, the result should be similar.

PP-109
Identification of the large conductance Ca^{2+}-activated potassium channel in rat brain mitochondria

M. Glab[1], G. M Wilczynski[2] and **A. Szewczyk**[3]
[1]*Laboratory of Intracellular Ion Channels, Department of Cellular Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland,* [2]*Department of Pathology, Medical University of Warsaw, Warsaw, Poland,* [3]*Laboratory of Intracellular Ion Channels, Department of Cellular Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland. E-mail: m.glab@nencki.gov.pl*

A large amount of evidence has implicated mitochondria as the potential target for cytoprotective strategies. It has been shown that increased mitochondrial $K+$ uptake may induce protection in different models of cell death. Electrogenic $K+$ uptake into mitochondria could be catalysed by $K+$ selective channels such as mitochondrial ATP-regulated potassium channel (mitoKATP channel) and mitochondrial large conductance Ca^{2+}-activated potassium channel (mitoBKCa channel). The mitoBKCa channel was described in human glioma cells LN229 and guinea pig ventricular cells. It has been shown that the pharmacological preconditioning with the use of BKCa channel opener NS1619 protects heart against infarction. Hence, the aim of our studies was to test

the presence of mitoBKCa channel in rat brain mitochondria. We employed immunohistochemical studies performed on paraffin-embedded rat brain sections with the use of specific antibodies against alpha and beta 4 subunit of BKCa channel and anti-COX antibody as a mitochondrial marker. Double-label immunohistofluorescence experiments revealed that the distribution of immunoreactivity generated by beta 4 subunit antibody colocalizes with the mitochondria. We have shown that the punctate mitochondrial beta 4 immunoreactivity is preferentially expressed in neurons with much less dense staining in glial cells. Taken together, these results suggest the presence of novel K + channel in rat brain mitochondria.

PP-110
Oestrogen signalling and human breast cancer: potential role of voltage-gated sodium channels

I. Ozerlat and **M. B. A. Djamgoz**
Division of Cell and Molecular Biology, Imperial College London, London, UK. E-mail: i.ozerlat@imperial.ac.uk

The role of oestrogen (ES) in human breast cancer (BCa) progression is complex. There are contradictory reports, and hormone-based therapies fail after a few years. ES was previously reported to regulate ion channel expression/activity. Voltage-gated sodium channel (VGSC) subtype Nav1.5 was reported to be upregulated in metastatic BCa. However, the mechanism regulating VGSC expression is not known. This study investigated a possible ES-VGSC interaction in human BCa cells. ERα-transfected MDA-MB-231 (MDA-ERα) cells had >60% lower motility compared with native cells ($P = 0.03$; $n = 4$). Treatment of MDA-ERα cells with antioestrogen ICI-182, 780 for >72 h increased their motility and migration by 18% and 32%, respectively ($P < 0.05$; $n > 5$). Interestingly, MDA-ERα cells were 42% less adhesive than native cells. The data would imply that ERα signalling could indeed have a complex role in BCa, appearing to suppress metastasis as regards motility/migration, but enhance it in relation to adhesion. Since VGSC activity is known to enhance metastasis of BCa cells, the data raise the possibility of a link between ES and VGSC activity. Indeed, the promoter region of Nav1.5 was found to include putative ES response element binding sites. The possible effect of ES on VGSC expression/activity is currently under investigation. The outcome of this study promises to help improve hormone-based therapy in BCa and raise the possibility of using ion channel drugs in novel therapies.

PP-111
Calcium signalling in the pre-capillary arterioles and its modulation by pH

L. Borisova[1], D. Eisner[2], S. Wray[1] and T. Burdyga[1]
[1]The Physiological Laboratory, University of Liverpool, Liverpool, UK, [2]Unit of Cardiac Physiology, University of Manchester, Manchester, UK. E-mail: L.Borysova@liv.ac.uk

Ca^{2+} play a key role in control of vasomotion in arterioles and other blood vessels, however mechanisms of Ca^{2+} signalling in the pre-capillary arterioles (PA) are poorly understood. Endothelial cells (EC) of the PA showed spontaneous heterogeneous Ca^{2+} events, which were resistant to removal of extracellular Ca^{2+}, nifedipine and ryanodine. Both spontaneous and carbachol-induced Ca^{2+} oscillations in EC were blocked by CPA, 2-APB or U73122. Intracellular alkalinization (IA) produced quick and reversible inhibition of spontaneous Ca^{2+} events in EC. Vascular smooth muscle cells (VSMC) at rest generated Ca^{2+} sparks which were fully blocked by ryanodine, augmented by 0.5–1 mM caffeine. IA increased the frequency and the amplitude of Ca^{2+} sparks and potentiated caffeine-induced Ca^{2+} waves. Phenylephrine-induced (10 μM) and endothelin-1-induced (1–20 nM) Ca^{2+} waves and vasomotion were fully blocked by 2-APB or U73122 and were little affected by ryanodine. IA abolished agonist-induced Ca^{2+} waves. These data suggest that in EC of the PA spontaneous and carbachol induced Ca^{2+} events are dependent exclusively on Ca^{2+} release from the SR via IP_3Rs while in VSMC both IP_3Rs and RyRs channels are involved with the latter playing a pivotal role. Also, the data obtained suggest that IA has a specific inhibitory action on Ca^{2+} release mediated by IP_3R but not RyRs channels.

PP-112
SRC kinase and voltage-gated sodium channel signalling: effects on human breast cancer cell migration

P. Uysal Onganer and M. Djamgoz
Division of Cell and Molecular Biology, Imperial College, London, UK. E-mail: p.onganer@imperial.ac.uk

A high level of voltage-gated sodium channel (VGSC) expression has been detected in human metastatic breast cancer (BCa) and VGSC activity has been shown to potentiate metastatic cell behaviours (MCBs). However, the mechanism(s) responsible for the VGSC upregulation in BCa is unknown. In this study, we investigated src, a non-receptor tyrosine kinase, and its possible interaction with VGSC activity in metastatic BCa. The experiments were carried out on the strongly metastatic human BCa MDA-MB-231 cell line. Cells were incubated in the src kinase blocker PP2 and/or the specific VGSC blocker tetrodotoxin (TTX) during transwell migration assays. The number of cells migrating over the 6 h was determined by MTT. Results were compiled as the means (± SEMs) of eight repeats of drug versus control readings from individual dishes. TTX (10 μM) by 35 ± 2% ($P < 0.01$) and PP2 (10 μM) by 46 ± 3% ($P < 0.01$) reduced migration. Co-treatment with PP2 + TTX suppressed migration by 28 ± 2% ($P = 0.02$). Importantly, there was no statistical difference between the effects of TTX and TTX + PP2 ($P = 0.07$). It is concluded (1) that src kinase and VGSC activity both are involved in enhancing the MCB of MDA-MB-231 cells and (2) that VGSC is downstream to src kinase, consistent with the following basic scheme: Src → VGSC → MCB. It is possible that a growth factor, such as epidermal growth factor (EGF) is involved as the primary signal upstream of src kinase.

PP-113
Basolateral CL-channels reside in lipid rafts in human airway epithelium

V. Duta and M. Duszyk
Department of Physiology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Canada.
E-mail: vduta@ualberta.ca

It has been proposed that basolateral Cl-channels in human airway epithelium act as a molecular switch between HCO3- and Cl-secretion. Additional work from our group identified bestrophin as a key player in the basolateral Cl-conductance of human airway epithelial cells. Lipid rafts are plasma membrane microdomains, involved in regulation of different signalling processes and characterized by an increased content of cholesterol. Since basolateral Cl-channels are highly regulated proteins, particularly by inflammatory mediators, we hypothesized that they might reside in lipid rafts. Short-circuit current (Isc) measurements showed that the removal of cholesterol by basolateral methyl-

beta-cyclodextrin blocked the response to 4,4'-diisothiocyanatostylbene-2,2'-disulfonic acid (DIDS) after S-nitrosoglutathione (GSNO), a nitric oxide donor, indicating that basolateral Cl-channels reside in lipid rafts. Co-immunoprecipitation and confocal microscopy studies demonstrated that bestrophin physically interacts with caveolin, a marker of lipid rafts. Moreover, pretreatment with short-interference RNA designed against caveolin, decreased Isc response to basolateral DIDS, in the presence of GSNO. The results of our study suggest that airway epithelial cells could regulate anion secretion via modifying content of plasma membrane cholesterol and affecting integrity of lipid rafts.
Acknowledgment: Funded by the Canadian Institutes of Health Research and the Canadian Cystic Fibrosis Foundation.

PP-114
Role of voltage-gated sodium channels in breast cancer metastasis and the effect of oestrogen on VGSC function

R. Fahrioglu Yamaci[1], H. Kaya[2], **M. B. A Djamgoz**[3] and E. Battaloglu[1]
[1]Bogazici University Department of Molecular Biology and Genetics, Bebek, Istanbul, Turkey, [2]Faculty of Medicine, Department of Pathology, Marmara University, Altunizade, Istanbul, Turkey, [3]Imperial College, Science, Technology and Medicine, London, UK. E-mail: rezanfahrioglu@yahoo.com

The study involves analysis of a novel voltage-gated sodium channel (VGSC) splice variant, $nNa_v1.5$ protein expression in breast cancer and correlation with lymph node metastasis (LNM) and oestrogen receptor (ER) status. Presence of $nNa_v1.5$ protein and LNM was observed in 34% of breast cancer cases confirming the correlation. Future metastasis was proposed for $nNa_v1.5(+)$/LNM(–) cases (24%) and $nNa_v1.5(+)$ patients with no LNM data (24%).In none of the $nNa_v1.5(-)$ cases LNM was positive (18%), $nNa_v1.5$ expression was observed in 53% of ER(+) and 16% of ER(–) patients. Because of the significant difference between the groups ($P \leq 0.005$) correlation between $nNa_v1.5$ expression and ER is proposed within the cases. Regulation of VGSC-metastasis correlation by oestrogen (E2) was tested on breast cancer cells. E2 increased the motility of VGSC(+)/ER(–) metastatic MDA-MB231 cells($P \leq 0.05$) but decreased in VGSC(–)/ER(+) non-metastatic MCF7 cells($P \leq 0.001$). Treatment with E2 + VGSC blocker, tetrodotoxin (TTX) has decreased motility in both cell lines ($P \leq 0.001$). In ERα-transfected MDA-MB231 cells, E2 first decreased then increased the motility ($P \leq 0.001$). The motility was decreased by TTX and TTX + E2 at all times. These results have confirmed positive correlation between $nNa_v1.5$ expression-breast cancer metastasis. We have also provided evidence for the effect of oestrogen on motility of breast cancer cells by regulating VGSC function.

PP-115
Isoform-specific distribution of plasma membrane calcium pumps from pig cerebellum in lipid raft microdomains

M. R. Sepúlveda[1], M. Berrocal-Carrillo[1], M. Gasset[2] and A. M. Durán[1]
[1]Dept. Bioquímica y Biología Molecular y Genética, Facultad de Ciencias, Universidad de Extremadura. Avda. de Elvas, Badajoz, Spain, [2]Instituto de Química-Física Rocasolano, Consejo Superior de Investigaciones Científicas. C/Serrano, Madrid, Spain. E-mail: rosarios@unex.es

We have analysed the association of the synaptosomal plasma membrane Ca^{2+}-ATPase (PMCA) from pig cerebellum with membrane microdomains known as lipid rafts, characterized by their high content in cholesterol and sphingolipids. The isoform PMCA4 was localized exclusively in rafts isolated by floatation in Nycodenz density gradients of ice-cold Brij 96 extracts. The colocalization with the raft markers cholesterol, ganglioside GM1, and PrP^c corroborated this PMCA4 distribution, while the PMCA isoforms 1, 2 and 3 were found in the detergent-soluble fractions, with the majority of the membrane proteins. Activity assays confirmed the bimodal distribution of the PMCA isoforms in the density gradient, with a lower activity for PMCA4 and greater stimulation by calmodulin than for the other isoforms. Besides, we have investigated molecular determinants of rafts association, showing the contribution of palmitoylation by PMCA4 sensivity to hydroxylamine. The preferential localization of PMCA4 in an ordered membrane microenvironment as rafts suggests a possible special role for this isoform in events tightly regulated of Ca^{2+} signalling.

PP-116
A yeast model for polycystic kidney disease ion channels

C. Palmer, E. Aydar and M. Djamgoz
Division of Cell and Molecular Biology, Imperial College, London, UK. E-mail: christopher.palmer@ic.ac.uk

Autosomal dominant polycystic kidney disease is characterized by the formation of large fluid-filled cysts in kidneys caused by abnormal differentiation and proliferation of kidney tubular epithelial cells, which result in chronic renal failure in 50% of patients by the age of 60. In 85% of patients the causative mutation is located in the PKD1 gene. The majority of the remaining mutations are located in the PKD2 gene. We have investigated a polycystic kidney disease related ion channel gene homologue in Schizosaccharomyces pombe (pkd2). The gene is essential and the protein encoded by this gene is localized to the golgi and plasma membrane and is involved in cell growth, membrane trafficking, cell size/shape determination and cell wall synthesis. Our most recent results indicate that: (a) plasma membrane localization of pkd2 increases during cell growth or following cell wall damage; (b) Pkd2 depletion results in a plasma membrane trafficking defect; (c) Pkd2 interacts with a synaptotagmin-like protein suggesting a role in membrane trafficking and (d) Internalization of pkd2 from the plasma membrane is a rapid process which is Ca^{2+}-dependent and inhibited by gadolinium and enhanced by membrane amphipaths. A model for the role of this pkd2 channel in yeast cell physiology is presented and discussed in light of our knowledge of polycystic kidney disease.

PP-117
Maxi-chloride channel a possible conductive pathway for taurine in human placenta

G. Riquelme and C. Vallejos
ICBM, Faculty of Medicine, University of Chile, Santiago, Chile. E-mail: griquelm@ med.uchile.cl

Taurine (Tau), the most abundant aminoacid (aa) in foetal blood is highly concentrated in human placenta. Tau is involved in neurological development and in volume regulation of placenta, is inadequately synthesized by the foetus and must be supplied by the mother, suggesting the presence of an efficient Tau-transport across apical syncytiotrophoblast membrane (MVM). In addition to carriers, an ionic channel may be a conductive pathway for Tau in human placenta. One candidate is a voltage-dependent Maxi-Cl channel from MVM, with a conductance over 200 pS and multiple substrates. Our aim was to study whether this

channel could be a Tau conductive pathway in the MVM. Purified human placental MVM were reconstituted into giant liposomes suitable for patch clamp recordings. Typical Maxi-Cl channel activity was detected in symmetrical Chloride (Cl) solutions. Aspartate (Asp), glutamate (Glut) and Tau solutions were used in the bath of the excised patches to detected single channel currents carried by these anions. Permeability ratios (P) were estimated from the shift in reversal potential of current-voltage curves after anion replacement. P values to Asp, Glut and Tau over Cl were 0.31, 0.42 and 0.40 respectively. In Tau symmetric conditions using equivalent Cl concentrations, the slope conductance was 62 pS. Data shows that Tau and others aa diffuse through the Maxi-Cl channel which may be important for volume regulation and for nutrients transport in placentas (FONDECYT 1040546).

PP-118
Calcium signals are affected by ciprofloxacin as a consequence of reduction of mitochondrial DNA content in Jurkat cells

J. Duszynski, R. Koziel and **K. Zablocki**
Nencki Institute of Experimental Biology, Warsaw, Poland. E-mail: j.duszynski@nencki.gov.pl

Effects of ciprofloxacin on mtDNA content, oxygen consumption, mitochondrial membrane potential, cellular ATP formation and capacitative Ca^{2+} entry into Jurkat cells were investigated. In cells incubated for several days with 25 μg/ml ciprofloxacin 60% reduction of mtDNA content, inhibition of the respiratory chain and significant decrease in mitochondrial membrane potential were observed. These changes led to a decrease in calcium buffering capacity of mitochondria what, in turn, resulted in a gradual inhibition of the capacitative Ca^{2+} entry. On the 4th, 7th and 11th day of incubation with ciprofloxacin the initial rate of Ca^{2+} entry was reduced by 33%, 50% and 50%, respectively. Ciprofloxacin caused a transient decrease in the cellular capability for ATP formation. In the cells incubated for 15 min with glucose, pyruvate and glutamine as exogenous fuel, ciprofloxacin reduced ATP content by 16% and 35% on the 4th and the 7th day of incubation with the drug, respectively. However, on the 11th day of incubation with this drug cellular ATP formation recovered. In conclusion, long-term exposure of Jurkat cells to ciprofloxacin at 25-μg/ml concentration seriously affects cellular energy metabolism and calcium homeostasis.

PP-119
Inhibition of the cardiac Na^+/Ca^{2+} exchanger by raised intracellular Ca^{2+}

T. M. Kang, J. E. Lee, Y. H. Nguyen and K. N. Mamatova
Department of Physiology, Sungkyunkwan University School of Medicine, Suwon, Korea. E-mail: tmkang@yurim.skku.ac.kr

The cardiac Na^+/Ca^{2+} exchanger is a bi-directional Ca^{2+} transporter that mainly extrudes Ca^{2+} during diastolic phase. We explored the inhibitory control of intracellular Ca^{2+} on the reverse-NCX1 activity by the measurement of $[Ca^{2+}]i$ transients and the conventional whole-cell current. In NCX1-expressing BHK cells, the amplitudes of $[Ca^{2+}]i$ transients and outward exchanger currents were gradually diminished when cells were repetitively activated by Na^+-free solution. The run-down of reverse-NCX1 current was dependent on $[Ca^{2+}]i$ and was attenuated as the currents were limited by Ni^{2+} or a low extracellular Ca^{2+}. To raise the $[Ca^{2+}]i$, capsaicin was treated in TRPV1-transfected NCX1/BHK cells, and it significantly inhibited the reverse-NCX1 current. PKC activation by Ca^{2+} influx through reverse-NCX was recorded by the trans-location of PKC substrate MARCKS and PKCβII. In fura-2 loaded BHK cells, the run-down of $[Ca^{2+}]i$ transients was reversibly inhibited by PKC inhibitors, staurosporine and H7. On the contrary to the results from fura 2-loaded cells, run-down of whole-cell NCX current could not be inhibited by the PKC inhibitors. Taken together, the inhibition of reverse-NCX1 current by raised $[Ca^{2+}]i$ could be explained in part by PKC activation. However, the possible roles of unknown diffusible factor(s) and the endocytosis of NCX1 should be explored.

Acknowledgment: This work was supported by the Korea Research Foundation Grant funded by the Korean Government (KRF-2005-041-E00016).

PP-120
Cytosolic and mitochondrial calcium transient during the release of histamine induced by beta-1,3-glucan in bone marrow mast cells

J. B. Youm, D. V. Cuong, N. Kim, S. Kang, M. Warda, H. Kim, T. M. Khoa, T. Kim and J. Han
Department of Physiology and Biophysics, Mitochondrial Signalling Laboratory, Busan, Korea. E-mail: jaeboum@gmail.com

The present study attempted to determine whether beta-1,3-glucan (BG) induces histamine release and to investigate the role of calcium transients in the histamine release. C57 black mouse bone marrow cells were collected and cultured in Iscove Modified Dulbecco Medium (IMDM) containing interleukin-3 and stem cell factors to selectively harvest mast cells after 5 weeks of culture. Mast cells were identified by toluidine blue staining, c-kit and FcεRI. Histamine contents and calcium transients were measured by spectrofluorometric assay and fluorescent confocal microscopy. BG induced histamine release and calcium transients of cytosol and mitochondria in the mast cells in a time- and dose-dependent manner. BG also induced hyperpolarization of mitochondrial membrane potential. Pretreatment with IgE inhibited BG-induced histamine release in a dose-dependent fashion. BG-induced histamine release was affected by calcium-free conditions. TMB-8, an intracellular calcium antagonist, dose-dependently inhibited the histamine release under these conditions. The results demonstrated that beta-1,3-glucan induces histamine release, which is associated with concomitant cytosolic and mitochondrial calcium transients.

PP-121
Expressional and functional profile of *TRPC* gene family in aging rat aorta

Y. Erac[1], C. Selli[1], I. T. Aydin[2], B. Kosova[3], C. K. Akcali[2] and M. Tosun[1]
[1]Pharmacology, Ege University, College of Pharmacy, Izmir, Turkey, [2]Molecular Biology and Genetics, Bilkent University, Ankara, Turkey, [3]Medical Biology, College of Medicine, Izmir, Turkey. E-mail: yasemin.erac@ege.edu.tr

This study investigates the expressional and functional profile of canonical transient receptor potential (TRPC) channel proteins (C1,3,4,5 and 6) in aging rat aorta. The expressions of TRPCs from smooth muscle (SM) and endothelial tissues of young and old rats were examined at mRNA and protein level by real-time PCR and western blot, respectively. In addition, acetycholine

(ACh) and cyclopiazonic acid (CPA) concentration-response curves were obtained in intact tissues. Our results showed that in both groups C1 was the predominant TRP homologue expressed in SM and endothelium. C3, C4 and C6 were expressed at lower levels and C5 at trace amounts. Interestingly during aging, C1 and C3 expressions in SM increased 130% and 24%, respectively; with no change in C6. In endothelium, while the expression of C3 and C6 elevated, C1 decreased. In aged rats, ACh and CPA relaxations were reduced with no change in max. phenylephrine (PE) contractions. In conclusion, the tissue-specific reciprocal changes in C1 expression may have an impact in vascular aging. Impaired ACh relaxation in old rats may be associated with the decreased endothelial C1, a candidate for endothelial store-operated calcium entry. Furthermore, unaltered PE contractions coinciding with the stable levels of C6 expression support the early report on C6 of being an essential component of the α1-adrenergic-receptor-activated calcium-permeable cation channel.

Acknowledgment: Supported by TUBITAK SBAG-2735 (MT).

PP-122
Identifying the promoter region of the human Nav1.7 voltage-gated sodium channel gene (scn9a)

J. Diss[1], M. Calissano[1], M. Djamgoz[2] and D. Latchman[1]
[1]MMBU, Institute of Child Health, University College London, London, UK, [2]Department of Biological Sciences, Imperial College London, London, UK. E-mail: j.diss@ich.ucl.ac.uk

The Nav1.7 voltage-gated sodium channel is mainly expressed in dorsal root ganglion (DRG) neurons and sympathetic ganglia of the peripheral nervous system (PNS). It plays a major role in nociception and its expression is upregulated in animal pain models. Nav1.7 levels are also strongly upregulated in metastatic prostate cancer cells *in vitro* and in prostate cancer patients *in vivo*. To analyse the mechanisms regulating physiological and pathophysiological Nav1.7 expression, we identified Nav1.7 transcriptional start sites using RT-PCR techniques including 5' rapid amplification of cDNA ends (5' RACE) with RNA from sensory neuron and prostate cancer cell lines. Identified 5' UTR exons map to a region of genomic DNA located ˜69 kb upstream of the first coding exon which lacks obvious TATA boxes, but possesses multiple consecutive GAGA boxes within a well-defined CpG island. When inserted into a basic luciferase vector this region produced luciferase expression in both sensory neuron/neuroblastoma and strongly (but not weakly) metastatic prostate cancer cell lines. The luciferase construct also responded to known regulators of Nav1.7 in sensory neurons. This study provides the initial description of the SCN9A (Nav1.7) functional promoter. Characterization of this promoter is likely to assist in the determination of key factors responsible for upregulation of Nav1.7 in pain and prostate cancer, and the development of strategies to control this pathophysiological expression.

PP-123
Antidromic potential signals and the receptor function

P. Nuhan
Hacettepe University, Ankara, Turkey.
E-mail: npurali@hacettepe.edu.tr

Concept of dynamic polarization has been used to describe the development of the neuronal function. Receptor or currents, generated in the dendritic region, evoke receptor potential, spreading passively to soma. Eventually, action potential, generated in axon hillock, propagates in the direction of the axon to the presynaptic site. However, the action potential can passively propagate back to the dendrites. In the present work the consequences of the antidromic action potentials in the receptor responses has been investigated comparatively in the rapidly and the slowly adapting receptor neurones. The receptor responses were recorded when the driving force of the current stimulus was constant and when the on line recorded membrane potential was allowed to influence the driving force for the receptor current. In the slowly adapting neuron membrane potential reduced the magnitude of the driving force for the receptor current, and kept the receptor responses within the active range even when large stimulations were used. Irrespective of stimulus magnitude, adaptation of the impulse response was not observed. In the rapidly adapting neuron, membrane potential substantially influenced the adaptive properties. Membrane potential has been shown to modulate the receptor response so that the rapid adaptation is facilitated. Thus, it was concluded that the antidromic action potentials should may be an important mechanism contributing to the adaptive properties of the receptors.

PP-124
Na+/Ca^{2+} exchanger contributes to sarcoplasmic reticulum Ca^{2+} refilling

I. B. Bal, Y. Sara and R. Onur
Department of Pharmacology, Hacettepe University, Ankara, Turkey. E-mail: burakbal@hacettepe.edu.tr

In skeletal muscles, Ca^{2+} efflux is carried out via Ca^{2+}-ATPase and Na^{+}-Ca^{2+} exchanger (NCX) while L-type Ca^{2+} channels do not contribute to the Ca^{2+} influx. Although Ca^{2+} entry pathway in skeletal muscle cells is still not well understood, store operated calcium entry (SOCE) is thought to play an important role. NCX, is reported to pump out Ca^{2+} ions in exchange of Na+ ions in normal mode and under certain conditions it operates in reverse mode where it accumulates Ca^{2+} in cytoplasm. In our study, contribution of NCX to SOCE is investigated. Mechanical recordings were obtained from diaphragm strips of Wistar rats. Ca^{2+} depletion was achieved by incubating in Ca^{2+}-free media. SOCE was induced by reintroduction of 2 mM Ca^{2+}. Increase in basal tone was used as a marker of Ca^{2+} entry. Area under caffeine contracture curve (AUCCC) was used as a measure of sarcoplasmic reticulum (SR) Ca^{2+} content. In Ca^{2+} depleted muscles Ca^{2+} administration induced SOCE and increased the basal tone. Selective NCX inhibitors, KB-R7943 and benzamil reduced this increase in basal tone, suggesting NCX operating in reverse mode contributes to SOCE. On the other hand, both inhibitors increased the AUCCC indicating NCX, reverts back to normal mode and decreased the final Ca^{2+} content of stores. In conclusion, NCX activity by contributing both SOCE and Ca efflux takes part in determination of final status of SR Ca^{2+} content.

Signalling and Apoptosis

PP-125
Research of neutrophils reaction on the lipopolysaccharides by atomic force microscopy

S. N. Pleskova[1], Y. Y. Gushina[1] and M. B. Zvonkova[2]
[1]*Research and Educational Center for Physics of solid state nanostructures, Laboratory of Scanning Probe Microscopy in medical and biological research, Nizhni Novgorod State University, Nizhny Novgorod, Russia,* [2]*Department of physiology and biochemistry of humans and animals, Nizhni Novgorod State University, Nizhny Novgorod, Russia. E-mail: pleskova@mail.ru*

The neutrophils morphology changes under a lipopolysaccharide in real time have been studied by Atomic Force Microscopy (AFM). In present paper it has established the change of cells membranes rigid and the change of volume and height of neutrophils after addition of LPS. Young's module was used for estimated the elastic properties of neutrophils' membranes. Young's module of intact cells was 2.1 ± 0.7 kPa. It was change significantly after addition of lipopolysaccharide. We were scanning the cells before and after LPS addition without FS–spectroscopy. During the first 30-min after LPS addition we observed swelling of the cells. The volume and height of cells change constantly after addition LPS. We observed the increase cells parameters only to 30 min. However, the cells parameters unstable after 1 h: cell now enlarges now reduces sizes. We suppose this is connected with forming and separation of the 'apoptosis like bodies'. The same result was obtained with Young's module during 2 h. After addition LPS we observed oscillation the values of Young's module. So, Young's module change significantly at the observations time. Probably change biochemical structures cause the increase of cells membranes rigid.

PP-126
Unusual apoptosis induced in heart tissue in anoxia, mechanisms of retaining cytochrome C

A. Tonshin[1], N. Lobysheva[1], **O. Podgorny**[2] and L. Yaguzhinsky[1]
[1]*Belozersky Institute, Moscow State University, Moscow, Russia,* [2]*Laboratory of Experimental Neurobiology, Koltzov Institute of Developmental Biology, Russian Academy of Science, Moscow, Russia. E-mail: atonshin@mail.ru*

The features of anoxia influence on isolated rat heart tissue slices were studied. Internucleosomal DNA fragmentation was detected as apoptosis hallmark. The program of cell death was found by two independent methods to proceed without cytochrome C release. The absence of cytochrome C release was proven to be independent from electrostatic interaction of this protein with the inner mitochondrial membrane. However, the outer membrane in mitochondria isolated from apoptotic tissue was found to be permeable to cytochrome C. The possibility of retaining cytochrome C independently from electrostatic interaction with inner membrane in mitochondria with permeabilized outer membrane during heart tissue apoptosis is discussed. The functional activity of mitochondria isolated from apoptotic tissue was characterized. Maximal respiration rate was not changed significantly, but loss of membrane potential and oxidative phosphorylation dysfunction was found. These changes were found to be dependent from PTP induction. CsA addition in tissue slices incubation media was found to prevent these mitochondrial functional damages but failed to prevent outer membrane permeabilisation for cytochrome C in isolated mitochondria. The induction of PTP was found to be a necessary step for cell death program execution. Inhibition of DNA cleaving by spermine was demonstrated. This inhibition was shown to be independent from spermine ability to prevent PTP induction.

PP-127
Modifications of ras alter content of secreted homocysteine by pc12 cells

M. Sepashvili, E. Zhuravliova, T. Barbakadze, N. Narmania and D. G. Mikeladze
Laboratory of Neurochemistry, Institute of Physiology, Tbilisi, Georgia. E-mail: maiasefa@yahoo.com

PC12 pheochromocytoma cells expressing a dominant inhibitory mutant of Ha-ras (M-M17-26) and PC12 cells transfected with normal c-rasH (M-CR3B) has been used to investigate the role of nitrosylation and farnesylation of Ras on the production of homocysteine and the activities of the redox-sensitive transcription factors NF-kB and c-Fos. We found that under serum and nerve growth factor withdrawal conditions undifferentiated apoptotic M-CR3B cells accumulated more homocysteine, than M-M17-26 cells and the production of homocysteine decreased under the action of manumycin and increased in the presence of L-NAME. Furthermore, we have shown that manumycin increased the activity of c-Fos in the M-CR3B cells and decreased the activity of NF-kB, while L-NAME reduced the activities of both transcription factors, and accelerated apoptosis of M-CR3B cells. In contrast to the M-CR3B cells, in M-M17-26 cells manumycin did not change the activity of c-Fos, nor the activity of NF-kB. We conclude that trophic factor withdrawal stimulates Ras, which apparently through the Rac/NADPH oxidase system induces permanent oxidative stress, modulates the activities of NF-kB and c-Fos, induces production of homocysteine and accelerates apoptosis. Nitrosylation of Ras is necessary for maintaining the survival of PC12 cells, while farnesylation of Ras stimulates apoptosis under withdrawal conditions.

PP-128
Thyroid hormone effects on tumour cell apoptosis

T. S. Saatov and A. A. Abduvaliev
Institute of Biochemistry, Uzbekistan Academy of Sciences. E-mail: saatov@uzsci.net

The work was initiated to study effects of thyroxine (T4) on both normal and tumour cell proliferation and apoptosis. The study was conducted on experimental rat models of the melanoma B-16 entwined strains, the colon adenocarcinoma (ACATOL) as well as in the experiments with the rat thymocytes. In *in vitro* experiments T 4 (10–4 M) was shown to cause ACATOL cells apoptosis-like death. *In vivo* confident inhibition of melanoma B-16 growth under the effect of T 4 was established. The apoptotic to mitotic index ratio was 8.19 to be the evidence for high rate of tumour regression. Study on T 4 effect on 3 H-thymidine incorporation into the thymic cells showed that the concentrations of the hormone 10–4 to 10–16 M significantly inhibit and the one of 10–8 M insignificantly stimulates the label incorporation into the rat thymocytes. We studied mechanism of apoptosis stimulation by thyroxine on the level of fast processes in the mitochondria. 3H-diazepam was shown to specifically bind with mitochondria with high affinity (Kd = 0.3 mkM), the binding being inhibited by thyroxine with King50% = 10 mkM. T4 could be supposed to increase the MTP-pore conductivity due to binding with

benzodiazepam receptor of the pore. We have managed to prove DNA fragmentation and cytochrome c release from the mitochondria into cytosol in tumour cells under the effect of T4.
Conclusions: Definite concentrations of T4 were shown to suppress proliferation, but induce both normal and tumour cell apoptosis.

PP-129
Pro-apoptotic effect of the hydrogen sulfide (h_2s) in isolated pancreatic acinar cells

Y. Cao, S. Adhikari, P. K. Moore and M. Bhatia
National University of Singapore. E-mail: mbhatia@nus.edu.sg

H_2S has recently attracted lots of interest as a novel gaseous mediator involved in physiological and pathophysiological events. However little is known, about the effect of H_2S on the modulation of cell apoptosis. In the current study, we investigated the pro-apoptotic effect of the H_2S donor, NaHS in isolated mouse pancreatic acini. We demonstrated that treatment of isolated mouse pancreatic acini with physiologically relevant concentrations of NaHS for 3 hours did not induce necrosis as verified with propidium iodide staining, but caused phosphatidylserine (PS) externalisation of pancreatic acinar cells, as shown by annexin V binding. In NaHS-treated pancreatic acini, caspase 3, 8 and 9 activities were significantly activated as evidenced by fluorimetric assay, and their activity was observed to be greatly attenuated when the corresponding caspase inhibitor was used. Inhibition of these caspases attenuated the apoptosis as shown by decreased annexin V-FITC binding. Moreover, loss of mitochondrial membrane potential was observed by fluorescence microscopy and quantitative analysis. Release of cytochrome C by mitochondria was determined using a semi-quantitative sandwich ELISA kit. These results show, for the first time, that low concentrations of H_2S induce apoptosis in pancreatic acinar cells *in vitro*, mediated by PS externalization, caspase cascade, loss of mitochondrial membrane potential and release of mitochondrial cytochrome C.

PP-130
Oestrogen-induced apoptosis and VEGF signalling pathways in tamoxifen-resistant breast cancer cells

A. Scherbakov[1], Y. Lobanova[2], E. Gershtein[1] and M. Krasil'nikov[2]
[1]Laboratory of Clinical Biochemistry, Russian N. N. Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Moscow, Russia, [2]Laboratory of Molecular Endocrinology, Russian N. N. Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Moscow, Russia. E-mail: scherba@yandex.ru

Paradoxical induction of apoptosis by oestrogen has been described previously for oestrogen-deprived and antioestrogen-resistant breast cancer cells. We analysed possible interrelations between cell sensitization to oestrogen apoptotic action and activation of mitogenic signalling in antioestrogen-resistant cells. Using a long-term treatment of MCF-7 breast cancer cells with antioestrogen tamoxifen, we have developed a new subline, designated MCF-7/T1, that was characterized by high level of resistance to tamoxifen and increased expression of VEGFR-2 and VEGF-A. The importance of the VEGF/VEGFR signalling in the autocrine regulation of cell growth was indicated by the ability of VEGF inhibitor (Flt-1/Fc chimera) to suppress the phosphorylation of MAP kinases as well as to inhibit the oestrogen-independent growth of MCF-7 cells. We have found that sensitization of tamoxifen-resistant cells to oestrogen-induced apoptosis required additional continuous cultivation in steroid-depleted medium and did not depend on the activity of VEGF pathway. Estradiol treatment resulted in a marked increase in p53 level in both parent and MCF-7/T1 cell lines suggesting that p53 might be involved in oestrogen apoptotic action. However, only tamoxifen-resistant cells underwent oestrogen-induced apoptosis providing an important support for the existence of disturbances in the anti-apoptoti machinery in the resistant cells formed independently of the acquired ability to oestrogen-independent growth.

PP-131
HSF1 down regulates expression of hsp70.2 gene prior to the induction of apoptosis in spermatocytes

N. Vydra, E. Malusecka, V. Dudaladava, D. Scieglinska, Z. Krawczyk and W. Widlak
Department of Tumour Biology, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland. E-mail: nvydra@yahoo.co.uk

HSF1 is a transcription factor that up-regulates expression of hsp (heat shock protein) genes under stress conditions. Heat shock proteins (HSP) protect somatic cells from stress-induced protein damages and cell death. However, expression of constitutively active heat shock transcription factor 1 (HSF1) in mouse spermatocytes induces apoptosis and leads to male infertility. Previous studies have also reported a similar phenotype in mice lacking a functional spermatocyte-specific Hsp70.2/Hst70 gene. We report here that prior to the onset of massive apoptosis caused by expression of active HSF1 in spermatocytes a marked reduction in HSP70.2 protein and mRNA levels occurs, and that the protein relocalizes from a predominant cytoplasmic to a nuclear position. During later developmental stages cells undergoing HSF1-induced apoptosis are essentially deficient in HSP70.2, which suggests a functional relationship between down-regulation of Hsp70.2/Hst70 and the degeneration of seminiferous epithelium caused by activation of HSF1. The DNA sequences responding to repression were mapped to the immediate promoter region of the Hsp70.2/Hst70 gene, but none of them were associated with HSF1. Most probably the Hsp70.2/Hst70 gene promoter is negatively regulated by factor(s) induced by activated HSF1, which remain to be identified.

PP-132
Modulation of JIP1-JNK signalling module by the vaccinia-related kinase-2 (vrk2)

S. Blanco and P. A. Lazo
Centro de Investigación del Cáncer, Instituto de Biología Molecular y Celular del Cáncer, CSIC-Universidad de Salamanca, Salamanca, Spain. E-mail: sblanco@usal.es

VRK2 kinase belongs to a family of Ser-Thr kinases of unknown function. *Vrk2* gene generates by alternative splicing two isoforms, VRK2A has a C-terminal hydrophobic region that anchors the protein to membranes in the ER and mitochondria, whereas VRK2B is diffusely detected in both cytoplasm and nucleus. The scaffold JIP1 (JNK interacting protein 1) forms a complex with upstream MAPKs of the JNK pathway such as MLKs, MKK7 and JNK as well, fostering the activation of JNK. Here we report that JIP1 also interacts with TAK1, another MAP3Ks which activates JNK and regarding the human kinome is distantly related with the MLK family. Endogenous JIP1 colocalizes with exogenous VRK2A and VRK2B *in vivo*. Furthermore, the two VRK2 isoforms interact by its N-terminal domain with JIP1 *in vitro*, binding the C-terminal region of JIP1. The kinase activity of VRK2 is not necessary for the interaction, but both VRK2

isoforms phosphorylate JIP1 in its N-terminal region, and do not affect the JNK interaction domain of JIP1. VRK2A, but not VRK2B, also interacts by its C-terminal domain with TAK1. VRK2 interaction with JIP1 does not interfere with individual interactions between JIP1 with JNK or TAK1. Nevertheless, both isoforms inhibit the activation of JNK by TAK1 and DFO, a drug which induces hynoxia/anoxia. Therefore, we postulate that the VRK2 kinases are components of a new signalling pathway likely to play a role in normal proliferation regulating some stress responses.

PP-133
Characteristics of the cytotoxic action of treflan on root cells of barley

Yu. Kozhuro[1], E. Sheval[2], N. Poleshuk[2] and V. Polyakov[2]
[1]*Department of Genetics, Belarus State University, Minsk, Belarus,* [2]*A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia. E-mail: kazhura@tut.by*

A study of the herbicide treflan action on root cells of Hordeum vulgare cv. Visit was performed. The presence of treflan during the seed germination led to retardation of the root growth, and this retardation was correlated with the treflan concentration. The metaphase block and appearance of a lot of multinucleated cells were revealed in treflan treated roots. To determine the mechanism through which the herbicide exerts its toxicity, light and electron microscopy analysis was conducted. There were partially developed cell walls inside all multinucleated cells, and it appears that the irregulation of cytoskeleton observed using antibody to β-tubulin is a reason for this phenomenon. We also observed an impaired development of root hairs in unusual sites near the root tip. The hair cells as well as cells of the differentiation zone started to death at 4th day of development. The cytoplasm of these cells was condensed, the latest stages of death were characterized by cytoplasm shrinkage inside partially destroyed cell walls. These morphological events correlated with the internucleosomal fragmentation of DNA. In the fraction degraded DNA the two types of fragments having the sizes 720 and 1080 bp. distinctly prevailed. Also the activation of two proteases with a molecular mass of ˜60 kDa was detected. The morphological and biochemical features revealed allow interpreting the phenomenon described as a programmed cell death selectively induced in differentiated root cells.

PP-134
Nucleolar localization of phosphatidylinositol 4-kinase pi4k230 in various mammalian cells

A. Kakuk[1], **E. Friedländer**[2], Gy. Vereb[2], E. Petrasch-Parwez[3], T. Balla[4], L. Heilmeyer[5], G. Gergely[1] and Gy. Vereb[1]
[1]*Department of Medical Chemistry, Medical and Health Science Center, Faculty of Medicine, University of Debrecen, Debrecen, Egyetem tér 1, Hungary,* [2]*Department of Biophysics and Cell Biology, Medical and Health Science Center, Faculty of Medicine, University of Debrecen, Debrecen, Egyetem tér 1, Hungary,* [3]*Institut für Anatomie, Abteilung für Neuroanatomie und Molekulare Hirnforschung, Ruhr-Universität Bochum, Bochum, Germany,* [4]*Endocrinology and Reproduction Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA,* [5]*Institut für Physiologische Chemie, Abteilung für Biochemie Supramolekularer Systeme, Ruhr-Universität Bochum, Bochum, Germany.*
E-mail: elza@jaguar.dote.hu

Immunofluorescent detection of the subcellular localization of phosphatidylinositol 4-kinase PI4K230 gave prominent signals in the nucleolus of several ethanol fixed neural and non-neural cells in addition to the cytoplasmic and nuclear immunofluorescence. The nucleolar localization of PI4K230 was detected in slices from different regions of the rat brain, too. On the other hand, PI4K230 could not be detected in the nucleolus of formaldehyde fixed cells. Nucleolar PI4K was detected by four different monospecific polyclonal antibodies directed to distinct peptide regions of PI4K230 and produced in different species using direct and indirect immunofluorescence. The masking effect of PFA fixation was prevented by a short (10 min) washing with phosphate buffered saline (PBS) prior to fixation, moreover it could be reverted by unmasking the epitopes with heating the formaldehyde fixed cells in a citrate buffer, pH 6.0. Pretreatment of cultured cells with RNAse removed PI4K230 from the nucleolus supporting its association with nucleolar RNA. In the cell lines tested, nucleolar PI4K230 disappeared when cultured cells became confluent. Treatment of COS-7 cells with siRNA specific for PI4K230 strongly reduced the cytoplasmic immunoreactivity and caused a redistribution removing it from the nucleolus, while leaving the fluorescence apparently unchanged in the nucleoplasm. All these observations suggest the dynamic, possibly functional association of PI4K230 with nucleolar structures.

PP-135
Immunohistochemical assessment of metallothionein as an apoptotic marker in chronic hepatitis c

C. Stanciulescu[1], C. Pisoschi[1], M. Banita[2] and **N. Mitrea**[3]
[1]*Biochemistry Department, University of Medicine and Pharmacy Craiova, Romania,* [2]*Histology Department, University of Medicine and Pharmacy Craiova, Romania,* [3]*Biochemistry Department, University of Medicine and Pharmacy 'Carol Davila' Bucharest, Romania. E-mail: camiparsot@yahoo.com*

Many studies are focused on the role of apoptosis in death of liver cell and are increasing evidence that this phenomenon could be an important mechanism also in chronic viral hepatitis C as a defence against viral infection. Metallothionein (MT) is a cystein-rich protein with important biological functions in homeostatic regulation of metals, scavenging of free radical. Reactive oxygen species are involved in apoptosis and may play a role in the pathogenesis of hepatitis C. Our aim was to evaluate the expression of metallotionein in liver biopsies from patients with chronic viral hepatitis C and its correlation with apoptosis. Apoptosis was studied on 18 liver biopsies by Tunel method. Immunohistochemical reactions for metallothionein were performed on 3 μ paraffin sections using a mouse monoclonal anti-metallothionein diluted 1:50, the avidin-biotin complex to amplify the immune reaction and diaminobenzidine as a revelator. Tunel method revealed an increased number of apoptotic liver cells in chronic viral hepatitis C. The hepatocytes showed a strong cytoplasmatic and nuclear staining for metallothionein in six cases, a weak cytoplasmatic immunostaining in nine cases and for three of them the immunoreaction were negative. These data assume the link between hepatocyte apoptosis and the expression of liver metallothionein, so this could be a possible marker for apoptosis in chronic viral hepatitis C.

PP-136
Novel tamoxifen derivatives induced mitochondria-involved apoptosis in non-oestrogen receptor cell

Y. Nagahara[1], I. Shiina[2], T. Shinomiya[3], M. Tanaka[1] and M. Ikekita[4]
[1]Department of Biotechnology, Tokyo Denki University, Saitama, Japan, [2]Department of Applied Chemistry, Tokyo University of Science, Tokyo, Japan, [3]Department of Radio Isotopes and Biosafety Research, National Research Institute for Child Health and Development, Tokyo, Japan, [4]Department of Applied Bio. Sciences, Tokyo University of Sci., Chiba, Japan
E-mail: yunagahara@b.dendai.ac.jp

Oestrogen promotes secondary sexual characteristics by binding to oestrogen receptor (ER). Moreover, oestrogen facilitates proliferation of ER positive carcinoma, such as breast carcinoma. Tamoxifen (TAM), a nonsteroidal triphenylethylene derivative, inhibits oestrogen binding to ER and blocks cell proliferation. Accordingly, TAM is now widely used as anti-breast carcinoma drug. On the other hand, some reports revealed that TAM was also effective to certain ER negative carcinomas, which induced apoptosis to those cells. Consequently, TAM might be powerful tool for affecting various carcinomas, and so that potent TAM derivatives are needed for better use. However, how TAM and their derivatives induce apoptosis to ER negative cells were uncertain. We synthesized a number of TAM derivatives and evaluated the cytotoxic effect to ER negative human T lymphoma Jurkat. Treatment of TAM and their derivatives decreased cell viability and fragmented DNA. Some derivative reduced cell viability in lower dose than TAM. Using specific peptide substrates, activation of caspases, specially caspase-3 and caspase-9 was observed. A pan caspases inhibitor, Z-CH_2-Asp-DCB inhibited DNA fragmentation, suggesting that TAM derivative-induced apoptosis was caspase dependent. Moreover, overexpression of Bcl-2 completely blocked TAM derivative-induced caspase activation and DNA fragmentation. Our results suggested that TAM and their derivatives induced apoptosis in a mitochondria-involved pathway.

PP-137
Alkalosis induces anti-apoptotic events via the MAPK signalling pathways in rat cardiac myoblasts

K. Stathopoulou, C. Gaitanaki and I. Beis
Department of Animal and Human Physiology, School of Biology, Faculty of Sciences, University of Athens, Panepistimioupolis, Athens, Greece. E-mail: kstath@biol.uoa.gr

The acid-base balance is one of the most important physiological parameters, affecting the cell function. In the present study we examined the effect of alkalosis on ERK (Extracellular Regulated Kinases) and JNK (c-Jun N-terminal kinases) signalling pathways in H9c2 cardiac myoblasts. Treatment with alkaline medium (pH 8.5) induced a moderate and prolonged ERK1/2, but a strong and transient JNK1/2 phosphorylation. These activations were partially attenuated by the Na^+/H^+ exchanger inhibitors amiloride or HOE642 (a kind gift from Aventis Pharma, Deutschland). Also, alkalosis induced a significant c-Jun phosphorylation in a JNK dependent manner as shown by using the selective JNK inhibitor SP600125. This result correlated well with electrophoretic mobility shift assay experiments, in which cell exposure to pH 8.5 caused increased binding at oligonucleotides containing the AP1 (Activator Protein 1) consensus sequence, which was abrogated by SP600125. In addition, long-term exposure to alkalosis induced the Bcl-2 phosphorylation at Ser-70, which is catalysed by JNKs and is implicated in anti-apoptotic cellular events. In parallel, cell viability experiments revealed that cells exposed to alkalosis for the same time periods showed enhanced survival. All the above results support that alkalosis induces signal transduction pathways that are implicated in cell survival, possibly via Bcl-2. Ms. Stathopoulou is a State Scholarships Foundation fellowship recipient.

PP-138
dUTpase MRNA silencing triggers apoptosis in cancer cells

J. Kovari, I. Zagyva, G. Merenyi and B. G. Vertessy
Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest, Hungary.
E-mail: julia.kovari@freemail.hu

dUTPase has a preventive role in DNA repair by reducing dUTP/dTTP ratio in cell. Lack of the enzyme triggers formation of uracil-DNA, which activates excision repair and leads to thymine-less cell death. The present aim is to reduce or zero the cellular dUTPase level in different cancer cells by mRNA silencing to analyse the ensuing apoptotic pathway. Four siRNAs were designed to trigger the simultaneous degradation of both nuclear and mitochondrial (DUT-N and DUT-M) isoforms of dUTPase mRNAs. First, the cells are transfected with an inducible expression vector containing the Drosophila melanogaster dUTPase gene which is turned on at the beginning. The second step is the transfection of the silencing plasmid from which cells produce siRNAs causing degradation of the physiological human dUTPase mRNAs. After cancelling of human dUTPase, D. mel. dUTPase protects cells from apoptosis. Cut-off of the plasmid responsible for D. mel. dUTPase production leads to apoptosis. Results showed that transfection of He-La cells only with human dUTPase silencing plasmids led to a radical reduction of DUT-N mRNA, but the enzyme protein level was unchanged. One of the silencing plasmids triggered the total degradation of DUT-M mRNA and caused phenotype alteration. The second silencing plasmid specifically degraded DUT-N mRNA. The last two silencing plasmids caused apoptosis in He-La cells. Transfected DU145 cells also suffered apoptosis by three of the silencing plasmids.

PP-139
Modifications in the human T cell proteome induced by intracellular HIV-1 Tat protein expression

M. Coiras[1], L. E. Camafeita[2], T. Urena[1], J. A. Lopez[2], F. Caballero[1], B. Fernandez[1], M. R. Lopez-Huertas[1], M. Perez-Olmeda[1] and J. Alcami[1]
[1]AIDS Immunopathology Unit, National Centre of Microbiology, Instituto de Salud Carlos III, Madrid, Spain, [2]Proteomic Unit, Fundación Centro Nacional de Investigaciones Cardiovasculares Carlos III, Madrid, Spain. E-mail: mcoiras@isciii.es

HIV-1 establishes long-term infection of CD4+ T cells, which become long-lived reservoirs. Persistent infection decreases CD4+ number. However, infected T cells are quite protected from apoptosis. HIV-1 transactivating protein Tat is necessary for viral replication and is a potent activator of viral gene expression and regulator of cell gene expression. Soluble Tat is highly toxic since it causes dramatic cytoskeletal rearrangements. Thus, it could be considered a pro-apoptotic factor. However, there are contradictory evidences that Tat could also present anti-apoptotic properties.

Objectives: (a) Development of a Jurkat (JJ) cell line stably transfected with Tat and (b) Analysis by mass spectrometry (MS) of the JJ-Tat proteome.
Results: (a) Tat protein presented nuclear localization in JJ-Tat cells, and was fully functional; (b) Tat expression resulted in protection from Tunicamycin-induced apoptosis and (c) Analyses by DIGE/MS revealed a down-regulation of some cytoskeletal proteins (CP).
Conclusions: (a) Because some viral proteins cause cytoskeletal reorganizations that can induce apoptosis, down-expression of these CP could maintain the cellular integrity. This could be involved in the survival of long-term reservoirs of HIV-infected CD4+ T cells; (b) Expression of these CP could also be critical for the actin cytoskeletal reorganizations necessary to initiate HIV-induced membrane fusion events, viral assembly and budding. This could be important to avoid the re-infection of T cells.

PP-140
Characterization of the nuclease responsible for uracil-DNA signalling in *drosophila melanogaster*

M. Pukancsik[1], A. Bekesi[1], F. Felfoldi[2], I. Zagyva[1], **K. Medzihradszky F.**[3] and B. Vertessy G.[1]
[1]*Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest, Hungary,* [2]*MolCat Ltd., Budapest, Hungary,* [3]*Proteomics Laboratory, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary. E-mail: p_marcsi@enzim.hu*

The fruitfly genome lacks the otherwise common uracil-DNA glycosylase and may tolerate uracil incorporation in its DNA. This incorporation is prevented by the enzyme dUTPase that removes dUTP from the DNA polymerisation pathway. In cells deficient in one or both of these enzymic activities, the uracil content of DNA is significantly increased. In Drosophila larval stages dUTPase presence is confined to the imaginal disks suggesting correlation between presence of dUTPase in larval/imaginal tissues and cell survival. All cells containing dUTPase (i.e. the imaginal disk cells) survive the pupal stages and became differentiated into fly organs while all cells lacking dUTPase are sentenced to death during metamorphosis (cf Békési et al., 2004, JBC 279: 22362–70). Here, we set out to show the presence of uracil-DNA in Drosophila larval tissues. We compared genomic DNA from larvae and imago after treating them with UDE (uracil-N-glycosylase) and APE (AP endonuclease), the members of BER mechanism. In larval genomic DNA notable degradation was detectable. We identified a uracil-DNA specific nuclease (UDE) by uracil-DNA pull-down experiments with no detectable homology to other proteins except a group of sequences present in genomes of other pupating insects (Békési et al., submitted). The protein was investigated by CD and fluorescence spectroscopies as well as by proteolysis. Results suggest secondary structural elements and flexible segments within the protein.

PP-141
Thermal stress induces anti-apoptotic events via the p38-MAPK pathway in *M. galloprovincialis*

E. Gourgou, C. Gaitanaki and I. Beis
School of Biology, Department of Animal &Human Physiology University of Athens, Athens, Greece. E-mail: elgour@biol.uoa.gr

Thermal stress is a common kind of environmental stress for marine invertebrates, due to their special habitat. In the present study, we investigated the possible effect of thermal stress on the p38-MAPK signalling pathway, in *Mytilus galloprovincialis* gill tissue. The stressful stimulus was applied to the whole organism. Hypothermia (4°C) induced a moderate p38-MAPK phosphorylation, whereas hyperthermia (30°C) induced p38-MAPK phosphorylation maximized at 60min. As far as the possible synergistic effects of hyperthermia and Cu^{2+} is concerned, it was revealed that these two stressful stimuli act co-operatively, inducing an almost double p38-MAPK activation. Furthermore, investigating the possibility of pro-apoptotic or anti-apoptotic events occurring as a result of the p38-MAPK activation, it was revealed that hyperthermia, also in combination with Cu^{2+}, may lead to anti-apoptotic events possibly via the induction of Hsp70 over-expression in the gill tissue. This was revealed by detection of the protein itself, as well as by RT-PCR concerning the Hsp70 mRNA. Activation of caspase-3, known for its apoptotic action, was not detected. The specificity of the pathway was confirmed by the fact that using SB204580, specific inhibitor of p38-MAPK, induction of HSP70 was abolished. Therefore, thermal stress seems to activate anti-apoptotic events in gill tissue of *M. galloprovincialis*.

PP-142
Apoptosis correction in myocardial infarction with liposomal form of isosorbide dinitrate

Z. Bulentayeva[1], B. Bekmanov[1], A. Mansharipova[2] and R. Bersimbaev[1]
[1]*Molecular genetics, Institute of General Genetics and Cytology, Almaty, Kazakhstan,* [2]*Institute of Cardiology and Internal Diseases, Almaty. Kazakhstan. E-mail: zeinepb@nursat.kz*

Apoptosis plays crucial role in the number of cellular processes. Several studies established that apoptosis occurs in various cardiac pathologies, such as ischaemia, myocardial infarction. Several apoptosis regulation molecules have been found in last decade, but still the exact role of most of them did not revealed. Nitric oxide (NO) is a key molecule involved in the pathophysiology of heart. The aim of our work was to investigate the NO role in development of stress-induced myocardial infarction in rats. Obtained data suggest that stress significantly decreases NO levels in blood and myocardial tissue, whereas NO-donor (izoket) significantly increases NO level in myocardial tissue. Izoket and progesterone inhibit the stress-induced apoptosis. Also it was demonstrated that izoket-mediated apoptosis inhibition was associated with reduction of an infarction area and improvement of cardiomyocyte structure. The apoptosis inhibition directly depended from duration of the izoket injections. Evaluation of O^{2-}, $ONOO^{-}$, OH^{-} (reactive oxygen specious, ROS) concentrations in blood and myocardial tissue samples revealed that in samples from control group levels of all parameters are significantly lower than in other experimental groups and parameters from stress and progesterone groups were almost equally high. We suggested that NO plays important role in apoptosis inhibition in myocardial tissue and it might be associated with decrease of ROS level.

PP-143
Mammalian cell death and differentiation mediated by forkhead transcription factor FOXO subfamily

K. sakamoto, K. Munekata, H. Kim, Y. Miyaguchi, Y. Katada, T. Nakamura and T. Kojima
Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan. E-mail: sakamoto@biol.tsukuba.ac.jp

The forkhead transcription factor FOXO subfamily is widely accepted as a regulator of cell cycle arrest, apoptosis, survival,

and differentiation by regulating expression of a number of target genes under the control of phosphorylation by Akt and deacetylation by Sirtuin. To analyse the effect of oxidative stress for FOXO subfamily, H2O2 and Glucose oxidase were added into HepG2, He-La and others, resulting in the elevation of FOXO1a expression and subsequent increase of cell death in dose- and time-dependent manners. In response to H2O2 stimuli, nuclear translocation of FOXO1a and its transcription activity were synergistically induced, yielded to increase the expression of pro-apoptotic genes, Bim and BCL6. To ensure the inducible effect of apoptosis by FOXO1 subfamily, cells stably expressing dominant negative FOXO3a were incubated with H2O2, resulting in the loss of induction of cell death. Further protein analysis revealed that exogenously introduced p53 could associate with FOXO3a under the control of oxidative stress, and thereby, suppressed transcription activity of FOXO3a. Furthermore, RT-PCR revealed that expression of FOXO4 was clearly upregulated during the differentiation of mouse 3T3-L1 into adipocyte. Because FOXO4-RNAi introduced 3T3-L1 failed to differentiate, FOXO subfamily potentially involves in a crucial role in adipogenic development, as well as in mammalian cell apoptosis.

PP-144
Sphingosylphosphorylcholine as an antiproliferative agent in thyroid cancer cells

E. Afrasiabi and K. Tornquist
Department of Biology, Abo Akademi University, Turku, Finland. E-mail: emad.afrasiabi@abo.fi

Among the group of bioactive sphingolipids, sphingosylphosphorylcholine (SPC) has been known to induce both antiproliferative and proliferative effects on cells depending on receptor expression and cell type. In a recent study we showed that a thyroid anaplastic cancer cell line (FRO) expresses two putative SPC receptor types; namely GPR4 and OGR1. In the present investigation we show, using a thymidine incorporation, MTT assay, and cell counting, that SPC reduced cell proliferation in a concentration dependent manner. The effect was pertussis toxin insensitive suggesting that other G proteins than Gi/G0 are involved in this signalling cascade. The effect of SPC was also independent of PLC, PKC, PI3 kinase, MAP kinase, p38 kinase, or jun kinase. Application of SPC to the cells induced a rapid (<30 min) rounding of the cells. However, DAPI staining and DNA-ladder analysis could not reveal any apoptotic effects of SPC. Furthermore, when cells treated with SPC for 24 h were washed and replated, they continued to grow, albeit slower than control cells. Flow cytometry analysis revealed a significant increase in the population of cells in the G2 phase, and a reduction in S phase after a 24 h treatment of the cells with SPC. Taken together, SPC seems to be an effective suppressor of thyroid cancer cell proliferation. Further investigations are needed to clarify the mechanisms underlying these effects.

PP-145
Modulation of apoptotic signals with esterification of selenium and vitamin E in binary compounds

P. S. Vraka, M. N. Rikkou, C. Drouza, A. D. Keramidas and **A. D. Odysseos**
Department of Chemistry, University of Cyprus, Nicosia, Cyprus. E-mail: odysseos@ucy.ac.cy

Redox-silent vitamin E epitomised by α-tocopheryl succinate elicits augmented pro-apoptotic signals activated by monoesterification of dicarboxylic acids with the phenol oxygen of aromatic rings. Organoselenium structure is critical in signal modulation. In order to obtain an insight into the synergism between vitamin E and selenium and enhance apoptotic activity, we have reported a new strategy introducing phenylselenyl and succinate moieties on vitamin E functional domains. Here in, we introduce a new series of pro-apoptotic vitamin E-selenium esters epitomised by α-tocopheryl selenyl diacetate. While the novel esters retained the antioxidant and free radical scavenging capacity of both selenium and vitamin E, they inhibited significantly prostate cancer cell growth when tested against their thionyl, succinate and phenylselenyl succinate analogues. Apoptotic features were disclosed in cells treated with diacetate and succinate esters but not with vitamin E derivatives or selenodiacetic acid. Caspase-3 enzymatic activity was significantly augmented in selenyl diacetic over succinate, thionyl diacetic and phenylselenyl succinate esters. Cancer cells were resistant to phenylselenyl acid or selenodiacetic acid, suggesting that apoptosis induction is attributed to the structural changes imposed onto the functional domain of these esters rather than to selenium and that ester stability plays a key role in the modulation of selenium bioavailability at cellular level.

PP-146
Nitric oxide induces a novel type of regulated, necrosis-like cell death in human carcinoma

S. Kocturk[1], A. Richter[2], D. Guner[2] and P. T. Daniel[2]
[1]Dokuz Eylul University Medical Faculty, Department of Biochemistry, Izmir, Turkey, [2]University Medical Center Charite, Berlin, Germany. E-mail: semra.kocturk@deu.edu.tr

Nitric oxide (NO) is a pleiotropic second messenger that has been implied in a broad range of cellular responses including apoptosis. To clarify the signalling requirements of NO, we employed a set of tumour cell mutants that carry distinct defects in the intrinsic apoptosis signalling cascade Here, we show that NO donor S-Nitroso-N-acetylpenicillamine (SNAP) trigger cell death via the intrinsic, pathway in p53 proficient HCT116 colon carcinoma and p53/Rb double mutant DU145 prostate carcinoma cells. Apoptosis induction was associated with mitochondrial membrane potential dissipation and release of cytochrome C. However, SNAP induced cell death equally efficient in Bax deleted HCT116 cells as compared with Bax expressing HCT116 wild type cells. Similar data were obtained in Bax-mutated DU145 cells and Bax re-expressing DU145 transfectants. Likewise, inhibition of pan-caspase activities by the use of zVAD-fmk failed to interfere with apoptosis induced by NO. Flow cytometric analyses showed the early occurrence of a PI/Annexin-V-FITC double positive cell population. This indicates that cell death proceeds via a primarily necrosis-like mechanism. Nevertheless, cell death was inhibitable by overexpression of the anti-apoptotic Bcl-xL. This establishes a novel, caspase independent and necrosis like mode of cell death induction by NO that occurs through a regulated, Bcl-xL inhibitable mechanism.

PP-147
PMA activates NFκB in a sphingosine kinase dependent manner

T. Blom and K. Törnquist
Department of Biology, Abo Akademi University, Turku, Finland. E-mail: tblom@abo.fi

Sphingosine-1-phosphate (S1P) regulates several cellular functions, e.g. motility and survival, either by acting as an intracellular second messenger or by binding to G-protein coupled S1P-receptors (S1PR). To date there is no clear consensus on the intracellular versus extracellular roles of S1P in calcium signalling and

promotion of survival. Phorbol 12-myristate 13-acetate (PMA) has been shown to activate both the survival-promoting transcription factor NFκB and sphingosine kinase. We have studied the role of sphingosine kinase in PMA-induced activation of NFκB (p65). We found that PMA-induced NFκB binding was dependent on intracellular calcium and on S1P synthesis. NFκB was activated by exogenous S1P at nanomolar concentrations, suggesting the involvement of G-protein coupled S1P-receptors. This was supported by the finding that NFκB p65 was activated also by dihydro-S1P, which may bind and signal through S1PR but has no known intracellular effect. Exogenously added S1P induced cytoplasmic calcium oscillations, whereas PMA did not have any measurable effect on the intracellular calcium concentration. The Gαq inhibitor U73122 attenuated the S1P-induced calcium oscillations without affecting NFκB p65-activation, suggesting that S1P activates at least two divergent signalling pathways. We conclude that PMA stimulates p65 binding by a sphingosine kinase dependent mechanism, likely through autocrine S1P signalling.

PP-148
Phospholipase D2 acts as an important regulator in nitric oxide synthesis in raw 264.7 cells

S. Y. Park, J. H. Cho, D. Y. Oh, H. J. Choi and **J. S. Han**
Department of Biochemistry and Molecular Biology, College of Medicine, Hanyang University, Seoul, Korea.
E-mail: ttokttoki@hotmail.com

The purpose of this study was to identify the role of phospholipase D2 (PLD2) in lipopolysaccharide (LPS)-induced nitric oxide (NO) synthesis. LPS enhanced NO synthesis and inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) expressions in macrophage cell line, Raw 264.7 cells. We found that the expressions of PLDs were increased when we stimulated Raw 264.7 cells with LPS. By blocking of PLD activity using 1-butanol, NO synthesis and expressions of iNOS and COX-2 were decreased. To confirm the role of PLD in NO synthesis in macrophages, we made stable cell lines transfected by PLD1 and PLD2, and their dominant negative forms, respectively. Interestingly, we found that only PLD2 overexpression, not PLD1, increased NO synthesis and expressions of iNOS and COX-2. Overexpression of dominant negative form of PLD2 (DN-PLD2) completely blocked LPS-induced NO production, while DN-PLD1 did not effect on LPS-induced NO synthesis. The activity of PLDs is established through phosphatidic acid (PA). Therefore, we investigated whether PA could increase NO synthesis and expressions of iNOS and COX-2. NO synthesis and iNOS and COX-2 expressions were increased by PA treatment. Taken together, the present study suggest that NO synthesis is regulated by PLD-dependent manner, and among PLD isozymes, PLD2 acts as an important regulator in NO production signal in Raw 264.7 cells.

PP-149
Interplay between PI3K/AKT and MAPK signalling pathways in DNA damaging drug-induced apoptosis

E. R. Lee, J. Y. Ahn, Y. J. Kang, B. W. Kim and S. G. Cho
Department of Animal Biotechnology, Bio/Molecular Informatics Center, IBST, and RCTCP, Konkuk University, Seoul, Korea.
E-mail: ssangoo@konkuk.ac.kr

Specific apoptosis pathways implicated in the action of an anticancer drug need to be characterized to improve the benefits of chemotherapy. Despite of many reports on the DNA damaging drug-mediated chemotherapy, more straight forward study is required to unravel the detailed molecular mechanism of its apoptotic effect. Doxorubicin, a DNA damaging anticancer drug, induced apoptosis of NIH3T3 cells in dose and time-dependent manner. Prior to cell death, both Akt and p38 MAPK were transiently phosphorylated and then almost completely inactivated, while ERK1/2 and JNK showed sustained activation in response to the drug treatment. Suppression of PI3K/Akt and p38 MAPK activities by specific inhibitors accelerated and enhanced doxorubicin-induced apoptosis. Inhibition of PI3K/Akt activation had significant effect on ERK phosphorylation, suggesting that Akt signalling pathway negatively regulates ERK activation. We were also able to find that PI3K/Akt inactivation and sustained ERK activation were intimately associated with the etoposide-induced apoptosis in HaCaT cells. Taken together, our results demonstrate that interplay between Akt signalling pathway and ERK activation has key role in the apoptosis induced by DNA damaging drugs such as doxorubicin and etoposide both in NIH3T3 fibroblasts and HaCaT keratinocytes.

PP-150
Eriodictyol protects UV-induced apoptosis in human keratinocyte mainly by regulating p38 MAPK signalling pathway

E. R. Lee, J. Y. Ahn, Y. J. Kang, J. H. Kim and S. G. Cho
Department of Animal Biotechnology, Bio/Molecular Informatics Center, IBST, and RCTCP, Konkuk University, Seoul, Korea.
E-mail: ssangoo@konkuk.ac.kr

Increase in the adverse effects of solar ultraviolet (UV) radiation enhances the need for novel chemoprevention strategies. Here, we investigated the effect of flavonoids on UVC-induced apoptosis in HaCaT keratinocytes. Out of decades of flavonoids, 5,7,3',4'-tetrahydroxy flavanone (eriodictyol) and 3,4'-dihydroxyflavone were found to exert slight but significant stimulatory effect on cell viability. Eriodictyol most effectively suppressed the UV-induced cell death of HaCaT keratinocytes, concomitant with the inhibition of PARP or pro-caspase-3 cleavage. We could know that p38 MAPK and ATF2 activation was suppressed during UVC-induced apoptosis of keratinocytes, and treatment with eriodictyol induced a significant induction of p38 MAPK and ATF2 phosphorylation. Inhibition of p38 MAPK activity by addition of specific inhibitor, SB202190, or over-expression of dominant-negative mutant form of p38 MAPK resulted in suppression of the anti-apoptotic effect of eriodictyol. Moreover, eriodictyol exerted an apparent suppressive effect on the UVC-induced ROS generation in HaCaT keratinocytes. Taken together, these findings suggest that eriodictyol protects keratinocytes from UVC-induced apoptosis by activating the p38 MAPK signalling pathway, supporting the distinct structure-activity relationship (SAR) between several flavonoids, including 3,4'-dihydroxy flavone and 5,7,3',4'-tetrahydroxy flavanone.

PP-151
Phospholipase D1 is a key factor for decidualization in human endometrial stromal cells

M. S. Yoon[1], J. B. Koo[1], K. J. Oh[1], J. H. Hwang[2] and **J. S. Han**[1]
[1]Department of Biochemistry & Molecular Biology, College of Medicine, Hanyang University, Seoul, Korea, [2]Department of Obstetrics and Gynecology, College of Medicine, Hanyang University, Seoul, Korea. E-mail: androusy@hanyang.ac.kr

Using a primary cell culture system of human endometrial stromal cells (ES cells), we investigated the role of phospholipase D

(PLD) in 8-Br-cAMP-induced decidualization, which is a morphological and biological differentiation process. When treated with 0.5 mM 8-Br-cAMP for 6 days, ES cells were transformed into a decidualized morphology and produced significant amounts of prolactin (PRL). Simultaneously, the activity and expression level of PLD1 also increased. Moreover, Overexpression of dominant negative (DN)-PLD1 inhibited the morphological changes induced by 0.5 mM 8-Br-cAMP, whereas overexpression of the wild form of PLD1 induced morphological changes in the absence of 0.5 mM 8-Br-cAMP treatment. Moreover, 8-Br-cAMP activated ERK1/2 in time-dependent manner during decidualization, and the blockage of MEK inhibited partially 8-Br-cAMP-induced PLD expression. Treatment of ES cells with Rp-isomer reduced partially PLD1 expression level, but it did not change ERK1/2 activation, implying that PKA is involved in PLD1 expression by ERK-independent pathway. Inhibition of Ras decreased ERK1/2 activation and partially PLD1 expression. In addition, cotreatment with Rp-isomer and manumycin completely inhibited PLD1 expression, suggesting that PKA and Ras/ERK independently regulate PLD1 expression. Taken together, these results suggest that upregulation of PLD1 is essential for decidualization of ES cells and PLD1 expression is mediated by PKA and Ras/ERK, separately.

PP-152
Cytotoxic drugs (All-Trans-R.A. and hydroxyurea) on caspase-3 activity in pregnant rat (liver and kidney)

H. Frangou[1], S. Massouridou[1], **E.-N. Emmanouil**[2] and E. Nikoloussis[2]

[1]Department of General Biology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece, [2]Department of Histology, Embryology and Anthropology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece. E-mail: frangou@med.auth.gr

Hydroxyurea is a teratogenic drug causing birth defects in a variety of experimental animals. All-Trans-R.A. is a Retinoid analogue which affects many biological processes. All-Trans-R.A. and HU induce apoptosis in some cell types. Caspase-3 is an active caspase, 'effector', which degrades intracellular substrates during apoptosis. To further understanding the apoptotic molecular mechanisms of All-Trans-R.A. and HU on rat liver and kidneys, caspase-3 activity was tested by colorimetric assay on subcellular fractions. Pregnant rats (230–260 g) were treated on Gestational days (GD), 9th, 10th with: (a) corn oil, (b) All-Trans-R.A. 50 mg/kb weight, (c) NaCl 9% and (d) HU 4.56 mg/kb weight. The animals were sacrificed at 17th day of their pregnancy. The subcellular fractions of liver and kidney parenchyma were taken according to the method of Nordlie and Lardy, and the caspase-3 activity was determined by a colorimetric assay (kit, Sigma). Caspase-3 activity was found increased only in renal nuclear fraction after HU administration. There was not any evidence of statistically difference from All-Trans-R.A. treated tissues as correlated with the control tissues. In pregnant rat liver the caspase-3 activity was decreased after treatment with the above drugs in crude as well as in subcellular fractions. Our findings suggest: (a) HU may induce apoptosis in pregnant rat kidney cells via caspase-3 process, and (b) All-Trans-R.A. may produce downregulation of this process.

PP-153
Corticotropin-releasing hormone induces apoptosis of brain microglia in culture: involvement of mitochondrial apoptotic pathway

J. Ock, S. Kim and **K. Suk**

Department of Pharmacology, School of Medicine, Kyungpook National University, Daegu, Korea. E-mail: ksuk@knu.ac.kr

Neuropeptides are short-chain peptides found in brain tissue, with some functioning as neurotransmitters and others functioning as hormones. Neuropeptides may directly or indirectly modulate glial functions in the central nervous system (CNS). In the current study, effects of various neuropeptides on the viability and inflammatory activation of cultured microglia have been investigated. While vasoactive intestinal peptide, substance P, cholecystokinin, or neuropeptide Y did not affect microglial cell viability, corticotropin-releasing hormone (CRH) induced a classical apoptosis of mouse microglia in culture as evidenced by nuclear condensation and fragmentation, TUNEL staining, and cleavage of caspase-3 and poly (ADP-ribose) polymerase (PARP) protein. CRH, however, did not influence nitric oxide production or inflammatory gene expression including cytokines and chemokines, indicating that CRH did not affect the inflammatory activation of microglia. The CRH-induced microglial apoptosis appeared to involve a mitochondrial pathway and reactive oxygen species based on mitochondrial membrane potential change, caspase-9 activation, and the sensitivity to antioxidants. Taken together, our results indicate that the stress neuropeptide CRH may regulate neuroinflammation by inducing the apoptosis of microglia, the major cellular source of inflammatory mediators in CNS.

PP-154
FAS-ligand-mediated 'reverse signalling' in autoimmunity: the possible impact

J. A. Trizna, E. S. Telegina, K. A. Popovich, N. A. Rabaya and O. V. Proussakova

Institute of Theoretical and Experimental Biophysics RAS, Pushchino, Russia. E-mail: triznaj@mail.ru

It is known that in the blood of autoimmune patients the content amount of Fas-receptor is elevated. We analysed the content of Fas-receptor and its oligomerization state in serum of patients suffering from autoimmune diseases. In serum of patients with the level of Fas expression 3-5-times higher than control level (up to 10 ng/ml), the major part of Fas-antigen was found as high molecular fraction (150–200 kDa). In serum of healthy individuals, soluble Fas was exclusively in monomeric form. Serum of patients with rheumatoid arthritis showed anti- proliferative effect on dividing cell culture. Immune-histochemical analysis of affected synovia demonstrated high expression of FAS-ligand. At the same time in peripheral blood lymphocytes of patients, high level of soluble Fas was observed. Since synoviocytes, being one of the cellular targets in autoimmune diseases, in particular rheumatoid arthritis, demonstrate high level of Fas-ligand expression, one can suggest that specifically cytotoxic 'reverse signalling' plays important role in pathogenesis of immune diseases.

PP-155
Apoptosis of Caco-2 cells caused by ADAP in relation to expression of p53, c-ras, c-mos and caspase 3

S. Marczi[1] and Lj. Glavas-Obrovac[2]
[1]Scientific Unit for Clinical-Medical Research, Clinical Hospital Osijek, Osijek, Croatia, [2]Department of Nuclear Medicine, Radiation Protection and Pathophysiology, Clinical Hospital Osijek, Osijek, Croatia. E-mail: saskamarczi@yahoo.com

4-Methyl-2,7-diamino-5,10-diphenyl-4,9-diazapyrenium hydrogensulfate (ADAP) is a DNA and RNA intercalator. Cytotoxicity of ADAP was analysed using the MTT assay. ADAP caused strong growth inhibitory effects on human colon carcinoma (Caco-2) cells in concentrations 1 and 10 μM, in comparison to human normal cells WI38. Apoptotic characteristics of treated Caco-2 cells (1 μM ADAP for 1 h) were observed using annexin-V-fluorescein. Immunoband depletion assay, performed with 0.1 mM ADAP on Caco-2 cells, showed that ADAP is not a topoisomeraseII poison. The mRNA expression of the genes p53, c-Ki-ras, c-N-ras, c-H-ras, c-mos and caspase 3, in Caco-2 cells treated with 1 μM ADAP, was examined by RT-PCR. The expression of all analysed genes, except for c-H-ras and p53, was dependent on the incubation time (3, 12 and 24 h). Transcripts for c-H-ras were not detected in treated Caco-2 cells when compared to control nontreated cells, and amount of the p53 mRNA in treated cells were similar to control cells.

Conclusion: Apoptosis of the Caco-2 cells treated with ADAP was a result of the mode of action different than inhibiting the topoisomeraseIIα enzyme. ADAP caused the increased expression of c-Ki-ras, c-N-ras, c-mos and caspase 3 gene products, which pointed out the possible role of these genes in apoptotic cell death of the treated Caco-2 cells.

PP-156
Molecular mechanism of methylating agent-induced apoptosis: identification of novel chemosensitivity markers

V. Pletsa, A. Korillou, M. Patrinou-Georgoula, S. A. Kyrtopoulos and A. Guialis
Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, Athens, Greece. E-mail: vpletsa@eie.gr

Methylating agents are a widely used class of anticancer drugs. The most biologically significant DNA methylation adduct is O6-methylguanine (O6-meG) repaired by O6 methylguanine DNA methyltransferase (MGMT). Inefficient repair and thus O6-meG accumulation can lead to cell death via apoptosis. The molecular mechanism is not fully elucidated and it seems to be cell type dependent. RNA binding proteins is an abundant superfamily of multifunctional proteins implicated in almost every aspect of RNA metabolism. Reports on their qualitative and quantitative modifications during apoptosis as well as their interactions with apoptosis-associated elements indicate their potent implication in the control of apoptotic cascade. An O6-methylguanine-DNA-methyltransferase inducible He-La cell line was treated with N-methyl-N-nitrosourea (MNU), a model methylating agent, under varying expression levels of MGMT. Our results show that: (a) Even low levels of MGMT confer significant protection against MNU-induced cell death; (b) Apoptosis is a late event prominent after 72 h as shown by FACS analysis, caspase-7 and not caspase-3 activation, PARP cleavage and DNA fragmentation and (c) Poly(A) polymerase cleavage is detected during apoptosis in He-La Tet On cells lacking MGMT activity (novel chemosensitivity marker?). The specific apoptotic pathway involved, MNU-induced apoptosis in other cell lines and modifications of additional RNA binding proteins are currently under investigation.

PP-157
Androgen receptor (ar) expression and apoptosis in the reproductive tissues of the porcine embryo

M. Somczyńska, K. Knapczyk, A. Hejmej, M. Duda and B. Bilinska
Laboratory of Endocrinology and Tissue Culture, Institute of Zoology, Jagiellonian University, Krakow, Poland. E-mail: slom@zuk.iz.uj.edu.pl

Each mammalian foetus has a potential to develop both male and female reproductive systems. Early in mammalian foetal development, the gonads are ambi-sexual and both the Mullerian and Wolffian ducts are present in the urogenital ridge. Development of sex-specific phenotype depends on androgens and anti-Mullerian hormone during the phase of organogenesis of the urogenital tract. Testosterone promotes the differentiation of the mesonephric (Wolffian) structures while dihydrotestosterone is essential for the development of external genitalia. Apoptosis plays a predominant role in the establishment of the female gonadal germ cell pool leading to the developmental tissue modelling. The process of apoptosis is regulated by many factors, including steroid hormones that act via specific receptors. The aim of this study was to define the expression pattern and time of the appearance of ARs in male and female foetal gonads and mesonephroic ducts as well as in non-reproductive tissues, and to localize TUNEL-positive cells on the same sections. Tissues of embryos obtained from day 18 to 90 of gestation were used for the detection of both, ARs and apoptotic cells, by means of immunohistochemistry and TUNEL assay, respectively. The cells of foetal gonads, kidneys, and lungs were positive for ARs and apoptosis. The detection of AR in foetal tissues at different days of gestation gives a further perspective in the understanding of its role in the target tissues during foetal development.

PP-158
Sphingolipids interact with calmodulin: new roles in signal transduction?

E. Kovacs and K. Liliom
Institute of Enzymology, Hungarian Academy of Sciences, Budapest, Hungary. E-mail: erika.kovacs@enzim.hu

The structurally simplest sphingolipids, including sphingosine, sphingosine-1-phosphate and ceramide, besides being structural components of biological membranes and intermediates of sphingolipid metabolism, have recently been shown to be important modulators of basic cellular processes like growth, apoptosis and motility. These lipids activate numerous signal transduction pathways, in which their levels change in a highly regulated temporal and spatial manner. The facts that sphingosine-1-phosphate and sphingosylphosphorylcholine can mobilize calcium from internal sources, that sphingosine kinase binds to and is possibly regulated by calmodulin, and that sphingosine is an inhibitor of several calmodulin-dependent enzymes, suggested to us that these members of the sphingolipid family might interact with calmodulin. To characterize the interaction of both Ca^{2+}-saturated and apocalmodulin with sphingosine, sphingosine-1-phosphate and ceramide, *in vitro* binding assays were carried out. Changes in fluorescence of the tyrosine residues of calmodulin as well as of dansyl-labelled calmodulin, and circular dichroism spectra of the

protein were measured. The functional effect of the interactions was tested in a calmodulin-dependent phosphodiesterase assay system. Our results show that these sphingolipids bind to calmodulin specifically, and differentially inhibit its ability to activate the enzyme phosphodiesterase.

PP-159
Mitochondrial localization of human cell death-inducing DFFA (DNA fragmentation factor a)-like effector-a

J. Santorova, K. Janouchova, K. Smolkova, M. Zackova, L. Hlavata and P. Jezek
Department of 75 Institute of Physiology Academy of Sciences, Prague, Czech Republic. E-mail: jezek@biomed.cas.cz

CIDEa,b,3 proteins are related to both N terminals of the heterodimeric DNA fragmentation factor DFF, consisting of the 40 kDa caspase-3-activated nuclease (DFF40/CAD) and its 45 kDa inhibitor (DFF45/CAD). CIDE-induced apoptosis could bypass caspase-dependent apoptosis, since the N-domain of CIDE binds to the domain on DFF45 opposing its inhibitory effect on DFF40. Revealed mitochondrial localization of CIDEs might serve as sequestering potentially danger proteins and preventing them to induce permanent apoptosis. Hypothetical, yet not demonstrated export of CIDEs from mitochondria could initiate apoptosis due to certain death-signal reflecting the state of mitochondria. We have confirmed mitochondrial localization of recombinant human CIDEa in W303 *S. cerevisiae* yeast and human CIDEa or CIDEa-fused with a red fluorescent protein (RFP-CIDEa) in selected culture cells, such as embryonic kidney 293T cells, hepatocellular carcinoma HEPG2 cells, and insulinoma INS1-E cells. The CIDEa import into the inner membrane was proven by immunodetection of fractionated mitochondria and its identity was verified by Western blotting and by MALDI-TOF-assisted peptide mapping of the trypsinized samples of isolated yeast mitochondria. RFP-CIDEa transfected to cultured cells was co-localized with mitotrackers and with mitochondrially-targeted green fluorescent protein. Our results prove definitively the mitochondrial location of CIDEa.

Acknowledgment: Supported by GACR grant 301/05/0221.

PP-160
Role of lysophosphatidic acid in rat uterine leiomyoma cells proliferation

E. Billon, Z. Tanfin and P. Robin
IBBMC, CNRS, Orsay, France.
E-mail: emmanuelle.billon@erc.u-psud.fr

Uterine fibroids (leiomyomas) are benign tumours of the uterine smooth muscle. Many hormones and growth factors are involved in leiomyoma growth through paracrine and autocrine mechanisms. Recently, the important role of phospholipids as major regulators of tumour development has emerged. Lysophosphatidic acid (LPA) is a bioactive lysophospholipid which stimulates cell proliferation, migration and survival, by acting via G-protein-coupled receptors. LPA is involved in the development and spreading of many tumours, including those of the reproductive system. Therefore we investigated the potential role of LPA and the signalling pathways involved in the proliferation of tumoural uterine smooth muscle cells (ELT-3). We found that LPA induced ELT-3 proliferation, via the activation of the MAP kinases ERK1/2. Using pharmacological and molecular approaches we identified the receptors involved in these effects. Quantification of LPA production, by an enzymatic test, showed that ELT-3 cells did not synthetize LPA but produced lysophosphatidylcholine (LPC) that can be converted into LPA by a lysoPLD activity when present in the medium. Indeed, incubation of ELT-3 cells in the presence of S. chromofuscus phospholipase D, which possesses a lysophospholipase D activity, led to the production of LPA that triggered ERK1/2 activation and cell proliferation. Our data underline the pathophysiological role of LPA in uterine leiomyoma development.

PP-161
ERK1/2 phosphorylation in human dendritic cells in response to chemokines

D. Oz Arslan[1], B. Kan[1] and A. A. Maghazachi[2]
[1]Department of Biophysics, School of Medicine, Marmara University, Istanbul, Turkey, [2]Department Of Anatomy, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway. E-mail: ozdevrim01@yahoo.com

Dendritic cells (DCs) are the most potent antigen presenting cells in the immune system. These cells uptake antigens in peripheral tissues which leads to their activation and migration into secondary lymphoid organs. Responsiveness to the chemokines CCL19, CCL20, CCL5 and expression of the corresponding receptors are important components of the DC recruitment process. Stimulation with chemokines via G protein-coupled receptors leads to downstream signalling events, involving small GTPases, phosphotinositide 3-kinases, protein tyrosine kinases and MAP kinases (MAPKs). In this study, immature and mature DCs (iDCs and mDCs) were generated *in vitro* from monocytes of healthy donor were treated with various chemokines and the phosphorylation state of ERK1/2 was determined using Western blot analysis. Treatment of iDCs with CCL20 and CCL5 resulted in phosphorylation of ERK2. In mDCs ERK2 was phosphorylated upon exposure to CCL19. We also demonstrated the expression of CCR7, the receptor of CCL19, in mDCs. Treatment of mDCs with pertussis toxin suggested the involvement of Gi/o in the CCL19-induced phosphorylation of ERK2. In conclusion, our findings indicate that MAPK signalling pathways may be key regulators of DC stimulation with chemokines. Understanding the molecular mechanisms underlying DC chemotaxis will provide further insight into the role of these cells in the immune response.

Acknowledgment: D. Oz Arslan was funded by Norway Research Council.

PP-162
Short tail coenzyme q induced p53-dependent apoptosis

M. Ikekita[1], Y. Esaka[1], Y. Nagahara[2], Y. Hasome[1] and T. Shinomiya[3]
[1]Department of Applied Bio. Sciences, Tokyo University of Sci., Chiba, Japan, [2]Department of Biotechnology, Tokyo Denki University, Saitama, Japan, [3]Department of Radio Isotopes and Biosafety Research, National Research Institute for Child Health and Development, Tokyo, Japan. E-mail: masalab@rs.noda.tus.ac.jp

Coenzyme Q functions as an electron carrier and reversibly changes to either an oxidized (CoQ), intermediate (CoQ-), or reduced ($CoQH_2$) form within a biomembrane. The $CoQH_2$ form also acts as an antioxidant and prevents cell death, and thus has been successfully used as a supplement. However, effect of CoQ form treatment to cells is not well understood. CoQ contains hydrophobic isoprenoid chain and this chain length varies among species and organs. In the present study, we examined the effect of CoQ and its isoplenoid side chain length variants on the growth of the cells having different p53 statuses. Treatment with CoQs having shorter isoprenoid chains, especially CoQ_2, induced cell

viability decrease and caspase-3 activity increase in p53-point mutated BALL-1 cells, whereas treatment with longer isoprenoid chains did not. However, CoQ_2 did not induce apoptosis in either a p53 wild-type MOLT-4F or a p53 null HL-60. Western blotting analysis revealed that this CoQ_2 induced apoptosis was dependent on p53 protein levels. Moreover, fluorescent probe study and using phosphorylated p53 specific antibody revealed that CoQ_2 induced reactive oxygen species (ROS) and subsequent phosphorylation of p53. An antioxidant, L-ascorbic acid, inhibited CoQ_2 induced apoptotic stimuli. Overall, these results suggested that short tail CoQ induces ROS generation and further p53-dependent apoptosis.

PP-163
Phosphatidylinositol lipid regulates NF-l assembly

S-K. Kim[1], T. K. Kwon[2], D. S. Min[3], Y. H. Lee[4] and **J-S. Chang**[1]
[1]Department of Life Science, College of Natural Science, Daejin University, Kyeonggido, Korea, [2]Department of Immunology, College of Medicine, Keimyung University, Taegu, Korea, [3]Department of Molecular Biology, College of Natural Science, Pusan National University, Busan, Korea, [4]Division of Molecular and Life Science, College of Science and Technology, Hanyang University, Kyeonggido, Korea. E-mail: jchang@daejin.ac.kr

Phospholipase C-gamma1 (PLC-gamma1) has two pleckstrin homology (PH) domains: an amino-terminal domain and a split PH domain. Phosphatidylinositolphosphate (PtdInsP) is a ubiquitous membrane phospholipid that plays diverse roles in cell growth and differentiation. We found that the PLC-gamma1 PH domain directly binds to neurofilament light chain (NF-L). To clarify the regulation mechanism for NF-L self-assembly, we examined the effects of the PLC-gamma1 PH domain-associated PtdInsPs on NF-L self-assembly. Our results revealed that PtdInsPs negatively regulate NF-L self-assembly *in vitro*. Further experiments showed that PtdInsPs directly bind the positively charged Arg54 of murine NF-L to inhibit self-assembly. Expressions of mutant NF-L proteins lacking binding affinity to PtdInsP rescued PtdInsP-induced inhibition of self-assembly *in vitro* and showed accelerated intermediate filament formation in human adrenal carcinoma SW13 (Vim-) cells, suggesting that a positively charged Arg54 appeared to play a critical role in NF-L self-assembly. These results collectively suggest that PtdInsPs mediate neuronal differentiation by regulating NF-L self-assembly.

PP-164
Mithramycin A sensitizes trail-mediated apoptosis by down-regulation of XIAP gene promoter through Sp1 sites in renal cancer cells

E. M. Jung[1], T. J. Lee[1], J. H. Lim[1], J-W. Park[1], D. S Min[2], J-S Chang[3], **Y. H. Lee**[4] and T. K. Kwon[1]
[1]Department of Immunology, School of Medicine, Keimyung University, Taegu, Korea, [2]Department of Molecular Biology, College of Natural Science, Pusan National University, Korea, [3]Department of Life Science, Daejin University, Pochon-gun, Kyeonggido, Korea, [4]Division of Molecular & Life science, College of Science & Technology, Hanyang University, Ansan, Korea. E-mail: kwontk@dsmc.or.kr

Cancer therapy traditionally combines cytotoxic agents, or a cytotoxic agent with a biologically active agent. Mithramycin A is a DNA-binding antitumour agent, which has been used clinically in several cancer therapies and Paget's disease. The antitumour property of mithramycin A is probably associated with its inhibitory effects on replication and transcription of tumour cells. In this study, we demonstrate for the first time mithramycin A selectively down-regulated XIAP gene expression. Mithramycin A-induced down-regulation of XIAP involves the putative Sp1 sites within the XIAP promoter region. Using a combination of the chromatin Immunoprecipitation (ChIP) assay and the luciferase reporter assay, we found that two specific Sp1 sites (located at –144 bp and –25 bp relative to the transcription start site) are required for mithramycin A-mediated inhibition of the XIAP promoter. In addition, we show that mithramycin A considerably increases the direct cytotoxic effect of tumour necrosis factor (TNF)-α-related apoptosis-inducing ligand (TRAIL) on renal cancer cells *in vitro*. The sensitization to TRAIL-induced apoptosis was prevented by the broad caspase inhibitor zVAD-fmk, overexpression of crm-A and XIAP, whereas overexpression of Bcl-2 had no effect. Taken together, the present studies suggest that mithramycin A may be an effective sensitizer of TRAIL-induced apoptosis in human renal cancer cells.

PP-165
On the role of tryptophan 323 residue of Xiap Bir3 domain on its inhibitory activity of caspase-9 and binding to SMAC type peptide

A. Mohamad-Gholy[1], A. MacKenzie[2] and J. Davoodi[1]
*[1]Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran, [2]Apoptosis Research Center, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada.
E-mail: mohamadgholy@ibb.ut.ac.ir*

The ability of the wild type xiap Bir3 domain as well as its Trp 323 mutation to Ser on inhibition of human caspase-9 and binding to AVPFVASLPN (SMAC type peptide) was investigated. According to the published X-Ray structure, Trp323 from Bir3 forms a hydrogen bond with Gln245 from caspase-9 as well as the proline in the P3 position of the Smac-type peptide located at the N-terminus of the small subunit of caspase-9. In order to investigate the role of W323 on these interactions, this residue was mutated to Serine. Circular dichroism studies showed that the protein maintained its native conformation. Furthermore, compared to the wild type protein, the thermal stability of the mutant protein remained essentially unchanged indicating that W323S mutation did not hamper proper folding of the protein. The binding of the wild type Bir3 as well as its mutant to smac-type peptide was also investigated by fluorescence spectroscopy. Addition of the peptide to Xiap Bir3 caused quenching of the emission spectra at 340 nm with 50% quenching at 15 μM, while for the mutant 50% quenching occurred at 39 μM. This indicates that Trp323 plays a very important role in binding to Smac-type peptide. The inhibition of caspase-9 by wild type Bir3 and its mutant was also compared. While the wild type protein potently inhibited the enzyme, the mutant failed to do so. These results indicate that Trp323 of Bir3 plays a pivotal for both in recognition of Smac type peptide as well as binding to caspase-9.

PP-166
Cell cycle-dependent expression of cIAP2 at G2/M phase contributes cell survival during mitotic cell cycle arrest

H. S. Jin and Tae H. Lee
Department of Biology, College of Science, Yonsei University, Seoul, South Korea. E-mail: hsjin@yonsei.ac.kr

cIAP2, a member of the inhibitor of apoptosis protein (IAP) gene family, is a primarily NF-kappaB-inducible gene for cells to

respond quickly to different apoptotic stimuli. A recent study using cDNA microarray technology has suggested that cIAP2 transcription can be regulated in a cell cycle-dependent manner. However, the mechanism for such regulation is unknown. In this study, we confirmed the cell cycle dependence of cIAP2 expression at both mRNA and protein levels, and found that a bipartite CDE (cell cycle-dependent element)/CHR (cell cycle gene homology region) element in the cIAP2 promoter was responsible for cIAP2 gene activation in G2/M phase. Selective downregulation of cIAP2 caused nocodazole-blocked mitotic cells to become susceptible to apoptosis, indicating that G2/M-specfic expression of cIAP2 contributes to survival of mitotically-arrested cells. We also showed that cell cycle-dependent, G2/M-specific cIAP2 expression was further enhanced by NF-kappaB activation, and p53 activation in A549 (p53+/+) cells or introduction of p53 into p53-null H1299 cells repressed NF-kappaB-induced cIAP2 transcription. Our studies document cIAP2 as the only G2/M-specific gene that is activated by NF-kappaB, and will help in understanding the molecular basis of cIAP2 overexpression in a variety of human cancers.

PP-167
Counteracting role of TGF-β and TNF on Fas-mediated apoptosis of FDC

Tae H. Lee, Sun-Mi Park and Min-Jung Kim
Department of Biology, College of Science, Yonsei University, Seoul, South Korea. E-mail: thlee@yonsei.ac.kr

Follicular dendritic cells (FDC) constitute the framework of germinal center (GC) in secondary lymphoid follicles, and the integrity of FDC networks is critically affected by cytokines present in the GC. We have previously shown that TNF promotes Fas-mediated apoptosis of HK cells, an established FDC-like cell line, by up-regulating Fas expression. However, in the developing GC, FDC death is not a hallmark of GC despite the presence of TNF and Fas ligand (FasL). Here, we report that TGF-β inhibits Fas-mediated apoptosis of HK cells by down-regulating the expression of surface Fas and caspase-8. The inhibitory effect of TGF-β can be observed when HK cells were simultaneously treated with TNF and TGF-β, indicating that TGF-β counteracts the effect of TNF in sensitizing cells to Fas-mediated apoptosis. Furthermore, the deprivation of TGF-β by injecting neutralizing TGF-β antibodies to the SRBC-immunized mice resulted in the sporadic appearance of FDC undergoing apoptosis in the lymphoid follicles, suggesting that TGF-β functions as a naturally occurring inhibitor that rescues FDCs which are predisposed to apoptosis. Our study documents a novel function of TGF-β in the maintenance of FDC networks.

PP-168
Photoactivation of mitochondria-targeted photosensitizer induce ROS production and cell death of He-La cells

D. S. Izyumov, O. Yu. Pletjushkina and B. V. Chernyak
Department of Bioenergetics, A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia. E-mail: denis_izyumov@mail.ru

It is widely accepted that reactive oxygen species (ROS), producing by mitochondrial respiratory chain takes part in human diseases. The effects of mitochondrial ROS were studied using Mitotracker Red (MR) as a photosensitizer, which was accumulated in mitochondria. We have shown that photoactivation of MR caused production of ROS. Immediately after illumination fluorescence of CM-DCF was revealed in mitochondria. After 5–10 min incubation in the dark, fluorescence became higher and spread homogenously in the cells. The rate of ROS production was stimulated with inhibitors of complex I and complex III of respiratory chain. This ROS production was prevented with diphenyleneiodonium (inhibitor of flavin-containing enzyme) and mitochondria-targeted antioxidant mitoQ. Mild illumination of MR-loaded cells caused cytochrome C release from mitochondria into cytosol and apoptosis. Apoptosis but not the release of cytochrome C was fully prevented with inhibitor of caspases zVADfmk. Increase in the fluence of illumination changed the features of cells death and led to necrosis during 3–5 h after illumination. Preincubation with zVADfmk and overexpression of Bcl-2 had no protective effect in this case. MitoQ protected against necrosis caused by strong illumination and revealed some features of apoptosis.

PP-169
Stress induced immune system changes; role of apoptosis

B. Pehlivanoglu, S. Bayrak, E. İleri and D. Balkanci
Department of Physiology, Hacettepe University Medical Faculty, Ankara, Turkey. E-mail: pbilge@hacettepe.edu.tr

Acute stress alters immune variables that are also affected by gender and menstrual cycle. Women are affected more from the stress-induced diseases. Involvement of the apoptosis in stress response has been studied recently. Our aim was to investigate the effect of mental stress on apoptosis of immune cells in men and women and at different phases of the cycleHealthy men (n = 13) and women (n = 9) volunteers at 18–25 years of age were subjected to Stroop colour-word interference and cold pressor tests. Women tested twice at follicular and luteal phases. Pre and post-test lymphocyte subsets and apoptotic T lymphocytes were determined flowcytometrically. Menstrual phase was assured by plasma oestrogen and progesterone levels. Stress response was evaluated by blood pressure and heart rate measurements throughout the test and plasma cortisol levels before and after the test. All the data were evaluated by appropriate statistical methods. Stress, decreased the CD4/CD8 ratio in men (P = 0). CD4+ cells were decreased in all groups after the test (P = 0). CD19+ cells were reduced in women at follicular phase ($P < 0.05$) and increased in men ($P < 0.01$), where as CD56+ cells were increased in women and decreased in men ($P < 0.02$). Annexin+ cells were higher in all groups after the test (P = 0).
Conclusion: Stress-related apoptosis of T cells may be an explanation of depressed cellular immunity Further studies can enlighten the diversity of immune responses to stress between men and women via apoptosis.

PP-170
Melatonin and its oxidation product inhibit FasL expression and protects T cells from activation-induced cell death

A. Pedrosa[1], R. Weinlich[2], G. Amarantes-Mendes[2] and A. Campa[1]
[1]Departamento de Análises Clínicas, Universidade de São Paulo, São Paulo, Brazil, [2]Departamento de Immunologia, Universidade de São Paulo, São Paulo, Brazil. E-mail: alziana@usp.br

Despite the increasing interest in the biological effects of melatonin on the immune system, the effect of this compound on T cells apoptosis remains largely unknown. This is particularly relevant in the context of activation-induced cell death (AICD) because: activated T cells are able to synthesize and to use melatonin and at the end of an immune response, apoptosis of effector T cells is

crucial to maintain homeostasis. Here we investigated the effect of melatonin and its oxidation product N-acetyl-n-formyl-metoxykynurenine (AFMK) on AICD. Apoptosis and FasL expression were measured in anti-CD3-stimulated T cell hybridoma (DO11.10) by flow cytometry and RT-PCR, respectively. At 1 mM, melatonin and AFMK inhibited approximately 70% of DNA fragmentation and the expression of FasL. The antioxidant activities of melatonin and AFMK have been considered as the operative mechanism for most of the biological effects triggered by melatonin. In comparison with the antioxidant N-acetyl-cysteine (NAC), melatonin and AFMK were more effective in inhibiting T cells death and Fas ligand expression, thus suggesting that the action of melatonin and AFMK include additional activities beyond their antioxidant property. The inhibition of T cell death induced by melatonin and AFMK may have important clinical implications, especially in cases in which melatonin is pharmacologically administered. In conclusion, melatonin and AFMK prevented anti-CD3-induced T cell death by blocking the Fas ligand expression.

PP-171
Expression of heparin affin regulatory peptide in glioma cells

E. Lampropoulou, A. Parthymou, E. Poimenidi, E. Giannopoulou and **E. Papadimitriou**
Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, Patras, Greece. E-mail: epapad@upatras.gr

Glioblastoma is the most frequent and malignant human brain tumour. None of the therapeutic approaches used up to date have significantly improved the clinical outcome of this disease and most patients with GBMs die in less than a year. Among the factors that may play a significant role in the progression of these tumours is cAMP, which seems to be decreased in high-grade tumours and significantly decreases glioma cell growth. Heparin affin regulatory peptide (HARP), also known as pleiotrophin or heparin-binding growth-associated molecule, is an 18-kDa secreted growth factor that has high affinity for heparin. HARP is expressed in glioblastomas, and although its role in glioma cell growth has not been clearly defined, preliminary results support an inhibitory effect on glioma cell growth and angiogenicity. Moreover, it seems that HARP expression is regulated by cAMP and plays a significant role in the inhibitory effect of cAMP on glioma cell proliferation.

Acknowledgment: We thank the Program Pythagoras for funding the above work.

PP-172
Apoptosis and cell proliferation in the gerbil ventral prostate after vasectomy

S. Pereira[1], O. A. Martins[2], F. E. Martinez[2] and **W. Mello Junior**[2]
[1]Biologia Celular, Instituto de Biologia, Universidade Estadual de Campinas, Unicamp, Campinas, Sao Paulo, Brazil, [2]Anatomia, Instituto de Biociencias, Universidade Estadual Paulista, Unesp, Botucatu. Sao Paulo, Brazil. E-mail: mellojr@ibb.unesp.br

Diseases such as cancer and benign prostatic hyperplasia are related to the disruption in the mechanism of regulating the balance between both processes of apoptosis and cell proliferation in the prostatic cells. That balance is controlled by androgens and growth factors. Vasectomy might interfere in action of transforming growth factors and in local production of growth factor, altering that balance. Thus this study evaluates the influence of the vasectomy on both processes of apoptosis and cell proliferation in the epithelium of the gerbil ventral prostate after vasectomy. The gerbils were divided into two experimental groups – vasectomized and sham-operated. Each experimental group was divided into two subgroups: Early (at 120 days of age) and Late (at 240 days of age). When the animals from all experimental groups attained 360 days of age, the ventral prostate of the gerbil was collected and processed histologically. It was evaluate indices of apoptosis (Fuelgen's reaction and anti-Caspase-9) and cell proliferation (anti-Ki67 and anti-PCNA) in the ventral prostate of the vasectomized and sham-operated gerbils. The indices of apoptosis did not alter significantly, and the indices of cell proliferation increased significantly in the gerbil ventral prostate after vasectomy. Thus, the vasectomy cause imbalance between both processes of apoptosis and cell proliferation in favour of cell proliferation in the epithelium of the gerbil ventral prostate.

PP-173
DQA induces human leukaemia cell death by an oxidative stress pathway

E. Galeano, A. I. García-Pérez and P. Sancho
Department of Biochemistry and Molecular Biology, University of Alcalá, Alcalá de Henares, Spain. E-mail: eva.galeano@uah.es

Delocalized lipophylic cations, such as dequalinium (DQA), comprise a new class of antitumoral agents which accumulate and are retained in the mitochondria of carcinoma cells due to a higher negative mitochondrial transmembrane potential of these cells than of normal cells. Mitochondria, which makes an integral contribution on the regulation of main cellular events, is an adequate target for tumour cell eradication. To understand the antitumoural properties of DQA, we are studying its effect on two human leukaemia cell lines: NB4, derived from acute promyelocytic leukaemia, and K562, derived from a chronic myeloid leukaemia in blastic crisis, which is resistant to treatments that induce apoptosis in other myeloid leukaemia cells. Previous studies have shown that DQA induces apoptosis or necrosis, respectively, on these cell lines by an unknown mechanism related to an early mitochondrial dysfunction which produces oxidative stress. Here, we have studied the implication of p38 and JNK pathways in cell death as a response to DQA-induced cell stress. Our results show a JNK activation by DQA under conditions that produce oxidative stress in both cell lines and apoptosis in NB4 cells. This study improves our knowledge of DQA intracellular mechanism for therapeutic purposes.

PP-174
Identification of a novel splice variant of BAX protein in human skin

T. Zuliani[1], Hl. Dougier[2], M. Verdier[1], M. Bonnet-Duquennoy[3], K. Lazou[3], R. Polakowska[4], M. Dumas[3] and Mh. Ratinaud[1]
[1]Lab. Physiologie Mitochondriale, Faculté de Médecine, Limoges, France, [2]UMR CNRS 6101, Limoges Cedex, France, [3]L. V. M. H. Recherche, Saint-Jean de Braye, France, [4]INSERM U459, Faculté de Médecine, Lille, France.
E-mail: mireille.verdier@unilim.fr

Pro- and anti-apoptotic members of the Bcl-2 family are fundamental in the control of apoptosis. Among these, Bax plays a key role. Its recruitment to mitochondria elicits the release of apoptotic proteins in the cytosol triggering caspase-dependent or independent apoptosis. For Bax, 9 isoforms (alpha, beta, gamma, ldelta, epsilon, lomega, lsigma, zeta and lpsi) have already been described in various tissues, but not in epidermal cells. Using a PCR amplification of Bax cDNA from human HaCat keratinocytes, a major 558 bp band and a 502 bp smaller one were detect matching, after sequencing, to Bax beta and gamma respectively. Interestingly, a

360 bp PCR-product was identified corresponding, after sequencing, to a new Bax splice variant named Bax lambda. It results to the splicing of exon 1 to exon 4 with the exclusion of exon 2 and 3, the 3' end being identical to Bax beta. RT-PCR on normal human cells demonstrated that Bax lambda is expressed in cultured normal human fibroblasts and keratinocytes. Moreover, undifferentiated basal epidermal cells (integrin β1 positive cells) expressed this variant since non-adherent differentiated cells do not. While the physiological significance of these findings remained to be elucidated, it highlighted the complexity of the regulation of Bax, particularly in keratinocyte apoptosis and differentiation.

PP-175
Intracellular signalling and histological examination of PACAP treatment on small bowel

A. Ferencz[1], B. Rácz[1], B. Gasz[1], K. Kalmár-Nagy[2], A. Tamás[3], D. Reglődi[3] and E. Róth[1]

[1]Department of Surgical Research and Techniques, University of Pecs, Pecs, Hungary, [2]Department of Surgery, University of Pecs, Pecs, Hungary, [3]Department of Anatomy, Neurohumoral Regulations Research Group of the Hungarian Academy of Sciences, University of Pecs, Pecs, Hungary. E-mail: andrea.ferencz@aok.pte.hu

Pituitary adenylate cyclase-activating polypeptide (PACAP) is present and plays a central role in the intestinal physiology. Until now the influences of PACAP on the small bowel ischemia/reperfusion (I/R) injury has not been examined. The aim of the present study was to investigate the possible protective effect of PACAP on the intestinal I/R model in rat. I/R groups (sham operated, 1 h I, 2 h I, 3 h I) and PACAP-treated groups were designed. During 3 h reperfusion PACAP intravenous perfusion were administrated. Small bowel biopsies were collected after laparotomy, at the end of the ischemia and reperfusion periods. We evaluated tissue damage on haematoxylin/eosin-stained sections. Furthermore, we investigated the activation of MAP kinases, caspase-3 pathways, and their possible role in protective effect of PACAP: Our histological results showed that ischemia caused destruction of the mucous layer, which was further deteriorated by the end of the reperfusion. 3 h PACAP treatment significantly protected the intestinal structure during early and late reperfusion. On the other hand proapoptotic pathways were increased in all I/R groups. In contrast, PACAP-treatment could decrease the activation these pathways. Our present results propose a possible protective effect of PACAP in I/R injury of the small intestine, which provides basis for further investigation to elucidate the mechanism of this protective effect.

Acknowledgment: Supported by Grants OTKAF046593 and OTKA046589.

PP-176
PACAP increases chondrocyte survival through acting on apoptosis signalling pathways

B. Rácz[1], D. Reglődi[2], B. Fodor[1], B. Gasz[1], L. Benkő[1], A. Ferencz[1], A. Tamás[2], F. Jr.Gallyas[3], E. Rőth[1] and B. Borsiczky[1]

[1]Department of Surgical Research and Techniques, University of Pecs, Pecs, Hungary, [2]Department of Anatomy, Neurohumoural Regulations Research Group of the Hungarian Academy of Sciences, University of Pecs, Pecs, Hungary, [3]Department of Biochemistry and Medical Chemistry, University of Pecs, Pecs, Hungary. E-mail: boglarka.racz@aok.pte.hu

Pituitary adenylate cyclase activating polypeptide (PACAP) has well-documented neuroprotective actions both *in vitro* and in different injury models *in vivo*. Protective effects have recently been shown also in non-neuronal systems. Osmotic stress activates intracellular secondary messenger signals that may contribute to the pathogenic alternations of different cells. The aim of the present study was to investigate the activation of MAP kinases, CREB and caspase-3 pathways in osmotic stress of chondrocytes, and their possible role in the protective effect of PACAP. Elevated proportion of apoptotic cells and decreased number of viable chondrocytes were found in the osmotic stress group. Increased phosphorylation of ERK1/2, p38 MAPK, caspase-3 and decreased phosphorylation of CREB were found in the chondrocytes treated by hyperosmotic medium, suggesting the activation of proapoptotic pathways. Treatment with PACAP increased cell survival in hyperosmotic stress. PACAP treatment increased the activation of CREB and decreased the activation of ERK1/2, p38 and caspase-3. These results demonstrate that the degenerative effect of hyperosmotic medium and the protective effect of PACAP involve complex kinase signalling pathways. Since increased osmotic loading frequently accompanies articular diseases it is suggested that the mentioned pathways play a role in the pathomechanism of such clinical conditions.

Acknowledgment: Supported by OTKA F046504, T-048851, T046589 and ETT 596/2003.

PP-177
PACAP attenuates excitotoxic retinal injury by influencing apoptotic pathways in neonatal rats

D. Reglődi[1], A. Tamás[1], P. Kiss[1], A. Lubics[1], B. Gasz[2], B. Borsiczky[2], F. Jr.Gallyas[3], G. Tóth[4], E. Rőth[2] and B. Rácz[2]

[1]Department of Anatomy, Neurohumoral Regulations Research Group of the Hungarian Academy of Sciences, University of Pecs, Pecs, Hungary, [2]Department of Surgical Research and Techniques, University of Pecs, Pecs, Hungary, [3]Department of Biochemistry and Medical Chemistry, University of Pecs, Pecs, Hungary, [4]Department of Medical Chemistry, University of Szeged, Szeged, Hungary. E-mail: dora.reglodi@aok.pte.hu

Pituitary adenylate cyclase activating polypeptide (PACAP) and its receptors are present in the retina and exert several distinct functions. Recently, we have shown that PACAP is protective against monosodium glutamate (MSG)-induced retinal degeneration. In the present study we studied the possible signal transduction pathways involved in the protective effect of intravitreal PACAP administration against apoptotic retinal degeneration induced by neonatal MSG treatment. MSG induced activation of proapoptotic signalling proteins and reduced the levels of antiapoptotic molecules in neonatal retinas. Co-treatment with PACAP attenuated the MSG-induced activation of caspase-3 and JNK, inhibited the MSG-induced cytosolic translocation of apoptosis inducing factor (AIF) and cytochrome c, and increased the level of phospho-bad, CREB and ERK1/2. Furthermore, PACAP treatment alone decreased cytosolic AIF and cytochrome c levels, while PACAP6-38 increased cytochrome C release, caspase-3 and JNK activity and decreased phospho-Bad activity. In summary, our results show that PACAP treatment attenuated the MSG-induced changes in apoptotic signalling molecules *in vivo* and suggest that also endogenously present PACAP has neuroprotective effects. These results may have further clinical implications in reducing glutamate-induced excitotoxicity in several ophtalmic diseases.

Acknowledgment: Supported by OTKA T046589, T048851, T048848, F046504, F048908, ETT596/2003 and MTA.

PP-178
Role of dual leucine zipper-bearing kinase (DLK) in tissue transglutaminase (TTG)-mediated apoptosis

K. Robitaille, A. Daviau and R. Blouin
Département de biologie, Faculté des sciences, Université de Sherbrooke, Sherbrooke, Québec, Canada.
E-mail: Karine.Robitaille@USherbrooke.ca

DLK is a key regulator of the c-Jun amino terminal kinase (JNK) signalling pathway. As opposed to other components of this pathway, very little is known about the physiological roles of DLK or about the mechanisms responsible for its own regulation in mammalian cells. Recent work from our laboratory has shown that DLK serves as an intracellular target of the Ca^{2+}-dependent cross-linking enzyme tTG in cells undergoing calphostin C-induced apoptosis. To extend these observations, we have examined what effects tTG-mediated cross-linking has on DLK activity and function. Our results indicated that cross-linked DLK behaves as a constitutively active protein, which has a priming effect on cell death initiated by calphostin C. This effect is entirely dependent on the kinase activity of DLK since a catalytically-inactive DLK mutant consistently failed to facilitate calphostin C-induced apoptosis, even if the oligomerization of the mutant and wild-type forms were comparable. These observations led us to propose that modulation of DLK activity by cross-linking may represent an important mechanism by which tTG regulates apoptosis. In the study, currently underway, we are examining whether tTG could modulate apoptosis in cells in which expression of endogenous DLK has been silenced by RNA interference. These experiments are of critical importance because deregulation of DLK activity by tTG-mediated cross-linking might contribute to cell death, which occurs in neurodegenerative disorders.

PP-179
Caspases are inactivated by tert-butylhydroperoxide

Y. Aksoy[1], K. Kesik[1], H. Canpınar[2] and D. Güç[2]
[1]*Biochemistry Department, Hacettepe University, Ankara, Turkey,* [2]*Medical Oncology Department, Hacettepe University, Ankara, Turkey. E-mail: yaseminb@hacettepe.edu.tr*

Apoptosis is a special form of cell death, which can be triggered by a variety of signals and pathophysiological conditions, including oxidative stress. Caspase-3 is a key executioner of apoptosis, whose activation is mediated by the initiator caspases, caspase-8 and caspase-9. Reduced Glutathione (GSH) plays a central physiological role in maintaining the homeostasis. In this study, tert-butyl hydroperoxide (t-BOOH) used to decrease GSH levels, and N-acetyl-L-cysteine (NAC) used as antioxidant. The primary objective of this study is to determine GSH levels and caspase-3, 8 and 9 activities at apoptosis in HL-60 cells with or without NAC. After preincubation of the cell with antioxidant NAC, regeneration of GSH occurs as control level. but without NAC the value of GSH is half of the control level. At the present of NAC 25% value of GSH loses at 0 min but this value increases and reaches the control value at 1 min. On the other hand, without NAC, GSH value loses as 76% of its first value. Early apoptosis estimated using Annexin V on the flow cytometry. After 4.5 h oxidants incubation of cells, cells undergo necrosis. Caspase 3, 8 and 9 activities were decreased after 5 h compared with control group in absence NAC. In presence NAC, enzyme activities were the same with control group during 24 h. We have thought that the cells have gone apoptosis with independent mechanism from caspase activation.

PP-180
Reduction of cisplatin toxicity in breast cancer cell lines by the quercetin

A. Demiroglu Zergeroglu[1], Z. Karakaya[1] and E. Kilic[2]
[1]*Department of Biology, Gebze Institute of Technology, Gebze, Kocaeli, Turkey,* [2]*Department of Biochemistry, Medical Faculty, Erciyes University, Kayseri. E-mail: ademiroglu@gyte.edu.tr*

It is well known that, Cisplatin (CP) is highly effective cytotoxic agents in the treatment of malignancies. In spite of this, CP's utility has been limited since it causes several reactions including renal toxicity, gastrointestinal toxicity and ototoxicity that link to the production of free radicals. More recently, experimental studies have provided evidence for anti-oxidative activities of flavonoids. Quercetin (QC) is the most common flavonoids exist in plant food. Combination of CP and QC might lead to a reduction of toxicity of CP. At the present study, the effects of QC on CP toxicity were studied in an *in vitro* model of two cultured breast cancer cell lines. While cell proliferation was induced dose- and time-dependently by QC itself, CP alone inhibited cell proliferation as time and dose dependent manner in both MCF-7 and T47-D cells. In combination, CP with a certain doses of QC abolished the anti-proliferative effect of CP. Our preliminary results indicate that QC may be overcome CP toxicity in breast cancer cell lines. Yet the exact mechanism of inhibition needs to be investigated further.

PP-181
Effect of herb extracts on Jurkat cells survival

S. Turek[1], I. Berdowska[1], I. Fecka[2], A. Marcinkowska[1], B. Zielinski[2], T. Wysocka[3] and T. Banas[2]
[1]*Wrocaw Medical University, Department of Pharmacognosy, Nankiera, Wroclaw, Poland,* [2]*Wrocaw Medical University, Department of Medical Biochemistry, Chalubińskiego, Wrocaw, Poland,* [3]*Department of Histology and Embryology, Wrocaw Medical University. E-mail: iza@bioch.am.wroc.pl*

Phytochemicals are able to exert disparate effects on living cells, some of them show proapoptotic, and others antiapoptotic properties. The aim of this study was to evaluate the influence of selected herb extracts on human leukaemia cells viability. Aqueous extracts were prepared from: Thymus serpyllum (A), Thymus vulgaris (B), Majorana hortensis (C), and Mentha piperita(D). Jurkat cells were cultured with 10–500 mg/ml concentrations of these extracts for 35 h., and tested with neutral comet assay and MTT cytotoxicity assay. None extracts exhibited proapoptotic activity within the lowest concentration range (10 and 50 mg/ml) since percent of apoptotic cells was 1–3% (comparable with control). Extract C caused apoptosis in 25% cells. Clear increase in apoptotic cells was noticed after the incubation of Jurkat cells with the highest concentration (500 mg/ml) of extracts A, B and C (60%, 40% and 90%, respectively). Extract D showed only minor effect on apoptosis, inducing it in 10% cells. The results obtained from MTT assay showed biological activity of the cells. Extracts A, B and C at 100 mg/ml inhibited biological activity of the cells by 10–20%, and at 500 mg/ml their cytotoxic effect was the greatest. Surprisingly, the influence of extract D was opposite, since even at the highest concentration it seemed to stimulate the activity of the cells by 20%. Therefore, extract D seems to show disparate effect on cell survival in comparison with three other herbs.

PP-182
Resveratrol and mitochondrial pathway of apoptosis

D. Hotnog[1], V. Roman[1], L. I. Brasoveanu[1], C. Billard[2] and J. P. Kolb[2]
[1]Center of Immunology, Institute of Virology 'St. S. Nicolau', Bucharest, Romania, [2]U736 INSERM 'Résistance et Survie des Cellules Tumorales', Paris, France. E-mail: immunoce@rdslink.ro

B-cell chronic lymphocytic leukaemia (B-CLL) is a neoplasic disorder characterized by defective apoptosis due, in part, to endogenous nitric oxide (NO) production. The major problem in treating leukaemia is the existence of leukaemic cells resistant to drugs consecutive to the development of antiapoptotic machinery. Death receptors belonging to TNF (tumour necrosis factor) superfamily play a central role in apoptosis because they can activate the caspases cascade, causing the apoptotic demise of the cell. TRAIL (TNF-related apoptosis-inducing ligand), a member of the TNF family, could be an alternative apoptosis inducer, due to its cytotoxic effects against human leukaemia, but not normal cells. There are evidences that suggest the existence of a cross-talk between intrinsic and extrinsic apoptotic pathways. Resveratrol (3, 4', 5-trihydroxy-trans-stilbene) was found to have pro-apoptotic and anti-proliferative effects in many tumour cell types. We demonstrated that resveratrol reduced NO production in B-CLL and elicited the decrease of the mitochondrial transmembrane potential. Therefore, in this study we investigated the effects of resveratrol on TRAIL pathway in the leukaemic EHEB cell line after the decrease of the mitochondrial transmembrane potential. Our results prompted us to consider that both TRAIL, through its pro-apoptotic action, and resveratrol, used as adjuvant, via its anti-proliferative and pro-apoptotic effects, could be used in leukaemia immunotherapy.

PP-183
PPAR-γ agonists induce apoptosis in B lymphocytes and B lymphoma lineage cells by an NF-κB-dependent mechanism

F. Akbiyik[1], D. M. Ray[2], S. H. Bernstein[3] and **R. P. Phipps**[2]
[1]Hacettepe University, Department of Biochemistry, [2]University of Rochester School of Medicine and Dentistry, Departments of Environmental Medicine, Microbiology and Immunology, The Lung Biology and Disease Program, [3]The Lymphoma Biology Program of the James P. Wilmot Cancer Center, Rochester/NY. E-mail: fakbiyik@hacettepe.edu.tr

Peroxisome Proliferator Activated Receptor-gamma (PPARγ) is a transcription factor important in lipid metabolism and is emerging as an important regulator of immunity and inflammation. 15-deoxy-D-12,14-Prostaglandin J2 (15d-PGJ2) is an endogenous ligand for the nuclear receptor PPARγ. Our group previously demonstrated that mouse B cells and B lymphoma cells express PPARγ and 15d-PGJ2 rapidly kills them by apoptosis. Broad-spectrum caspase inhibitors do not rescue these cells from apoptosis, but caspases 2, 3, 8, 9, and 10 are potently activated. 15d-PGJ2 inhibits NF-κB activation by preventing Inhibitor kappa B (IκB) degradation in B cells. Activation of B cells with CD40 ligand causes IkB degradation and NF-κB activation and partially rescues them from 15d-PGJ2 induced apoptosis. The ability of 15d-PGJ2 to kill B cells in the absence of potent survival signals supports an anti-inflammatory role for this PG. Apoptosis induction by PPARγ ligands may be important for immune regulation by killing B lymphocytes as a rapid means to dampen inflammation. Moreover, the ability of PPARγ agonists to kill malignant B lineage cells has implications for their use as anti-B lymphoma agents.

PP-184
BCL-2 function in hairy cell leukaemia cell lines resistant and sensitive against IFN-α

S. Berker Karauzum[1], D. Yasar[1], E. Dirice[1], N. Imir[2], L. Undar[3] and G. Luleci[1]
[1]Department of Medical Biology and Genetics, Faculty of Medicine, Akdeniz University, Antalya, Turkey, [2]Central Laboratory of Medical Faculty, Akdeniz University, Antalya, Turkey, [3]Dapartment of Oncology and Haematology, Faculty of Medicine, Akdeniz University, Antalya, Turkey. E-mail: sibelkarauzum@akdeniz.edu.tr

It is known that Daudi (Burkitt lymphoma) cells are very sensitive against IFN-alpha but the reasons are still unclear. In the other hand, Escol cells are resistant to IFN-alpha. Excuses behind these distinct responses of two cell lines were investigated in this study. The focus of this research was to determine whether Bcl-2 protein synthesis is different between these two cell lines, or not. We have indicated that in Escol cells Bcl-2 protein expression is very high whereas Daudi cells are lack of Bcl-2 protein using Western Blot analysis. Related to Bcl-2 protein synthesis, Caspase-1 an apoptosis-inducing enzyme was found to be inactive (not cleaved) in Escol cells and active (cleaved) in Daudi cells. Cleavage of Caspases is mediated through Bcl-2 cellular concentration so we treat Daudi cells with Bcl-2 expression vector proving them to express Bcl-2. It was observed that Daudi cells over expressing Bcl-2 are much more resistant against IFN-alpha then the precursor Daudi cells. No maturation of Caspase-1 in Daudi cells over expressing Bcl-2 was detected. While the Bcl-2 over expression closes up the pores mitochondria releasing of cytochrome-C, cleavage of Caspases and apoptosis can be blocked.

Embryonic Stem Cells

PP-185
Megakaryocyte specific ablation of the podocalyxin gene by the Cre lox-P system

S. Alonso-Martin, S. Larrucea, N. Butta, E. Arias-Salgado, A. Nowakowski and R. Parrilla
Physiopathology and Human Molecular Genetics, Consejo Superior de Investigaciones Cientificas, Madrid, Spain. E-mail: soniaalm@cib.csic.es

Podocalyxin (Pdxl) is a strongly sulfated transmembrane sialoprotein found in the polyanion coating of the podocytes of the glomerular epithelial cells. This protein, that is essential for a normal glomerular function, is also expressed in vascular endothelial cells, megakaryocytes and platelets. The surface exposure of Pdxl in activated platelets and its homology to intercellular adhesion ligands suggests that it might play a role in cellular recognition and/or adherence. To elucidate the role of Pdxl in platelets we analysed: firstly, the effect of its specific overexpression in platelets; secondly, the generation of a specific null platelet mice. The transgenic mice showed an apparently normal phenotype but a reduction of the bleeding time. We generated, by homologous recombination, stem cells and thereafter mice

carrying a recombinant conditional Pdxl allele with a DNA fragment encompassed by loxP sequences. To produce null megakaryocyte Pdxl mice we have also generated transgenic mice expressing specifically Cre recombinase in platelets. We analysed the activity of Cre in platelets by western blotting and by crossing these mice with ROSA26 mice. The siblings showed a distinct X-GAL staining of megakaryocytes and platelets. The crossing of mice carrying the conditional Pdxl allele with mice expressing platelet-Cre will give us a selective ablation of the platelet Pdxl gene.

PP-186
Mu and kappa opioids induce the differentiation of embryonic stem cells to neural progenitors

E. Kim, A. L. Clark, A. Kiss, J. W. Hahn, C. J. Coscia and **M. M. Belcheva**

Department of Biochemistry and Molecular Biology, St. Louis University School of Medicine, St. Louis, MO, USA. E-mail: Beltchem@slu.edu

Considerable effort has been devoted to characterizing the intrinsic and extrinsic factors that regulate proliferation and differentiation of embryonic stem (ES) cells. Neurotransmitters have been implicated in the regulation of stem cell fate. Since various neural precursors exhibit functional neurotransmitter receptors, including G protein coupled receptors (GPCRs), it is anticipated that they are involved in cell fate decisions. We detected μ opioid receptor (MOR-1) and κ opioid receptor (KOR) binding and immunoreactivity in ES cells and in retinoic acid (RA) induced ES cell-derived neural progenitors (NPs). These GPCRs are functional as DAMGO, a MOR selective agonist and U69.593, a KOR selective agonist induce a sustained activation of extracellular signal-regulated kinase (ERK) signalling in undifferentiated ES cells. Moreover, both opioids enhance cell division of undifferentiated ES cells via the ERK signalling pathway. Importantly, biochemical and immunofluorescence data suggest that DAMGO and U69.593 divert ES cells from self-renewal and coax the cells to differentiate to NPs. In RA-differentiated NPs, opioids induce a biphasic ERK activation that correlates with opioid-induced inhibition of NPs proliferation. Our findings suggest that opioids may have opposite effects on ES cell self-renewal and differentiation. Finally, opioid modulation of ERK activity may play an important role in ES cell fate decisions by directing the cells to specific lineages.

PP-187
Reverse transfection of human embryonic stem cells

A. E. Topolska[1], M. Dottori[1], P. Jamshidi[1], G. Kolle[2] and M. F. Pera[1]

[1]*Monash University and the Australian Stem Cell Centre, Melbourne, Australia,* [2]*Institute for Molecular Bioscience, University of Queensland, Brisbane, Australia. E-mail: Agnieszka.Topolska@med.monash.edu.au*

Human embryonic stem cells (hESC) are immortal and pluripotential cells derived from human blastocysts. They can be propagated indefinitely *in vitro* and can differentiate into any cell type in the human body. Our understanding of the molecular basis of hESC biology is limited, thus identification of factors involved in maintaining the undifferentiated state of hESC and in promoting differentiation of progenitors of specific cell lineages is one of the most important tasks in hESC research. As hESC are routinely difficult to maintain and manipulate *in vitro*, conventional methods of molecular modification have low efficiency rate in hESC. We have established a new high throughput technique, called Reverse Transfection, of studying hESC. It combines microarray and transfection technologies and utilises a designed bi-cistronic expression vector (pBOS-IRES-EGFP) carrying a reporter gene for enhanced green fluorescent protein. We have used Reverse Transfection to introduce into hESC biologically functional molecules from the human transcriptome, and have assessed the effects of expression of molecules such as CD30 and CD30V in the hESC. We believe Reverse Transfection is a useful tool for high throughput phenotypical analysis of hESC.

PP-188
Nodal signalling in the regulation of pluripotent state of human and mouse ES and EC cells

N. Y. Krasnikova and O. F. Gordeeva

Group 'Embryonic stem cells', Institute of Developmental Biology RAS, Moscow, Russia. E-mail: olgagordeeva@yandex.ru

Mechanisms of pluripotent state maintenance and lineages determination in embryogenesis and pluripotent cell lines are still unclear. Embryonic stem cell and embryonal teratocarcinoma cell lines provide unique experimental systems for studying the earliest events in cell lineage restriction from pluripotent cells. We studied gene expression of Nodal/Lefty signalling pathway at the early stages of differentiation of mouse ES (R1) and EC (F9) cells as well as human ES (ESM 01) and EC (PA1) cells. We revealed that expression profiles of ES and EC cells were identical for mouse and human cell lines accordingly. Characteristic profiles coincided for ES cells that grew in different culture conditions. We conclude that pluripotent population of ES and EC cells at the early stage of differentiation, which are maintained by Nodal/Lefty signalling pathway in the analogous manner despite their origin and species differences.

Acknowledgment: This study was supported by the Russian Foundation for Basic Research (grant No. 05-04-49185) and Fundamental Research Program of the Russian Academy of Sciences 'Molecular and Cellular Biology'.

PP-189
Neural differentiation of mouse embryonic stem cells

G. Cetinkaya and S. Arat

Tubitak, Research Institute for Genetic Engineering and Biotechnology. E-mail: sezen@gmbae.tubitak.gov.tr

Embryonic stem cells (ESCs) have extensive self-renewal capacity and are competent to differentiate into any cell types in the body. Here we reported different systems to obtain an efficient neural differentiation of ESCs. Murine ESCs were aggregated and cultured as suspension for 8 days in two different media containing bone morphogenetic protein antagonist noggin or retinoic acid. Embryoid bodies were allowed to spread out onto gelatinised surfaces in ES cell medium supplemented with a qualified serum for 2 days and then in neurobasal media supplemented with B27 for 6 days. At the end of 17th day of differentiation promotion, cell populations highly enriched for proliferating nestin, NCAM and GFAP positive cells were obtained in noggin supplemented groups. Despite the weak attachment and reduced proliferation capacity, RA treated EBs formed cell colonies that were positive to neural markers; nestin, NCAM and GFAP, as well. When retinoic acid and noggin were supplemented together neural marker expression did not show a remarkable difference than the groups mentioned previously. Vimentin expression was more significant

in the group cultured without noggin and retinoic acid, than any other neural markers. Results showed that noggin-dependent controlled conversion of ESCs into neural cells is valuable for the study of neurogenesis and stem cell biology.

Acknowledgment: This work has been supported by TUBITAK EC FP6 Coordination Office (Marie Curie Actions, TR-Access Mobility Scholarship).

PP-190
Proteomic identification of a new 3-nitrotyrosine-containing proteins in cardiomyocyte differentiation: tyrosine nitration of β2-tubulin

Y. S. Park[1], S. K. Kang[1], J. Y. Lim[1], W. H. Kim[1], S. J. Lee[2], K. P. Kim[2] and S. I. Park[1]
[1]*Department of Biomedical Sciences, NIH, Seoul, Korea,* [2]*Department of Molecular Biotechnology, Konkuk University, Seoul, Korea. E-mail: parksi@nih.go.kr*

Nitric oxide (NO) is a precursor of reactive nitrating species, which produce modified proteins containing 3-nitrotyrosine. In this study, we confirmed that NO is endogenously produced by eNOS expression and its activation (p-Ser 1179). The NO level increased from differentiation day 4 and peaked day 8 and returned to basal level. Concomitantly, the tyrosine nitration level increased as the cardiac differentiation proceeded. However, the treatment of NOS inhibitor, L-NAME, suppressed the accumulation of NO production and subsequent tyrosine nitration, finally leading to prevention of the beating. The detection of tyrosine nitration coincided with the cells expressing cardiac troponin I. Furthermore, we identified for the first time the nitrated protein and its tyrosine residues by LC-MS/MS, where the amino acid sequences of tryptic digestion peptides exactly matched to β2-tubulin and specific residues were at the Tyr 106 and Tyr 340. Taken together, present study provided the first evidence that the tyrosine nitration of β2-tubulin could be a novel mechanism involved in the NO-mediated cardiac differentiation.

Adult Stem Cells

PP-191
From stem cells to neurons in the adult brain: the role of BM88

L. Katsimpardi[1], P. Charneau[2], P. M. Lledo[3], R. Matsas[1] and **D. Thomaidou**[1]
[1]*Laboratory of Cellular and Molecular Neurobiology, Department of Biochemistry, Hellenic Pasteur Institute, Athens, Greece,* [2]*Group of Molecular Virology and Vectorology, Institute of Pasteur Paris, Paris, France,* [3]*Odor Perception and Memory, Institute of Pasteur Paris, Paris, France. E-mail: l.katsimpardi@pasteur.gr*

Until recently, it was believed that the adult brain was unable to generate any new neurons. However, it is now commonly known that neural stem cells (NSC) remain in two areas of the adult brain, the subventricular zone (SVZ) and the dentate gyrus of the hippocampus. Neuroblasts that originate from SVZ precursor cells migrate long distances through the rostral migratory stream (RMS) to populate the olfactory bulb (OB), where they differentiate into local interneurons and establish contacts with their neuronal targets. The neuronal protein BM88 has been previously shown to drive mouse neuroblastoma Neuro2a cells towards exit from the cell cycle and differentiation towards a neuronal phenotype. In this study we investigated the role of BM88 in postnatal neurogenesis. We explored its *in vivo* distribution and performed gain- and loss-of-function studies in neurosphere cultures and SVZ explants derived from postnatal day (P) 5 brain. Using lentiviral vector gene transfer we demonstrated that BM88 overexpression leads to cell cycle arrest and differentiation of neuronal precursors. In addition, it disrupts normal chain migration from SVZ explants. Conversely, BM88 knockdown by RNA interference enhances cell proliferation and decreases neuronal differentiation. Taken together our results demonstrate that BM88 is implicated in mechanisms of cell cycle exit and differentiation towards the neuronal lineage in postnatal neurogenesis.

PP-192
Effect of estrogen on apoptotic regulatory mechanisms in mesenchymal stem cell maintenance

E. Terzioglu, F. Ayaloglu and K. C. Akcali
Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey. E-mail: eceter@bilkent.edu.tr

Mesenchymal stem cells (MSC) are adult stem cells which can differentiate into fat, bone, cartilage, muscle, liver and nerve cells. They can self-renew and have a high proliferative capacity. Although there are a great number of studies on MSC, the mechanisms that regulate the self-renewal feature of MSC are not clear. Therefore our aim is to explore the possible mechanisms that are involved in MSC maintenance and proliferation. We focused on estrogen due to its role on cellular proliferation. When we compared the colony forming unit (CFU) activities of MSC isolated from ovariectomized (ovx) and normal rats, we found an induction up to 3-fold in the number of the colonies formed when MSC are treated with oestrogen. Among the potential regulatory mechanisms of estrogen action on MSC, apoptotic pathway is one of the strongest possibilities due to the known affect of estrogen on apoptosis. In particular the bcl-2 gene family, which consists of pro-apoptotic and anti-apoptotic members such as bcl-2, bax, bak, bcl-x_L, and bcl-w, is likely a target of oestrogen. Several of these genes show differential expression patterns at mRNA and protein levels during different days of MSC culture. In the presence and absence of estrogen, these expressions change relatively with the proliferation and colony-forming rate. These data suggest that estrogen has a major role in MSC maintenance and apoptosis is one of the main mechanisms in its regulation.

PP-193
Human myoblasts are immunoprivileged and survived in xenogeneic host without immunosuppression

C. F. Guo[1], K. H. Haider[2], L. Ye[3], S. J. Jiang[2], P. K. Law[4], P. Wong[5], W. S. N. Shim[5] and E. K. W. Sim[1]
[1]*Surgery, National university of Singapore, Singapore,* [2]*Department of Pathology and Laboratory Medicine, University of Cincinnati, USA,* [3]*National university medical institutes, National university of Singapore, Singapore,* [4]*Cell Transplants Singapore, Pte. Ltd., Singapore,* [5]*National Heart Center, Singapore.*
E-mail: g0305832@nus.edu.sg

The aim was to investigate the immunological state and survival profile of transplanted human skeletal myoblasts (HSM) in a rat myocardial infarction model. Left anterior descending artery-ligated female Wistar rats were randomised to medium group-1 ($n = 10$), HSM transplanted group-2 ($n = 35$) and cyclosporine-treated HSM transplanted group-3 ($n = 35$). One week later, 150 μl medium without cells or containing 3×106 male HSM was injected into the infarct area. After 10 min, 1, 4, 7 and 28 days, the hearts were stained against CD4+, CD8+ cells, and macrophages, and it showed macrophage infiltration was detected from day-1 and followed by CD4+, CD8+ lymphocytes from day-4, but subsided by day-28. By real-time PCR against Y chromosome, the total HSM calculated in group-2 were 5.37%, 9.01%, 17.23% and 23.63% on day-1, -4, -7 and -28 respectively; in group-3, the total HSM were 9.26%, 15.55%, 31.36% and 54.70% respectively. The major histocompatibility complex I (MHC I) of the HSM in host was down-regulated on day-28 as compared to day-7 by expression of MHC I locus-A, B, C. By echocardiography, ejection fraction (EF) in group-3 (52.50%) improved more than that in group-2 (49.00%, $P < 0.05$); both EFs were significantly improved than group-1. To conclude, the HSM are immunoprivileged and showed prolonged survival in infarcted hearts without cyclosporine treatment, likely due to the down-expression of MHC I. Cyclosporine enhanced the survival of xeno-HSM and improved heart performance.

PP-194
Effects of MGF on human muscle stem cell proliferation in dystrophic, ALS and healthy human muscle

K. Ates[1], S. Yang[2], A. Sinanan[3], G. Golspink[2] and M. Lewis[3]
[1]*Department of Anatomy and Developmental Biology, University College London, London, UK,* [2]*University Department of Surgery, University College London, London, UK,* [3]*Division of Biomaterials and Tissue Engineering, Eastman Dental Institute, University College London, London, UK. E-mail: k.ates@medsch.ucl.ac.uk*

Like many other genes, the IGF-I gene can be spliced to produce several isoforms and in human muscle, it expresses at least two main isoforms, a liver type, systemic form (IGF-I Ea) and an autocrine/paracrine form (IGF-I Ec). This has been named Mechano Growth Factor (MGF) because of its mechanosensitivity. The roles of these two splice variants of the IGF-I gene were studied by proliferation/differentiation assays, using human primary cell cultures from biopsied muscles from congenital muscular dystrophy (CMD), facioscapulohumeral dystrophy (FSHD) and amyotrophic lateral sclerosis (ALS) patients as well as healthy volunteers. Human primary muscle cells were treated with IGF-I Ea and MGF E domain peptides and immunocytochemistry techniques with Desmin, DAPI and FITC markers were used to detect proliferation state. CPK and BCA protein assays were also used to determine the differentiation state following peptide treatment. The results showed that MGF significantly increased muscle stem (satellite) cell proliferation in both healthy and severe muscle wasting disorders. MGF dramatically increased progenitor cell proliferation in CMD, FSHD and ALS primary cultures. This also confirmed that MGF had no effect on myotube formation but that it increases myoblast progenitor cell proliferation, whilst IGF-I increased differentiation. The study supports the use of MGF as a potential therapeutic agent for muscle degeneration in neuromuscular disorders such as CMD, FSHD and ALS.

PP-195
OCT3/4 is highly expressed in bladder cancer: further evidence for stem-cell origin of cancer hypothesis

S. J. Mowla[1], Y. Atlasi[1], **A. M. Ziaee**[2] and A. R. Bahrami[3]
[1]*Department of Genetics, Tarbiat Modares University, Iran,* [2]*Nephrology Research center, Shahid Beheshti University of Medical sciences, Iran,* [3]*Institute of Biotechnology and Tissue Engineering, Ferdowsi University of Mashhad, Iran.*
E-mail: sjmowla@modares.ac.ir

Oct3/4 is a key regulator of pluripotency in embryonic stem and germ cells. The expression of such genes is in correlation with tumorigenesis process and can affect some aspects of tumour behaviour such as tumour recurrence or resistance to current therapies. Here we have investigated the potential expression of oct3/4 in bladder cancer.

We used semi-quantitative RT-PCR to examine 30 tumoural, 13 matched non-tumoural and nine healthy control tissue samples. The expression of Oct3/4 at protein level was further determined by Dot blot and Western blot analysis and Immunohistochemical (IHC) approach has been used to determine the distribution of this protein in tumour sections.

Results: Oct3/4 expression was detected in all examined tumours but at much lower level in some non-tumoural samples and also in few healthy controls. Dot blot and Western blot analysis further confirmed the expression of Oct3/4 in tumoural biopsies. According to IHC results, Oct3/4 is primarily localized in the cytoplasm of tumoural cells, which is in contrast with our data on embryonic stem cells.

Conclusion: Regarding the anti-differentiation role of Oct3/4, the expression of the gene can be used as a potential tumour marker for diagnosis and/or prognosis of bladder tumours. Work is currently in progress in our lab to examine the correlation between the grade of malignancy and tumour recurrence with the level of oct3/4 expression.

Differentiation of Stem Cells

PP-196
Hypothalamic proline-rich polypeptide is regulator of haematopoietic stem cell colony formation

B. Bezirganyan[1], K. Davtyan[2] and A. Galoyan[1]
[1]*Laboratory of Neurohormones Biochemistry, H. Buniatian Institute of Biochemistry NAS RA, Yerevan, Armenia,* [2]*Laboratory of Neurohormones Biochemistry, H. Buniatian Institute of Biochemistry NAS RA, Laboratory of Immunology and Virology, Department of Pathophysiology, 'Armenicum' Research Center, Yerevan, Armenia. E-mail: kristina_bezirganyan@yahoo.com*

Hypothalamic AGAPEPAEPAQPGVY proline-rich polypeptide (PRP) is one of the C-terminal derived peptides from the neurophysin-vasopressin associated glycoprotein produced by N. Paraventricularis and N. Supraopticus of bovine neurohypophysis. We studied the influence of PRP on haematopoietic stem cell colony formation *in vivo and in vitro* as well as on doxorubicin-induced bone marrow (BM) cells apoptosis. We observed that an absolute content of mature granulocytes, lymphocytes and monocytes decreased in PRP-treated rat in comparison with untreated rats BM preparations. Colony Forming Unit (CFU) analysis of nucleated BM cells obtained from untreated and PRP-treated rats shown that in PRP-treated rats, the number of CFU at day 7 and day 14 in BM was dramatically increased in contrast to untreated animals BM. PRP in concentration range 500–1000 ng/ml increased CFU in BM cell culture obtained from untreated rats. However PRP does not affect the future increase of the number of colony forming cells *in vitro* in animals, received PRP before BM was harvested. PRP *in vitro* time- and dose-dependent manner increased BM early apoptosis of granulocytes and monocytes and there is no effect on BM lymphocytes. PRP *in vitro* down-modulate doxorubicin-induced apoptosis of BM granulocytes and monocytes. These observations suggest that PRP is a regulator of stem cell colony formation and doxorubicin-induced BM cells apoptosis.

PP-197
Comparative investigation of spontaneous and RA induced differentiation of embryonic stem cells

A. Tas[1], S. Arat[1] and **H. Dalcik**[2]
[1]*Research Institute for Genetic Engineering and Biotechnology, Gebze, Kocaeli, Turkey,* [2]*Department of Histology and Embryology, Kocaeli University, Kocaeli, Turkey. E-mail: sezen@gmbae.tubitak.gov.tr*

Mouse embryonic stem (ES) cells derived from inner cell mass of blastocysts can differentiate into various types of cells. In suspension culture, ES cells can form 3-D structures called embryoid bodies (EBs) and can differentiate spontaneously into different types of cells. In this study, we cultured ES cells as aggregates and then investigated their spontaneous and neuronal differentiation. In suspension culture, EBs diameters and morphologies were analysed. EBs were treated with Retinoic Acid (RA) to induce their neuronal differentiation. EBs had compact, circular and well-arranged morphologies until the 8th day of culture. After 8th day, they lost their normal morphologies and resembled to blastocysts. On the 10th day, a mass of tissue composed of beating cells were observed. When RA was administrated to the culture media, EBs did not loose their morphologies and beating of the tissue was not observed. The initial beating cell groups were observed at day 7 after attachment. On the 17th day, neurons and neuronal networks were visualized. Rhythmically contracting cardiac cells were easily recognized on the 17th day. In the 4(/4+ RA group, different neuronal cell types were observed. In this group, neuron precursor cells were appeared at day 2, neuron-like cells and astrocytes were appeared at day 7.We obtained cardiac cells in high level but on a limited scale of cells differentiated into neurons in spontaneous differentiation.Correspondence: S. Arat, sezen@gmbae.tubitak.gov.tr

PP-198
Differentiation of mesenchymal stem cells to functional cardiomyocytes in shorter time

F. Ayaloglu[1], E. Terzioglu[1], M. Ugur[2], S. Durdu[3], E. Ozcınar[3], A. R. Akar[3], U. Ozyurda[3] and K. C. Akcalı[1]
[1]*Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey,* [2]*Department of Biophysics, Ankara University, Ankara, Turkey,* [3]*Department of Cardiovascular Surgery, Ankara University, Ankara, Turkey. E-mail: fatmaay@bilkent.edu.tr*

Multipotent Mesenchymal Stem Cells (MSC) have capacity to differentiate into osteoblasts, chondrocytes, adipocytes, and also to cardiomyocytes upon induction with appropriate factors. MSCs do not encounter immune rejection in the host and this feature makes them promising agents in the treatment of several diseases. Although several methods are well established for *in vitro* isolation and expansion of MSC from bone marrow, the duration of culture may be a problem in cell-based therapies for acute myocardial infarction or acute hind-limb ischaemia. Our goal is to be able to obtain cardiomyocytes from rat MSC in less time. Previously we have shown that rat MSCs do not differentiate into any specific cell line, but show the characteristics of MSCs at both 9 and 14 days of culture. In this study, we examined the differentiation potential of undifferentiated MSCs to cardiomyocytes by azacytidine application at day 9 and 14. Our results show that, both at day 9 and 14, MSCs show specific electrophysiological features of contracting cells, such as the presence of cytoplasmic free Ca^{2+}. The expressions of nestin, SM22α and cardiac specific markers such as Troponin I and α Sarcomeric Actin are being analysed by RT-PCR, western analysis and immunochemical staining. Our data suggest that it is possible to differentiate MSCs to functional cardiomyocytes in a shorter time period, which could be a very useful tool for the patients awaiting urgent cell-based therapies.

PP-199
IL-7 expands the pre-B cell compartment in human B cell development

Y. Parrish, J. Rogerio, E. Sahakian, G. Huang, Q. L. Hao, L. W. Barsky, E. Zielinska, M. A. Price and **K. J. Payne**
Division of Research Immunology/BMT, Children's Hospital Los Angeles, Los Angeles, USA. E-mail: kpayne@chla.usc.edu

Human B cell development is thought to differ from that in mouse with respect to the requirement for IL-7. However, most studies of IL-7 in human B cell development were performed using fetal bone marrow (BM). Recent reports indicate that IL-7 is not essential for fetal B lymphopoiesis in mice. Here we examine the role of IL-7 in nonfetal human B cell development using a novel *in vitro* model based on co-culturing cord blood (CB) haematopoietic stem cells (HSCs) on primary stroma from adult

human BM. Addition of IL-7 to this co-culture model induces a 44-fold increase in production of human B cell precursors. IL-7-induced increases are dose-dependent, specific to CD19+ cells, and occur in the presence of human, but not murine stroma. Similar IL-7-induced increases were observed during *in vitro* B cell production from HSCs in adult human BM. IL-7 effects are mediated through increased proliferation among pro-B (CD19+CD34+) and pre-B (CD19+CD34-IgM-) cells and a trend toward increased cell survival at the pre-B stage. In the absence of IL-7 activity, pro-B and pre-B compartments generated from co-cultures of CB HSCs are of equal size. IL-7 induces a 5-fold expansion of the pro-B and a 60-fold expansion of the pre-B compartment *in vitro*, restoring the pre-B to pro-B ratio to that seen during *in vivo* B cell development in adult human BM. Our data provide evidence that IL-7 expands the pre-B compartment during human B cell development.

PP-200
Sphingosylphosphorylcholine induces differentiation of human mesenchymal stem cells into smooth muscle cells

E. S. Jeon, H. J. Moon, Y. M. Kim and **J. H. Kim**
Department of Physiology, Pusan National University College of Medicine, Busan, Republic of Korea. E-mail: jhkimst@pusan.ac.kr

Mesenchymal stem cells (MSCs) can differentiate into diverse cell types including adipogenic, osteogenic, chondrogenic, and myogenic lineages. In the present study, we demonstrated for the first time that sphingosylphosphorylcholine (SPC) induces differentiation of human adipose tissue-derived mesenchymal stem cells (hADSCs) to smooth muscle-like cell types. By using two-dimensional gel electrophoresis coupled to LC-MS/MS mass spectrometry analysis, Western blotting, or RT-PCR analysis, we demonstrated that SPC treatment increased the expression levels of several smooth muscle-specific genes, such as α-smooth muscle actin (α-SMA), calponin, SM22α, and SM-myosin heavy chain, as potent as TGF-β1 and –β3 in hADSCs. The SPC-induced expression of α-SMA was stereo-selective to D-erythro-SPC, but not L-threo-SPC, and was not duplicated by other lysophospholipids, including sphingosine-1-phosphate and lysophosphatidylcholine. Pretreatment of hADSCs with pertussis toxin (PTX) or U73122, a PLC inhibitor, attenuated the D-erythro-SPC-stimulated expression of α-SMA. In contrast, the TGF-β-induced expression of α-SMA was not affected by pretreatment with PTX or U73122. These results suggest that SPC treatment directs differentiation of hADSCs to smooth muscle-like cell types through a unique signalling pathway including Gi/o and PLC.

PP-201
Characterization of peptide stimulating differentiation of messenchymal stem cells into osteoblast

M. K. Shin[1], J. S. Chang[2], T. K. Kwon[3], Y. H. Jang[1], M. K. Kim[1], D. W. Kang[1], **Y. J. Park**[4], Y. S. Bae[5] and D. S. Min[1]
[1]*Molecular Biology, Pusan National University, Busan, Korea,* [2]*Department of Life Science, Daejin University, Pochon-gun, Kyeongggido, Korea,* [3]*Department of Immunology, School of Medicine, Keimyung University, Daegu, Korea,* [4]*Dental college, Seoul National University, Seoul, Korea,* [5]*Biochemistry, Dong-A University, Busan, Korea. E-mail: minds@pusan.ac.kr*

Osteoblast differentiation is a key aspect of bone formation and remodelling. We isolated the peptide stimulating differentiation of human messenchymal stem cells (hMSCs) into osteoblast. The peptide activated ERK and Akt signalling pathway in a time and dose-dependent manner. Interestingly, the peptide induced intracellular calcium increase in a dose-dependent, suggesting involvement of phospholipase C. Differentiation into osteoblast induced the expression of phospholipase D (PLD) in a time-dependent, and the peptide treatment upregulated PLD expression during differentiation. In addition, treatment of the cells with PLD inhibitor, 1-butanol, not 3-butanol, suppressed differentiation into osteoblast by measuring alkaline phosphatase activity which is known as a marker enzyme of osteoblastic differentiation. These results suggest that PLD is involved in the differentiation of hMSCs into osteoblast. This peptide stimulating differentiation into osteoblast will be helpful for future clinical application of MSCs Moreover, using a culture system that facilitates osteogenic differentiation of bone marrow-derived hMSCs, we analysed gene-expression profiles during the mineralization process. These profile should contribute to a better understanding of the process of mineralization in the matrix surrounding hMSCs.

Gene Therapy

PP-202
Novel hydrophobically modified chitosan derivatives as non-viral vectors for gene therapy

S. Ercelen[1], X. Zhang[1], G. Duportail[1], V. Babak[2] and Y. Mely[2]
[1]*Laboratoire de Pharmacologie et Physicochimie des Interactions Cellulaires et Moleculaires, UMR 7034 du CNRS, Faculté de Pharmacie, Université Louis Pasteur, Illkirch, France,*
[2]*A Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, INEOS RAS, Moscow, Russian Federation. E-mail: sebnem@aspirine.u-strasbg.fr*

Chitosans are linear polysaccharides of natural origin used to design non-viral vectors for gene delivery. However, though chitosan derivatives are non-toxic, their transfection efficiency is limited. In order to increase their transfection, novel low molecular weight (5 kDa) chitosan derivatives grafted with dodecenoyl (DDC) groups at 3 and 18% were synthesized. The results of the chitosan/DNA complexes at various N/P ratios, pH and salt conditions were monitored by dynamic light scattering, YOYO-1 fluorescence and fluorescence correlation spectroscopy (FCS). The smallest and more positively charged chitosan/DNA complexes were obtained at pH 5.8 and N/P = 5 in the absence of salt: a condition where the chitosan derivatives were fully protonated and in excess over the DNA phosphate groups. The average number of DNA molecules per 3% DDC-grafted chitosan/DNA complex was found to be about 15. To investigate the entry of chitosan/DNA complexes into cells, we monitored the interaction of the complex with model lipid DMPC (neutral) and DMPG (anionic) vesicles by fluorescence anisotropy. From the thermotropic anisotropy profiles, these derivatives were found to preferentially interact with the internal leaflet of the plasma membrane. The transfection efficiency and the low cytotoxicity of 3 % DDC-grafted chitosan are promising for the development of new chitosan derivatives.

PP-203
Differential transduction efficacy of adenoviruses containing NGR within cyclic and linear sequences in the HI-loop of fibre protein to aminopeptidase n and αvβ3 integrins

D. Majhen[1], J. Gabrilovac[2], J. Richardson[3], M. Eloit[3] and A. Ambriovic-Ristov[1]
[1]*Division of molecular biology, Ruder Boskovic Institute, Zagreb, Croatia,* [2]*Division of molecular medicine, Ruder Boskovic Institute, Zagreb, Croatia,* [3]*UMR 1161 Virologie INRA-AFSSA-ENVA, Ecole Nationale Vétérinaire,Maisons Alfort Cedex, France. E-mail: dmajhen@irb.hr*

The aim of this work was to design an gene therapy applicable Ad vector retargeted on the APN molecule expressed on angiogenetic cells via insertion of specific NGR motiv (asparagine-glycine-arginine) into the HI loop of a fibre protein. On the rhabdomyosarcoma (RD) cell line, which expresses APN but only very low levels of αvβ3 integrin and CAR receptor, all NGR-bearing Ad we have constructed exhibited moderately increased attachment and transduction efficacy in comparison to wild type virus. NGR-bearing Ad containing cyclic motifs were more efficient than those containing linear ones. The increased transduction efficacy of NGR-bearing Ads was completely abolished by the APN-specific peptide CNGRC and the integrin-specific peptide CRGDC. By measuring transduction efficacy on human laryngeal carcinoma cells with graded expression of αvβ3 integrin, we found that NGR-bearing Ad bound weakly to αvβ3 integrin. Treatment of RD cells with TGF-1 up-regulated APN and increased transduction efficacy with Ad bearing NGR within cyclic sequence while this effect is less pronounced for Ad bearing NGR within linear sequence and is likely to be predominantly consequence of moderately increased expression of αvβ3 integrin. Both, APN and αvβ3 integrin are up regulated in endothelial cells in angiogenesis, therefore NGR-bearing adenoviruses could be suitable vectors for tumour gene therapy.

PP-204
Polyethylenimine-based antisense oligodeoxynucleotides of IL-4 suppress the production of IL-4 in a murine model of airway inflammation

J. Seong[1], S. T. Kim[1], S. Kim[1], K. Lee[2] and **C. Kim**[1]
[1]*College of Pharmacy, Seoul National University, Seoul, Korea,* [2]*College of Medicine, Korea University, Seoul, Korea. E-mail: ckkim@plaza.snu.ac.kr*

Interleukin-4 (IL-4) plays a key role as an inflammatory mediator in allergic asthma. To develop a therapeutic agent which specifically inhibits production of IL-4, we designed antisense-oligonucleodies (AS-ODNs) against murine IL-4 mRNA. When tested *in vitro*, naked ODNs were biologically unstable and showed poor transfection efficiency. To improve its stability and maximize intracellular delivery, AS-ODNs were complexed with linear polyethylenimine (PEI). IL-4 AS-ODNs/PEI complexes, polyplexes, markedly improved the function of IL-4 AS-ODNs in inhibiting secretion of IL-4 in D10.G4.1 cell line. IL-4 AS-ODNs/PEI complexes were resistant to enzymatic degradation such as Dnase. Atomic force microscopy showed that polyplexes display spherical shape with an average diameter of 98 nm. *In vivo* study, the polyplexes of IL-4 AS-ODNs were effective in suppressing secretion of IL-4 in the bronchoalveolar lavage fluid and the level of IgE level in serum when tested in a murine model of airway inflammation. Histological study of lung tissues also shows that airway inflammation was alleviated in the IL-4 AS-ODNs/PEI complex treated mice. Taken together, our data clearly demonstrate that complexation of IL-4 AS-ODNs with PEI provides a potential therapeutic tool in controlling inflammation associated with allergic asthma and eosinophilia.

PP-205
Evaluation of the colloidal stability of cationic liposome-DNA complexes in biological fluids

A. Pelin Yargan[1], Turkan Eldem[1] and Ozgen Eser[2]
[1]*Pharmaceutical Biotechnology Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey,* [2]*Microbiology and Clinical Microbiology Department, Faculty of Medicine, Hacettepe University, Ankara, Turkey. E-mail: apelin@hacettepe.edu.tr; teldem@hacettepe.edu.tr; ozgen@tr.net*

Purpose: To investigate the colloidal stability of cationic liposome (CL)-DNA complexes (CLDC) in biological fluids.
Methods: DC-CHOL/DOPE (3:2 molar ratio) CL were condensed with DNA in 20 μl incubation volumes to form the CLDC1 and CLDC2 having charge ratios of 2 and 3.3. After confirming the complexation by dynamic light scattering (DLS) and electrophoresis, CLDC were incubated at 37°C in DMEM, DMEM + FBS (5%) and conditioned cell culture medium obtained from the retinal pigment epithelium cells (CM-RPE) and their colloidal stability were evaluated by DLS.
Results: After incubation in DMEM the size of CLDC1 was reduced to 776 nm (30 min) and increased to 2234 nm (3 h), whereas there was a slow decrease in the size of CLDC2 [2055 nm (30 min)/1847 nm (2 h)]. Although incubation in DMEM + FBS (5%) presented complete disintegration of both of the complexes, CM-RPE resulted in decrease in their sizes. The incubation in the CM-RPE diluted with water 1:1 and 1:3 ratios also decreased the sizes of CLDC1 to 944 nm (30 min)/1178 nm (2.5 h) and 1260 nm (30 min)/1116 nm (2.5 h) and CLDC2 to 2204 nm (30 min)/2472 nm (2.5 h) and 1470 nm (30 min)/1911 nm (2.5 h) respectively.
Conclusions: The composition of the biological fluids had great effect in altering the colloidal stability of CLDC that should be examined by taking the cell specific ECM (glycoaminoglycans) into considerations.
Acknowledgment: This study was granted by Hacettepe Univ. Scientific Research Unit (Proj. Nrs.02G047 and 02G018).

PP-206
Modification of Ca-phosphate/DNA complex preparation provides a tool for gene delivery *in vitro* and *in vivo*

S. Y. Kim, K. H. Lee and **D. J. Oh**
Department of Bioscience and Biotechnology, Sejong University, Seoul, Korea. E-mail: djoh@sejong.ac.kr

Calcium phosphate precipitation technique has been one of the most common methods to introduce genetic information into a variety of cells. This is an effective non-viral gene delivery system but is also widely used to produce viral vectors in industries. However, since a narrow window of parameter values used for Ca-phosphate/DNA complex preparation influences the transfection efficiency dramatically, the outcome differs among research groups, which has been a major issue in application of this technology. In this study, key parameters for preparation of Ca-phosphate/DNA complex were examined, and optimal conditions were suggested for highly efficient transfection. To secure the reproducibility for large-scale applications, the effects of long-term storage of the Ca-phosphate/DNA complex were also

examined. When pre-formed Ca-phosphate particles, which the target DNA was adsorbed on just before its use were used for gene delivery, stable and reproducible high transfection was obtained. This modified preparation also showed effective gene delivery when the Ca-phosphate/DNA complex was used in oral gene delivery. When mice were used in *in vivo* gene delivery, using a new technique based on DNA adsorption to pre-formed Ca-phosphate particles for preparation of Ca-phosphate/DNA complex, most of high level transduction was observed in the initial region of small intestine, which shows its potential use for gene therapy applications.

PP-207
Delivery of GM-CSF gene using chitosan complexes

E. Salva[1], B. Enneli[2] and J. Akbuga[1]
[1]*Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Marmara, Istanbul, Turkey,* [2]*Faculty of Pharmacy, University of Yeditepe, Istanbul, Turkey. E-mail: esalva@marmara.edu.tr*

Granulocyte macrophage colony-stimulating factor (GM-CSF) regulates proliferation, differentiation and function of hematopoietic progenitor cells. GM-CSF has short biological half life and it is necessary to administer frequent injections of high dose of GM-CSF , however this approach has been limited by the toxicity of this protein. Chitosan (CS), a natural cationic polysaccharide, has a high potential as a non-viral vector for gene delivery. In this study, we investigated the carrier ability of CS by the compaction of hGM-CSF gene into the CS complex and *in vitro* transfection efficiency of the complex. pORF-hGM-CSF pDNA was isolated by the alkaline lysis method. CS/DNA complexes were prepared by using different concentrations of CS and pDNA. The *in vitro* characterization (size and charge) was made. *In vitro* transfection studies were performed in He-La cell line. After transfection pORF-hGM-CSF expression was assayed by MTT kit. CS/pORF-hGM-CSF complexes can be obtained by using different ratio of N/P (0.1:1–10:1). The morphology of CS/pDNA complexes was dependent on the charge ratios and these were showed with the agarose gel electrophoresis. Full complexation was obtained with the ratio of 0.1:1. The better GM-CSF expression was found with 1:1. The dose of plasmid in complex is also important in protein expression. According to our results, the CS/pDNA complex is thought to have ability to be a good potential in the treatment of the disease with GM-CSF.

PP-208
Development of a novel delivery system for antisense oligonucleotides

B. Enneli, E. Salva and J. Akbuga
Department of Biotechnology, Faculty of Pharmacy, University of Marmara, Istanbul, Turkey. E-mail: benneli@yeditepe.edu.tr

Background: Antisense oligonucleotides (AsODN) are synthetic molecules that are able to inhibit gene expression and therefore they are potentially active for the treatment of viral infections, cancer or inflammatory diseases. However, because of their poor stability in biological medium and weak intracellular penetration, suitable delivery systems for antisense oligonucleotide is needed. The aim of this study is to use chitosan as a AsODN delivery system and to investigate the antisense activity of AsODN: chitosan complex in which chitosan is biodegradable, biocompatible and also non-toxic biopolymer.

Methods: The 15-mer phosphorothioate AsODN and phosphodiester AsODN were used. These AsODNs were complexed with low-molecular weight chitosan (0.25% and 0.5%) at different ratios (1:1, 1:6, 1:10) and complex formations were controlled by 0.7% agarose gel electrophoresis. The Dnase-I enzyme stability and serum stability of these complexes were studied.
Results: The complex formation was formed at the ratios of (AsODN:chitosan) 1:1, 1:6 and 1:10 by using phosphorothioate AsODN and low-molecular weight chitosan at two different concentrations. However, phosphodiester AsODN could not form complexes same as the phosphorothioate AsODN. In the presence of DNAse I enzyme, the complex stability was protected.
Conclusion: Chitosan-AsODN complexes appear to be promising candidates for delivery of AsODNs and these findings would render worthwhile of further investigation.

PP-209
Development of a functional genomic strategy for identification of novel cardioprotective genes

P. Panagopoulou, S. Psarras and Y. Capetanaki
Division of Cell Biology, Center of Basic Research, Foundation of Biomedical Research-Academy of Athens, Greece. E-mail: gpanagop@bioacademy.gr

There is strong evidence that a major cause of heart failure, the direct effect of many cardiomyopathies, is due to cardiomyocyte death. Despite the plethora of genes linked to cell death, there is limited information on cell death inhibitors, specifically in the myocardium. Our goal is to identify the maximum number of novel molecules with protective activity against cardiomyocyte death. The cardiomyocyte cultures are infected with a cDNA library cloned in an adenoviral vector and are subsequently exposed to conditions that cause cell death. The molecules enhancing cell survival will be identified with microchip array technology. To construct the cDNA library, polyA + RNA has been extracted and pooled from adult and neonatal mouse hearts and whole 11-day old embryos. To normalize the representation of the molecules for the benefit of low abundance transcripts, the Duplex Specific Nuclease enzyme strategy was used. The normalized cDNA is cloned in a plasmid vector that bears a loxP sequence and the plasmid library is then converted to an adenolibrary by *in vitro* Cre-mediated recombination with the adenoviral cosmid cS360loxP. The presence of several marker cDNAs is being monitored during the whole procedure to ensure the adequate representation of transcripts independent of their abundance and size. Specific conditions have been employed to induce high rates of cardiomyocyte cell death in cDNA containing and control cultures. The identified molecules will be presented.

PP-210
Chitosan-based nanoparticles for dermal gene delivery

S. Özbaş-Turan and J. Akbuğa
Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Marmara University, Istanbul, Turkey. E-mail: sozbasturan@yahoo.com

Recently, gene therapy has received significant attention. The main problem of gene therapy is the development of safe and efficient gene transfection system. Among the non-viral vectors, chitosan (CS) is a promising non-viral vector for gene transfer. Although CS has been widely investigated as a gene-carrier, there is very little information about the skin application of CS/DNA system.

In this study, pDNA-CS nanoparticles (NP) were evaluated for skin DNA delivery. NP were prepared according to earlier report. pSV-β-Gal was used as a reporter gene. The size charge and surface characteristics of NP were examined. pDNA release from NP was also studied. Primary human dermal fibroblast (HDF) cells and Swiss/NIH 3T3 cell lines were used for in *in vitro* transfection studies. The β-galactosidase was spectrophotometrically assayed with ONPG. The zeta potential of NP changed between 10.4–45.9 mV at pH 5.5 and 6.9–37.1 mV at pH 7.4. The size of NP is between 189.0–371.1 nm. Sustained DNA release was observed for 40–60 days in *in vitro* experiment. NP were sufficiently transfected in HDF cells and 3T3 cell lines. Post transfection, the β-Gal expression was determined during the 6 days. Expression level was increased depending on the incubation time.
In conclusion, CS nanoparticles showed *in vitro* transfection potential in HDF cells for pDNA. After this preliminary study, the potential of CS nanoparticles for dermal gene delivery will be investigated *in vivo* conditions.

PP-211
Supramolecular oligonucleotide complexes capable of efficient uptake by mammalian cells

O. Simonova[1], A. Vladimirova[1], D. Pyshnyi[2], M. Zenkova[1] and V. Vlassov[1]
[1]Laboratory ob biochemistry of nucleic acids, Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russian Federation, [2]Laboratory ob chemistry of nucleic acids, Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russian Federation. E-mail: olesya.simonova@niboch.nsc.ru

Oligonucleotides are perspective and powerful drugs for gene therapy of cancer and several viral diseases. The major obstacle limiting routine use and clinical implementation of oligonucleotide-based therapeutics is poor cellular uptake. In this study we proposed to use accumulation of oligonucleotides into long flexible structures for efficient delivery into mammalian cells. The formation of supramolecular complexes (up to 2000 bp in length) consistent of two types of phosphodiester oligonucleotides under physiological conditions was explored. We found that self-organization into such structures enhances the ability of oligonucleotides to bind with several human and mice cancer cells. The level of binding was shown to depend on used cell line, length and concentration of oligonucleotide complexes. To improve the efficiency of penetration through the cellular membrane we attached lipophilic cholesterol molecules to different components of the complex. Uptake and cellular distribution of supramolecular complexes formed by antisense oligonucleotides and cholesterol-modified vehicle-oligonucleotides were studied by fluorescent microscopy. We determined efficient delivery of oligonucleotide complexes into cytoplasm and nucleus of human 293 cells. The obtained results suggest that delivery of oligonucleotides into cells in the multimerized form could provide a simple and promising route for the improved uptake of pharmacologically active oligonucleotides by mammalian cells.

PP-212
Differential expression of trail and its receptors in patients with prostate carcinoma

A. D. Sanlioglu, I. T. Koksal, A. Ciftcioglu, M. Baykara, G. Luleci and **S. Sanlioglu**
Human Gene Therapy Unit, Faculty of Medicine, Akdeniz University, Antalya, Turkey. E-mail: sanlioglu@akdeniz.edu.tr

Because TNF-Related Apoptosis Inducing Ligand (TRAIL) selectively kills cancer cells without damaging normal cells, gene therapy approach using TRAIL is a feasible approach for the treatment of patients with cancer. However, recent publications suggest that significant portions of human tumours appear to be TRAIL resistant. Our recent studies demonstrate that TRAIL receptor composition is the major modulator of TRAIL sensitivity as demonstrated using prostate, breast and lung cancer cells. This study concerns the investigation of TRAIL and TRAIL receptor expression profiles during prostate carcinogenesis in order to evaluate their potential as biomarkers and also to predict the feasibility of a related gene therapy approach. Paraffin embedded prostate tissues of forty four patients with Benign Prostate Hyperplasia (BPH), twenty eight patients with Organ Confined Prostate Carcinoma (OCPCa) and twenty six patients with Advanced Prostate Carcinoma (APCa) are analysed using immuno-histochemical staining procedures. Significant levels of TRAIL-R4 decoy receptor expression are detected in patients with BPH, OCPCa and APCa. All TRAIL markers tested appear to be valuable markers in separating BPH patients from patients with OCPCa or APCa. Due to the presence of high levels of TRAIL-R4 expression in all patient groups, complementary gene therapy modalities might be needed to bypass potential TRAIL-R4-induced resistance.

PP-213
A DCR2 siRNA approach defeats trail resistance in lung cancer cells

C. Aydin[1], A. D. Sanlioglu[1], O. Ozbudak[2], L. Dertsiz[3], G. Ozbilim[4], C. A. Akdis[5] and S. Sanlioglu[1]
[1]Departments of Human Gene Therapy and Medical Biology-Genetics, Akdeniz University Faculty of Medicine, Antalya, Turkey, [2]Department of Pulmonary Diseases, Akdeniz University Faculty of Medicine, Antalya, Turkey, [3]Department of Thoracic Surgery, Akdeniz University Faculty of Medicine, Antalya, Turkey, [4]Department of Pathology, Akdeniz University Faculty of Medicine, Antalya, Turkey, [5]Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland. E-mail: cigdemaydin@akdeniz.edu.tr

Lung cancer causes the highest rate of cancer related deaths both in men and women. Chemotherapy and radiotherapy are inadequate in increasing patient survival as they both require p53 for their anti-tumour activity. Death ligands such as TRAIL induce apoptosis regardless of p53 status of cancer cells. In this study, an adenovirus delivery of TRAIL is tested as a potential gene therapy modality for patients with lung cancer. In our experimental settings, A549 lung cancer cells displayed complete resistance to TRAIL-induced cell death. However, a complementary gene therapy modality involving IKK inhibition sensitized lung cancer cells to TRAIL. While TRAIL-resistant A549 cells exhibited high levels of TRAIL-R4 decoy receptor expression, TRAIL-sensitive lung cancer cell lines (H441 and HBE) failed to express TRAIL-R4 on cell surface. Interestingly, a DcR2 siRNA approach targeting TRAIL-R4 receptor sensitized A549 cells to TRAIL-induced apoptosis.
Immunohistochemical analysis of ten patients with lung carcinoma demonstrated that high levels of TRAIL-R4 receptor expression is not a phenotype just restricted to A549 cell line. In conclusion, mainly TRAIL-R4 decoy receptor gene expression appeared to modulate TRAIL sensitivity in lung cancer cells.

PP-214
Adenovirus delivery of TRAIL effectively destroys synoviocytes of patients with rheumatoid arthritis

A. Bisgin[1], A. D. Sanlioglu[1], M. Ulker[2], S. Tuzuner[3], E. Terzioglu[2] and S. Sanlioglu[1]
[1]*Departments of Human Gene Therapy and Medical Biology-Genetics, Akdeniz University Faculty of Medicine, Antalya, Turkey,* [2]*Departments of Rheumatology – Allergy and Immunology, Akdeniz University Faculty of Medicine, Antalya, Turkey,* [3]*Department of Orthopaedic Surgery, Akdeniz University Faculty of Medicine, Antalya, Turkey. E-mail: atilbisgin@yahoo.co.uk*

Rheumatoid arthritis (RA) is characterized by the chronic inflammation of the synovial joints resulting from the hyperplasia of synovial cells and the infiltration of lymphocytes, macrophages and plasma cells. Recently, apoptosis induction of synovial cells through death ligands such as TNF, FasL and TRAIL has been explored as a treatment modality for RA. Thus, the primary objective of this study is the testing of the efficacy of adenovirus delivery of human TRAIL (Ad5hTRAIL) for the treatment of patients with RA. First, primary synovial cell cultures are established from eight patients with RA. Adenovirus permissiveness of synovial cells is determined by the infection of synoviocytes with AdEGFP vector. Ad5hTRAIL vector is used to determine TRAIL sensitivity of RA synoviocytes. In addition, real time RT-PCR assays followed by flow cytometric analyses is employed to detect TRAIL receptor profiles. Adenovirus vectors appear to be an ideal candidate for the gene delivery into synovial cells. While the presence of TRAIL death receptors are necessary for the induction of cell death, high levels of TRAIL-R4 decoy receptor expression on surface is correlated with TRAIL resistance. In conclusion, because adenovirus mediated delivery of hTRAIL kills synoviocytes isolated from patients with RA, this gene therapy approach might be valuable in the design of novel treatment strategies for patients with RA.

Cell Cycle Regulation

PP-215
Transcriptional silencing of protein kinase CKII is associated with cellular senescence

S. W. Ryu, J. H. Woo, Y. S. Lee, J. W. Park and **Y. S. Bae**
Department of Biochemistry, Kyungpook National University, Daegu, Korea. E-mail: ysbae@knu.ac.kr

Protein kinase CKII plays a critical role in cell growth and proliferation. In this study, we examine how CKII activity is regulated during cellular senescence. Our results demonstrate that CKII activity apparently decreases during both replicative and H2O2-induced senescence in human diploid fibroblast IMR-90 cells. The mRNA and protein levels of CKIIα decreases significantly during replicative and H2O2-induced senescence, while only slight reduction in those of CKIIβ is observed during replicative senescence. Treatment of IMR-90 cells with CKII inhibitors 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) and apigenin led cells to acquire a senescent phenotype as judged by the senescence-associated β-galactosidase marker and overexpression of p53 and p21Waf-1. Knockdown of CKIIα in IMR90 cells by RNA interference also dramatically induced the senescent phenotype. In parallel, CKII activity was transcriptional down-regulated in rat liver and testis with advancing age. Taken together, these results suggest that down-regulation of CKII activity is tightly associated not only with cellular senescence but also with organism aging.

PP-216
Effect of HSP70i on viability, mitosis, cell cycle and ploidy in cells treated with aneugenic drugs

M. Glowala-Kosinska, A. Mazurek, A. Fiszer-Kierzkowska and Z. Krawczyk
Department of Tumour Biology, Maria Skodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland. E-mail: mglowala@interia.pl

The inducible chaperone protein HSP70i is often constitutively and highly expressed in various cancers. The aim of this study was to determine whether overexpresion of this protein influences apoptosis, mitosis, cell cycle and ploidy in cells treated with aneugenic agents (benomyl, griseofulvin, paclitaxel, vinblastine). The study was performed on V79 hamster fibroblast cell lines either non-expressing (V79/hsp70– line) or constitutively expressing the HSP70i protein (V79/hsp70+ line). We found that the V79/hsp70+ cells were more resistant to apoptosis induced by benomyl, paclitaxel and vinblastine, but at higher concentrations of these drugs spindle defects were more frequent, and the fraction of micronucleated cells was higher in V79/hsp70+ line. After treatment with benomyl or vinblastine V79/hsp70– cells were partially blocked in G2/M phase, while V79/hsp70+ cells were entirely blocked and mitotic arrest was observed. These results suggest that cytotoxic mechanisms in V79/hsp70– and V79/hsp70+ cells substantially differ. V79/hsp70– cells shown a tendency to enter apoptosis, while more resistant V79/hsp70+ cells rather underwent a mitotic block, had more abnormal mitotic spindles and micronuclei. Our results indicate that the HSP70 overexpression may facilitate survival of cells, that otherwise would die because of highly disturbed mitoses. However the effect of this chaperone on cell cycle and ploidy is dependent on the kind of aneugenic drug and its concentration.

PP-217
The alfalfa CDKB2;1 is activated by wounding, and ethylene, in a non-cell division-dependent manner

M. Zhiponova[1], A. Pettkó-Szandtner[1], Z. Neer[1], S. Bottka[1], É. Stelkovics[2], T. Krenács[3], D. Dudits[1], A. Fehér[1] and **L Szilák**[4]
[1]*Insitute of Plant Biology, Biological Research Center, Szeged, Hungary,* [2]*Laboratory of Tumorpathology and Molecular Diagnostics, Institute for Biotechnology, Bay Zoltan Foundation for Applied Research, Szeged, Hungary,* [3]*First Institute of Pathology and Experimental Cancer Research, Semmelweis University of Medicine, Budapest, Hungary,* [4]*Szilak Laboratories, Bioinformatics & Molecule-design Ltd., Szeged, Hungary E-mail: laszlo.szilak@freemail.hu*

Cyclin-dependent serine/threonine kinases (CDKs) have pivotal roles in regulating the eukaryotic cell cycle. Plants possess a unique class of CDKs (B-type CDKs) with preferential protein accumulation at G2/M-phases, however, their exact functions are still enigmatic. We studied a 360 bp promoter region of the alfalfa (Medicago sativa) CDKB2;1 gene in transgenic plants and cell lines. The activity of the analysed promoter was characteristic

for proliferating regions in planta, and specific for cells in the G2/M-phases in synchronized cell cultures. Immunohistochemical analysis of transgenic root sections further confirmed the correlation of the expression of the CDKB2;1 promoter-linked reporter genes with the accumulation of the correspondent kinase. Auxin treatment activated the cell cycle and induced both the reporter and endogenous genes in transgenic leaf explants. Wounding, and the known as wound-response mediator and G2/M inhibitor ethylene, also activated CDKB2; 1 but in a non-cell cycle-dependent manner. In silico analysis of the promoter, indeed, revealed the presence of cis-elements indicating not only cell cycle- but wound- and ethylene-dependent regulation of this CDK gene. The presented data contribute to understand better the complex regulation of mitosis-specific CDK genes. Studying the G2/M regulation in plants increases the knowledge about the cell cycle progression control in other eukaryotic organisms, too.

PP-218
Identification of cell cycle regulation proteins in CD8+ T cells from HER-2/neu+ breast cancer patients

R. Süzme[1], F. Gürdöl[1], S. Sağlam[2] and G. Deniz[3]
[1]*Istanbul Faculty of Medicine, Department of Biochemistry, Istanbul University, Istanbul, Turkey,* [2]*Institute of Oncology, Istanbul University, Istanbul, Turkey,* [3]*Institute for Experimental Medical Research (DETAE), Department of Immunology, Istanbul University, Istanbul, Turkey. E-mail: raffi@istanbul.edu.tr*

Tumours are considered as immunogenic due to the presence of mononuclear infiltrates, composed of T cells, natural killer cells, and macrophages. Tumour antigens recognized by T lymphocytes are the major targets of anti-tumour immunity both in animals and in humans. Both human CD4+ and CD8+ T cells can respond to the products of some genes, i.e. mutated Ras, p53, and bcr-abl. An oncogene called HER-2/neu is not a mutated gene, but transforms cells when overexpressed. It encodes a cell-surface protein present in several types of carcinomas, and is able to stimulate both CD4+ and CD8+ cells. Cells that recognize a peptide derived from the HER-2/neu protein have been found among CD8+ T cells infiltrating breast tumours. The aim of this study was to examine whether the protective immune response generated through CD8+ cells is modulated by means of the cell cycle regulation proteins in breast cancer patients overexpressing HER-2/neu protein. The study was carried out in healthy women (1),cancer patients diagnosed as either Her-2/neu+ (2), or Her-2/neu− (3). CD8+ T cells were isolated from the blood samples using the negative magnetic isolation technique. Protein retinoblastoma (PRb), Cyclin D3 and cyclin-dependent kinase 4 (cdk4) were determined by the Western blotting. The presence of the regulatory proteins were evaluated with regard to expression of Her-2/neu protein in patients.

PP-219
Phylogenetic analysis and expression profiling of TFDP1 and TFDP1l in zebrafish

C. Sucularli, M. Ozturk and O. Konu
Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey. E-mail: cerens@bilkent.edu.tr

Zebrafish is an excellent model for comparative and functional genomics studies due to the extensive sequence and functional homology of zebrafish proteins with those of mammals. However, a large number of genes are found in duplicates in fish lineage while their mammalian homologues are present as a single copy. Such a duplication event has the potential to lead to diversification in function and expression. Transcription factor DP1, a heterodimerization partner of E2F family transcription factors, is a powerful regulator of cell proliferation. Although alternatively spliced in certain cases, TFDP1 exists as a single copy in mammals. On the other hand, tfdp1 and tfdp1-like (tfdp1l) in zebrafish are likely products of a duplication event although the phylogenetic analysis and expression profiling of them have yet to be performed. Accordingly, NCBI and ENSEMBL databases were used to extract nucleotide and protein sequences of zebrafish tfpd1 and tfdp1l, as well as tfdp1 homologs from mouse, human, Fugu, and Tetraodon species. Multiple sequence alignments and phylogenetic analyses were performed using ClustalW and Treefinder 2004. Total RNA from the liver, ovary, digestive tract, brain and gills of adult fish, and that from ZF4 cell line were subjected to RT-PCR reactions with specific primer pairs. TFDP1 and TFDP1L were ubiquitously yet differentially expressed in aforementioned adult tissues and in ZF4.

PP-220
Cytotoxic activity of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride

V. N. Shchukin, A. B. Moshnikova, O. V. Proussakova and A. Yu. Gorokhovatsky
Institute of Theoretical and Experimental Biophysics RAS, Pushchino, Russia. E-mail: samschukin@rambler.ru

Chemical cross-linking agents traditionally used for macromolecule complexing are capable of affecting cell metabolism, as dependent on the nature of the active groups and on the length of their mediating spacer. However, cytotoxic properties of these agents and the cytotoxic activity mechanisms remain poorly known. The current study was aimed to assay cellular effects of a cross-linker with 'zero'-length spacer, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC). When added to cultures of transformed cells, EDC induced a G2/M blockade followed by cell death. As revealed by the analysis of molecular targets, the cell cycle appeared to be deranged by EDC-induced interchain crosslinking within the double-stranded DNA. Administration of EDC to animals with experimental tumours resulted in their increased life span. An analysis of tumour cells from EDC-treated mice showed that this cross-linker induced a disturbance of tumour cell cytokinesis and hence, cell death. Thus, it has been shown that both *in vitro* and *in vivo*, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride exhibits cytotoxic activity, which may be of a potential therapeutic use.

PP-221
Modulation of cyclin B1 expression by cholesterol

M. A. Lasunción[1], **B. Ledo**[2], C. Fernández-Hernando[2], Y. Suárez[2], R. Busto[2], J. Sánchez-Wandelmer[2], C. Sánchez[2] and D. Gómez-Coronado[2]
[1]*Servicio de Bioquímica-Investigacón, Hospital Ramón y Cajal, Madrid, and Universidad de Alcalá, Spain,* [2]*Servicio de Bioquímica-Investigacón, Hospital Ramón y Cajal, Madrid, Spain. E-mail: miguel.a.lasuncion@hrc.es*

Cholesterol starvation results in cell cycle arrest at G2/M. In the present work we studied the changes in Ckd1-cyclin B1 complex activity and expression of its components upon cholesterol deprivation and restoration. HL-60 cells were cultured in cholesterol-free medium and treated with SKF 104976, an inhibitor of lanosterol 14α-demethylase. Then, the medium was supplemented

with vehicle or cholesterol, and the cell cycle was analysed by flow cytometry. In some instances, nocodazole was used to arrest cells in mitosis. Cdk1 activity was measured in cell extracts immunoprecipitated with cyclin B1 antibody. Cdk1 and cyclin B1 protein levels were measured by westernblot. Expression of cyclin A and cyclin B1 in cells at the different phases of the cell cycle was assessed by multiparametric flow cytometry after staining with FITC-labelled antibodies and propidium iodide. Cdk1, cyclin A and cyclin B1 mRNA levels were measured by RT-PCR analysis. Cholesterol starvation produced the expected arrest at G2/M, which was accompanied by a decline of both Cdk1 kinase activity and cyclin B1 expression. At this point, provision of cholesterol rapidly induced cyclin B1 gene transcription. Moreover, an increase in protein expression of cyclin B1, but not cyclin A, was detected in 4n cells. These results indicate that cholesterol specifically modulates cyclin B1 gene transcription at G2 phase, and allow proposing a regulatory role for cholesterol in G2-M transition in mammalian cells.

PP-222
APC/CCDC20 controls the ubiquitin-mediated degradation of p21 during early mitosis

V. Amador, P. Gonzalez-Santamaria and M. Pagano
Department of Pathology, MSB 599, New York University School of Medicine and NYU Cancer Institute, New York, NY, USA. E-mail: amadov01@med.nyu.edu

Temporally coordinated destruction of key cell cycle regulatory proteins by the ubiquitin-proteasome system represents an important regulatory mechanism to ensure that specific protein functions are turned off at the right time, in the right compartment and in a unidirectional fashion.
Degradation of the CDK inhibitor p21 is controlled during S-phase by an SCF ligase containing the F-box protein Skp2 as the substrate recognition factor. However, p21 re-accumulates in G2 and is then degraded again in early mitosis. With the purpose to find the responsible of p21 degradation in early mitosis, I investigated the ability of 35 different F-box proteins as well as Cdh1 and Cdc20 (two activators of the mitotic ubiquitin ligase APC/C) to bind endogenous p21 in cultured cells. Only Skp2 and Cdc20 were specifically co-immunoprecipitated with p21. p21 contains a conserved destruction box motif (D-box) characteristic of APC/C substrates. In agreement to that, I observed that Cdc20 silencing stabilizes p21. The principal goal of this project is to examine whether p21 may be targeted for degradation by the ubiquitin ligase APC/CCdc20 in early mitosis and how this contributes to the network that controls the precise temporal activation of Cdk1 and, consequently, the mitotic control.

PP-223
Influence of p27 protein on the cell proliferation

A. M. Mikecin and M. Grdisa
Division of Molecular Medicine, Institute Rudjer Boskovic, Zagreb, Croatia. E-mail: grdisa@irb.hr

Cell cycle is described as an interval between two cell divisions. It is regulated by cyclins and cyclin dependent kinases. It consists of four phases, G1, S, G2 and M phase. p27 protein (Cip/Kip family) binds cyclin E/cdk2 complex and inhibits G1/S transition. The aim of this research was to investigate the influence of p27 protein on proliferation of the cell lines.
For delivering proteins to the cells we used transduction, a method described as a direct delivery of the proteins/peptides and their complexes from extracellular matrix into the cell. We used MTT and WST test for monitoring proliferation of the cell lines and Western blotting for monitoring the expression of the proteins involved in cell proliferation and apoptosis. In RKO cell line, TAT fusion proteins (TAT-p27, TAT-pt p27 and TAT-N-p27) decreased expression of the cyclin D1 and E, which are involved in the regulation of cell cycle.
Furthermore, TAT fusion proteins increased fragmentation of PARP and caspase-3 expression. We have also shown that transduced fusion proteins do not influence the level of intracellular p27.
Our results have shown that influence of investigated fusion proteins on the cell cycle and apoptosis depends on the transduced protein and cell line in which the protein was transduced. Using method of the protein transduction it is possible to deliver physiologically functional protein into the cell, and therefore this method could find application in the tumour therapy.

PP-224
New synthetic genistein glycoside as potential antimitotic drug

A. Rusin[1], A. Gogler[1], J. Ramza[2], G. Grynkiewicz[3] and Z. Krawczyk[1]
[1]Department of Tumour Biology, Maria Skodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland, [2]Polpharma SA, Starogard Gdański, Poland, [3]Pharmaceutical Research Institute, Warsaw, Poland. E-mail: arusin@io.gliwice.pl

Genistein, isoflavone isolated from soy is a compound of particular significance in chemoprevention of many cancers. Biological activity of genistein may be enhanced by chemical synthesis of lipophilic glycosides In the presented poster we describe properties of a new synthetic genistein glycoside, which shows much more potent cytostatic and cytotoxic effect than genistein. We found that this compound inhibited proliferation and induced apoptosis in various cancer cell lines: DU145, LnCaP, AGS and Hct-116 in a time and dose dependent manner. Cell cycle analysis with the use of flow cytometry has shown that synthetic genistein glycoside caused G2/M block. Cytological analysis of effects on cytoskeleton shown this compound caused disruption of microtubules of mitotic spindles, arresting cell cycle in mitosis. Microtubules of interphase cells were only slightly affected. During prolonged treatment with synthetic genistein glycoside cells were able to escape from the mitotic block, however due to disorder of mitotic apparatus nuclear divisions were abnormal and overall survival ratio significantly declined. The tested compound does not undergo rapid biodegradation in cells or culture media and exerts its biological effects primarily as intact molecule. Our data show the mechanism of cytotoxicity of genistein and its new derivative is significantly different. To our knowledge, the tested compound is a first derivative of genistein with the potential to disrupt mitotic spindles.

PP-225
Survivin downregulation leads to chromosomal passenger complex defect and mitotic cell death of BCR-ABL expressing cells

K. Wolanin[1], R. Klinger[1], S. Vejda[2], S. McKenna[2], E. Sikora[1] and K. Piwocka[1]
[1]Nencki Institute of Experimental Biology, Warsaw, Poland, [2]Cork Cancer Research Centre, Biosciences Institute, University College Cork, Ireland. E-mail: k.wolanin@nencki.gov.pl

The Bcr-Abl oncoprotein is involved in the development and progression of chronic myeloid leukaemia. It is elevated at the blast

crisis stage of disease, which is highly drug resistant. We used a mouse cell line model, which was established to study the consequences of different levels of Bcr-Abl expression. We found that curcumin induces mitotic cell death, with associated G2M arrest, increased mitotic index and mitotic abnormalities leading to cell death. Curcumin treatment resulted in survivin downregulation and mislocalization of Aurora B, survivin's partner in the chromosomal passenger complex. We investigated whether survivin might be responsible for the disturbances in mitosis and mitotic catastrophe induction. We used RNA interference to downregulate survivin in high Bcr-Abl expressing cells, and examined whether this had similar effects on the cell cycle and viability. Cells treated with survivin siRNA initially exhibited G2M arrest followed by polyploidy and induction of cell death. In control mitotic cells, Aurora B was found in the centromeres of mitotic spindle chromosomes, central spindle midzone and midbody, corresponding to survivin localisation, in contrast to siRNA transfected cells, where survivin downregulation resulted in aberrant Aurora B localisation. In conclusion, our findings indicate that survivin might be a promising target for anticancer therapy of CML and mitotic catastrophe could be an alternative strategy to treat apoptosis-resistant CML cells.

PP-226
BUB3 acts differently from Bub1 and Bubr1 in the regulation of stable kinetochore-microtubule attachments

E. Logarinho, **T. Resende** and H. Bousbaa
CESPU, Instituto Superior de Ciências da Saúde – Norte, Grupo de Biologia Molecular e Celular, Rua Central de Gandra, Gandra PRD, Portugal. E-mail: hassan.bousbaa@iscsn.cespu.pt

Accurate chromosome segregation is ensured by spindle checkpoint mechanisms that monitor kinetochore (KT)-microtubule (MT) attachments and induce a mitotic delay until full metaphase alignment is achieved. Recent data have implicated the checkpoint proteins Bub1 and BubR1 in the regulation of stable KT-MT attachments. Unlike these proteins whose roles during mitosis have been well defined, Bub3 role has remained poorly studied. Here we show, using RNA interference, that besides its role in mediating spindle checkpoint signalling, Bub3 is also required for proper attachment of chromosomes to the spindle. Chromosomes in Bub3-depleted cells are able to congress and organize a metaphase plate that, however, exhibits a few permanently misaligned chromosomes. We have used three different assays to test for the KT attachment status in these cells. First we assayed for the presence of cold-stable microtubules, as KT microtubules are preferentially stabilized at 4°C. Secondly, we examined proteins whose KT localization depends strongly on MT attachment, dynactin and Clip170. Thirdly, we examined the ability of KT microtubules to form and drive chromosome movements after nocodazole washout. Altogether, the data from these assays show that functional KT-MT attachments are still established in the absence of Bub3, even though they are unstable. Furthermore, comparative analyses between Bub3, Bub1 and BubR1 phenotypes clearly show that Bub3 acts differently to mediate stable attachments.

PP-227
Signal transduction through the atos-atoc/az two component system towards poly (3-OH-butyrate) biosynthesis in *E. Coli*

M. C. Theodorou[1], E. C. Theodorou[1], C. A. Panagiotidis[2] and D. A. Kyriakidis[1]
[1]Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece, [2]Department of Pharmaceutical Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece. E-mail: marina_theodor@hotmail.com

The AtoS-AtoC/Az two-component system activates the atoDAEB operon expression upon acetoacetate induction for *E. coli* growth in short-chain fatty acids. It also enhances the poly (3-OH-butyrate) (cPHB) biosynthesis, upon acetoacetate induction as well as in the presence of spermidine. The response regulator of the system is the antizyme (Az) of ornithine decarboxylase and is the product of atoC gene. It belongs to the NtrC-NifA family of sigma54-RNA polymerase transcriptional activators. AtoC contains two putative phosphorylation sites, i.e. a conserved aspartic acid among the response regulators and a histidine residue in an H box consensus sequence, normally common to histidine kinases. We report here, that only phosphorylation-competent AtoC can lead to enhanced production of cPHB in *E. coli*, when overexpressed with AtoS. Specifically, upon acetoacetate induction, the mutation of Asp reduces cPHB accumulation, compared with cells expressing wild-type AtoC. The mutation of His residue has an even more pronounced effect. The relative effects of these mutations on cPHB accumulation are consistent with their effects on atoDAEB operon expression, i. e. the mutation of Asp has a more potent phenotype than the substitution of His, in the presence of spermidine. Introduction of both AtoC mutations render the system unresponsive to acetoacetate as well as polyamine, resulting in total abrogation of the AtoS-AtoC/Az overexpression effect phenotype to cPHB levels in *E. coli*.

PP-228
Phosphorylation of Tbx2 by the p38 map kinase regulates its stability, subcellular localisation and activity

A. Abrahams[1], C. Goding[2], M. I. Parker[1] and **S. Prince**[3]
*[1]Division of Medical Biochemistry, Faculty of Health Sciences, University of Cape Town, Observatory, Cape Town, South Africa, [2]Signalling and Development Laboratory, Marie Curie Research Institute, The Chart, Oxted, Surrey, RH8 OTL, UK, [3]Division of Cell Biology, Faculty of Health Sciences, University of Cape Town, Observatory, Cape Town, South Africa.
E-mail: sprince@curie.uct.ac.za*

T-box factors play a crucial role in embryonic development and recent work has provided an increasing body of evidence implicating the T-box family in cell cycle regulation and in cancer. For example, Tbx2 can suppress replicative senescence through repressing p19ARF and p21WAF1/CIP1/SDI1 gene expression and is over-expressed in some breast cancers, 50% of pancreatic cancer cell lines and in melanomas. The mechanism by which Tbx2 may contribute to cell cycle regulation and oncogenesis is, however, not known. To address this question we have focused on identifying signal transduction pathways that regulate the activity of Tbx2. Here we show, using western blot analysis, that Tbx2 is phosphorylated in response to stress-inducing agents such as UV irradiation and DOX treatment. Phosphorylation was shown to be mediated specifically by the stress-responsive p38 mitogen-activated protein (MAP) kinase. Furthermore, we have

identified the p38 target sites using site-directed mutagenesis and have shown that these sites are phosphorylated both *in vitro*, by p38 kinase assays, and *in vivo* using western blotting. In addition, we show that phosphorylation by the p38 kinase regulates Tbx2 stability, subcellular localisation and its ability to repress the p21 promoter. Taken together, these results have implications for our understanding of the role of Tbx2 in the cell cycle regulation, oncogenesis, as well as in development.

Therapeutic Enzymes

PP-229
The effects of doxycycline on peritoneal fibrosis resulted by chemical peritonitis in rats

Z. Cavdar[1], S. Yildiz[2], G. Oktay[3], S. Sarıoglu[4], O. Yilmaz[5], E. Guneli[5] and T. Camsari[2]
[1]*Biochemistry, Health Sciences Institute, Dokuz Eylul University, Izmir, Turkey,* [2]*Nephrology, Medical Faculty, Dokuz Eylul University, Izmir, Turkey,* [3]*Biochemistry, Medical Faculty, Dokuz Eylul University, Izmir, Turkey,* [4]*Pathology, Medical Faculty, Dokuz Eylul University, Izmir, Turkey,* [5]*Laboratory Animals Sciences, Medical Faculty, Dokuz Eylul University, Izmir, Turkey. E-mail: zahide.cavdar@deu.edu.tr*

Aim: Matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) play an important role in fibrosis. The aim of this study was to investigate the effects of MMP inhibitor, doxycycline (DOX), on peritoneal fibrosis resulted by chemical peritonitis appeared with chlorhexidine gluconate (CHX) in an animal model.
Methods: Twenty-one rats were divided into three groups ($n = 7$). CHX, CHX + DOX and saline were administered intraperitoneally to the first, second and third groups, respectively. At the end of study (14th day); in the peritoneal samples were evaluated in terms of thickness of the peritoneum, amount of the immature collagen with haemotoxylene-eosine and sirius red dye, respectively. MMP-2 and MMP-9 were analysed with gelatine zymography. TIMP-1, TIMP-2 and procollagen type I C-terminal peptide (PIPC) levels were detected with ELISA. All biochemical parameters were carried out in peritoneal lavage fluids.
Results: Histopathological evaluations and PIPC levels were found significantly different within the groups ($P < 0.05$). Active/pro-MMP-2 ratio was found significantly lower in CHX group than saline group ($P < 0.05$) but there was no significant difference in TIMP-2 levels. Although MMP-9 was not detected in all groups, TIMP-1 level was lower in CHX + DOX group as compared with saline group ($P < 0.05$).
Conclusion: In this study, MMP inhibition with DOX was observed inadequately since pro-MMP-2 levels were increased with CHX treatment during the fibrosis.

PP-230
A novel continuous electrophoresis method for purification of hen eggwhite lysozyme

H. R. Shahbazkia and M. Aminlari
Department of Biochemistry, School of veterinary medicine, Shiraz university, Shiraz, Iran. E-mail: shahbazkia@yahoo.com

Lysozyme has tremendous potential in clinical application for treatment of ulcers, infection, wounds and as a potentiator of some antibiotics. The expanding potential for application of lysozyme in the many fields of sciences dictates the urgency for developing efficient and simple methods for lysozyme purification.
The propose of this study was to develop a simple, efficient and very low cost electrophoretic method for purification of lysozyme from eggwhite.
Hen eggwhite was homogenized with equal volume of phosphate buffer (0.05 M, pH = 8.6). Fifty millilitre of this homogenized eggwhite was spilled into a beaker and the equal volume of phosphate buffer was spilled into the another same beaker. The anode electrode was put into the homogenized eggwhite beaker and cathode electrode was put into the buffer beaker. Solutions of two beakers were linked with five layers of whattman paper soaked with the phosphate buffer. Electrophoresis was performed with a 9–10 mA constant current. After 5 h the purification fold of lysozyme was 35.2 and lysozyme yield was 92%. SDS-PAGE results showed that the lysozyme separated by this method was 100% pure. In this method with the progress of time, lysozyme was concentrated in the cathode solution. In comparison with the other methods, that are multi steps and complex, this method is a single step and very simple.
Acknowledgment: This study was financially supported by the entrepreneurship center of Shiraz university, Shiraz, Iran.

PP-231
Cardiac markers in patients suspected on acute myocardial infarction and unstable angina

F. Bilal[1], N. Serdarevic[2] and J. Foco-Solak[3]
[1]*Department of Clinical Chemistry and Biochemistry, School of Medicine, University of Sarajevo, Bosnia and Herzegovina,* [2]*Department of Clinical Chemistry and Biochemistry, School of Medicine, University of Sarajevo, Bosnia and Herzegovina,* [3]*Department of Clinical Chemistry and Biochemistry, School of Medicine, University of Sarajevo, Bosnia and Herzegovina. E-mail: serdarevicnafija@yahoo.com*

The aim of this study was determination of creatin kinase MB (CK-MB), lactate dehydrogenase (LDH), aspartat aminotransferase (AST) and cardiac troponin I (cTnI) in patients suspected on acute myocardial infarction ($n = 30$) and unstable angina ($n = 30$). The patient's blood were taken after hospital in coming and during 48 hours after hospital treatment. The serum troponin I was determinated using AxSYM System (Abbott). The dry slide technology Vitros 250 Analyser (Ortho Clinical Diagnostic) was used for the determination of CK-MB, LDH and AST in serum. The serum concentration of troponin I in patients suspected on acute myocardial infarction were 35.08 ± 6.66 ng/ml after 12 h, 27.44 ± 5.11 ng/ml after 24 h and 11.26 ± 2.21 ng/ml after 48 h. The patients suspected on unstable angina have troponin I serum concentration 0.49 ± 0.39 ng/ml after 12 h, 0.76 ± 0.49 ng/ml after 24 h and 0.259 ± 0.09 ng/ml after 48 h. The patients suspected on acute myocardial infarction have serum concentration of CK-MB > 25 U/l, LDH > 460 U/l and AST > 48 U/l during 48 h. The biochemical heart markers CK-MB, LDH and AST serum concentration in patients with unstable angina during 48 h after hospital treatment were as at healthy individuals. Cardiac troponin I is seems to be the most heart-specific marker in patients with acute myocardial infarction and unstable angina.

PP-232
Modulation of hypotensive effects of bradykinin by cathepsin K

F. Lecaille[1], C. Vandier[2], E. Godat[1], V. Hervé-Grépinet[1], D. Brömme[3] and G. Lalmanach[1]
[1]*INSERM U618, Université François Rabelais, Tours, France,*
[2]*INSERM E-0211, Université François Rabelais, Tours, France,*
[3]*University of British Columbia, Vancouver, Canada.*
E-mail: fabien.lecaille@univ-tours.fr

Due to their proinflammatory properties, kinins – whose pharmacological effects are mediated by bradykinin (BK) receptors – are believed to play a key role in a variety of lung and heart diseases. They are implicated in inflammatory disorders, causing vasodilatation and contraction of smooth muscles. BK amount is regulated by kinases, such as neutral endopeptidase, carboxypeptidase N or ACE.
However evidence has been provided for the presence of alternative mechanisms of bradykinin generation and/or degradation [1, 2], supporting the idea that cysteine cathepsins may be involved in a such process. We demonstrated that cathepsin K, but not cathepsins B, L and S, is a potent kinin-degrading enzyme, which modulates BK-dependent contraction of bronchial smooth muscles, and impairs BK-induced transient falls in systemic blood pressure. Furthermore critical active site residues involved in the kininase activity of cathepsin K were identified. Based on these results, we suggest that cathepsin K is a new kinase, a unique property among mammalian cysteine cathepsins. Taken into account that kinins participate in the maintenance of cardiovascular homeostasis, inhibitors of cathepsin K in addition to ACE inhibitors (i.e. captopril) may have cardioprotective effects.

References:

[1] Puzer et al. Biol Chem 2005; 386: 699–704.
[2] Godat et al. Biochem J 2004; 383: 501–506.

PP-233
The genotype – phenotype relation of a detected glucose-6-phosphate dehydrogenase deficient case

E. Bilget Güven and K. Aksoy
Department of Biochemistry, Institution of Health Sciences, Çukurova University, Adana, Turkey.
E-mail: ebrukayra@yahoo.com

Glucose-6-phosphate dehydrogenase (G-6-PD; EC 1.1.1.49) is a cytoplasmic, hydrogen transfer enzyme mediating the reversible transfer of hydrogen from Glucose-6-phosphate (G-6-P) to nicotinamide adenine dinucleotide phosphate (NADP). Since G-6-PD occupies a central position in the assurance of the stability and viability for erythrocytes, the deficiency of this first and the key regulatory enzyme of the pentose phosphate pathway cause haemolytic anaemia. Among the reported 442 variants, G-6-PD Mediterranean (GdMed) mutation (563 C to T) is the most common one. GdMed is characterized with its low Km values for both of its substrates, migrating normally in electrophoretic media and can be synthesized in enough amounts but can not keep its stability *in vivo*.
In the light of all these facts, initially the enzyme kinetic study of the G-6-PD deficient samples gathered from the Çukurova region is achieved and then the molecular structures of these cases are examined by SSCP and sequence analysis. It is identified that one of the 13 samples analysed, 12-EÖ with '0' G-6-PD enzyme activity, is a GdMed variant although has different kinetic properties than the accepted values. Additionally, it is also presented that the same sample has a mutation (1311 C to T). All the detections about this sample in this research, which is investigating the genotype – phenotype relation, lead the thought that the enzyme might go under some post-transcriptional and / or post-translational modifications.

PP-234
Production of L-asparaginase, a chemotherapeutic enzyme, in bacteria expressing *Vitreoscilla* haemoglobin

B. Ates[1], **S. Gencer**[2], S. O. Erenler[2], M. Uckun[2], U. Ozer[2], I. Yilmaz[1] and H. Geckil[2]
[1]*Department of Chemistry, Inonu University, Malatya, Turkey,*
[2]*Department of Biology, Inonu University, Malatya, Turkey.*
E-mail: sgencer@inonu.edu.tr

L-asparaginase is an enzyme of high therapeutic value due to its use in treatment of leukaemia. The enzyme with this potential has only determined in several gram-negative bacteria. The biosynthesis of L-asparaginase is under catabolic regulation and is tightly controlled by the presence of oxygen. Present study was undertaken to determine how a highly efficient recombinant oxygen uptake system (the *Vitreoscilla* haemoglobin, VHb) affected the production of L-asparaginase in two gram-negative bacteria grown under various culture conditions. In VHb expressing *Pseudomonas aeruginosa* the level of the enzyme was higher than the host bacterium under both catabolite and non-catabolite repression, while in *Enterobacter aerogenes* VHb caused almost a total inhibition of L-asparaginase. In general, the L-asparaginase levels in *E. aerogenes* decreased when bacterium was grown in media supplemented with different carbohydrate sources. In *P. aeruginosa*, however, there was no such catabolite repression and in recombinant strain expressing VHb there was even a stimulatory effect of carbohydrates on L-asparaginase synthesis. The data prove that a critical level of dissolved oxygen is essential for L-asparaginase synthesis and we assume that this should be a consideration of major importance not fully recognized in the past.

PP-235
Effect of *Vitreoscilla* on L-lysine α-oxidase, a chemotherapeutic enzyme, from *Pseudomonas aeruginosa*

H. Kahraman, S. Gencer and H. Geckil
Department of Biology, Inonu University, Malatya, Turkey.
E-mail: hkahraman@inonu.edu.tr

L-lysine α-oxidase (LO) is one of a few microbial enzymes with therapeutic potential in certain cancers where it is used to 'starve' cells of an essential amino acid, L-lysine. The enzyme has been determined in several bacteria and fungi and its production is mainly regulated by carbon sources and oxygen. This study was concerned with the effect of *Vitreoscilla* haemoglobin (VHb) on the production of LO in *Pseudomonas aeruginosa*. Highest enzyme activity in both strains was determined in cultures at 24 and 48 h of incubation. In general, the recombinant strain (PaJC) expressing *Vitreoscilla* haemoglobin had higher LO activity than the host strain under both carbon catabolite and no-carbon catabolite repression conditions. Under no catabolite repression, PaJC strain had up to two-fold higher LO activity than the host strain. In the same nutritionally rich medium (LB) but supplemented with glucose, this difference was slight. For both strains, the repressive effect of glucose on LO synthesis was more

pronounced in minimal medium (MM). Cells in MM with 1% glucose had 2.5 to 4-fold lower LO activity than the cells in MM with 0.1% glucose. In conclusion, the beneficial effect of VHb for LO synthesis was apparent only under no catabolite repression conditions and application of such an oxygen uptake system might be advantageous in bioprocess that are finely balanced regarding the content and the type of carbon source.

PP-236
Bovine dipeptidyl peptidase II is binding adenosine deaminase

S. Sharoyan[1], A. Antonyan[1], S. Mardanyan[1], G. Lupidi[2], M. Cuccioloni[2] and M. Angeletti[2]
[1]*Laboratory of Nucleotides and Nucleosides, H. Buniatyan Institute of Biochemistry of Armenian NAS, Yerevan, Armenia,* [2]*Department of Biology, MCA, University of Camerino, Camerino, Italy. E-mail: biochem@ipia.sci.am*

Dipeptidyl peptidase II (DPPII) and dipeptidyl peptidase IV (DPPIV) are serine proteases that differ by their molecular size, pH-optimum, localization and, according to the publications, specific binding of only DPPIV to adenosine deaminase (ADA). Both enzymes are treatment targets at several diseases. We purified DPPIV and DPPII from bovine lung and kidney using the chromatographic methods. Our attention was attracted by the unexpected observation that DPPII preparation from both tissues posses ADA activity at all purification stages: higher in those from ADA-reach lung and lower–from kidney. During the purification of DPPII from lung, the increase of specific peptidase activity was accompanied with the decrease of specific ADA activity, indicating the stepwise dissociation of two enzymes. To study the ability of DPPII from kidney to bind externally added ADA, we incubated DPPII with the excess of ADA at 30° C during 2 h. The subsequent gel-filtration of this mixture through Sephadex demonstrated high-molecular shift and about 30-fold increase of the specific ADA activity of the protease peak in the elution diagram. The ability of DPPII to bind ADA was demonstrated also in the experiments using biosensor and fluorescence polarization methods. Kd of ADA binding to DPPII and DPPIV in the identical experiments is 800nM and 8nM respectively. So, the obtained data demonstrate the ability of DPPII from kidney to bind ADA, although with two orders lower affinity than DPPIV.

PP-237
Changes of drug metabolizing enzymes activities in senescent gilthead seabream (*Sparus aurata*)

H. A. Akdogan and A. Sen
Pamukkale University, Faculty of Arts and Sciences, Department of Biology, Denizli, Turkey. E-mail: hardag@pamukkale.edu.tr

Recently, fish are used frequently for endocrine, toxicological and carcinogenesis research as a model species. The gilthead seabream is among fish in common use as an experimental animal to investigate physiological processes or toxicities involving P450 metabolism. We have determined aminopyrene N-demethylase (AND), aniline 4-hydroxylase (AH), benzyloxyresorufin O-deethylase (BROD), caffeine N-demethylase (CND), coumarin hydroxylase (CH), erythromycin N-demethylase (END), ethoxyresorufin O-deethylase (EROD), lauric acid hydroxylase (LAH), methoxyresorufin O-deethylase (MROD), N-nitrosodimethylamine N-demethylase (NDMA-ND), penthoxyresorufin O-deethylase (PROD), p-nitrophenylhydroxylase (PNPH) and glutathione S-transferase (GST) as probe activities. In this study, male gilthead seabream (*Sparus aurata*) liver microsomes of different ages (ranging from 1.5 to 24 months) were used as sample. Despite the present work remains preliminary, results have demonstrated that a significant age-related decline in hepatic content of CYPs with selective reduction in CND and AND activities. In contrast, an incline in AH, END, NDMA-ND, and EROD activities were detected. Although further studies should be carried out, the present study suggests that ageing has a significant effect in activities of some but not all CYPs, which may account for interindividual variability in physiological responses to various endo- and/or exo-genous factors such as hormone and xenobiotics.

PP-238
The *in vitro* effects of some benzoxazolone derivatives on human leukocyte myeloperoxidase activity

A. Pabuccuoglu[1], Z. Soyer[2] and V. Pabuccuoglu[2]
[1]*Department of Biochemistry, Ege University Faculty of Pharmacy, Izmir, Turkey,* [2]*Department of Pharmaceutical Chemistry, Ege University Faculty of Pharmacy, Izmir, Turkey. E-mail: aysun.pabuccuoglu@ege.edu.tr*

Myeloperoxidase (MPO), a heme protein expressed by polymorphonuclear leukocytes, generates potent oxidants, which are proposed to contribute to tissue damage during inflammation and certain pathogenesis such as neurodegenerative disorders. MPO has become an important therapeutic target in recent years. It has been reported that the development of MPO inhibitor with high specificity and low toxicity is a challenging goal for the future. In this study, twenty ω-(2-oxo-3H-benzoxazol-3-yl)-N-phenylacetamide and propionamide derivatives having substituent of different lipophilic and electronic nature on the N-phenylring were synthesized to evaluate the inhibitory effects on leukocyte MPO chlorinating activity, since 2(3H)-benzoxazolone ring has produced diverse biological activities and many derivatives of 2(3H)-benzoxazolone have been known to possess analgesic and anti-inflammatory properties. The inhibitor effects of 2(3H)-benzoxazolone derivatives on MPO-chlorinating activity was determined on human leukocyte isolated from venous blood. The assay is based on the chlorination of taurine with hypochlorous acid produces taurine chloramines, which is measured by the reaction with 5-thio-2-nitrobenzoic acid. According to our assay system, the synthesized compounds, with one exception, exhibit varying inhibition percentage on the chlorination activity on MPO, depending on the nature and the position of the substituent on the N-phenyl ring and the aliphatic regions of title compounds.

PP-239
The importance of domains in enzyme activity of SHP-1

G. Arabaci[1] and D. Pei[2]
[1]*Department of Chemistry, Sakarya University, Sakarya, Turkey,* [2]*Department of Chemistry, The Ohio State, Ohio, USA. E-mail: agulnur@hotmail.com*

Signal transduction and other cellular processes are all controlled by two different events: chemical modifications and physical association of proteins involved. Protein Tyrosine Phospatases are among these proteins, which catalyse the specific hydrolysis

of phosphotyrosine in proteins releasing inorganic phosphate. The enzyme studied in this work is SHP-1, a Src homology 2 (SH2) domain-containing PTPase. The aim of this study is to show how important SH2 domains for SHP-1 activity. Here, the kinetics of irreversible inactivation caused by iodoaceticacid for SHP-1 and the catalytic domain of SHP-1 [SHP-1(DSH2)] were determined by using first order kinetics at pH 7.4 and 5.5. The Ki values show that the active sites of SHP-1 and SHP-1(DSH2) are different from each other and suggest that SH2 domains effects the active site of SHP-1. In addition to, that three different SHP-1 and SHP-1(DSH2) mutants (D419E, D419A and H420Q) were prepared by using site directed mutageneses. The kinetic studies of these mutants were also determined by using pNPP at pH 7.4 and calculated by Michaelis-Menten equations. The kcat/Km values of three mutants of SHP-1 (DSH2) had around 25-fold higher values relative to the same corresponding mutants of SHP-1. The results show that the real activity of SHP-1 comes from the active side of enzyme, which is not blocked, by the SH2 domains in the native enzyme. Thus, SH2 domains of SHP-1 play very important role for the enzyme activity in signal transduction.

PP-240
A comparative study on human serum paraoxonase 1 and arylesterase: effects of metals on enzyme activity

B. C. Demirdögen[1], A. Turkanoglu[1], Ç. Kalin[2], E. Sahin[2], E. Arinç[1] and O. Adali[1]
[1]*Biochemistry Graduate Programme and Department of Biological Sciences, Middle East Technical University, Ankara, Turkey,* [2]*Department of Biological Sciences, Middle East Technical University, Ankara, Turkey. E-mail: cbirsen@metu.edu.tr*

Human serum paraoxonase (PON1; EC 3.1.8.1/3.1.1.2), a high-density lipoprotein (HDL) associated ester hydrolase enzyme, protects LDL from oxidation by the hydrolysis of biologically active lipoperoxides. In addition, PON1 has been shown to play a role in the metabolism of pharmaceutical drugs. In this study, determination of biochemical properties and effect of metal ions on paraoxonase (PON) and arylesterase (ARE) activities was considered.
The rate of PON reaction was linear up to 9 mg of serum protein and for 12 min. The rate of ARE reaction was linear up to 1.2 mg of serum protein and for 200 min. The optimum temperature was found to be 50°C for PON and 45°C for ARE. The optimum pH for PON was found to be 11, while we observed two pH optimum peaks for ARE, which were pH 8 and 10. The apparent Vmax and Km values of human serum PON were found to be 238 U/l and 0.12 mM respectively, these numbers came out to be 217 U/ml and 1.39 mM for ARE. The effect of mercury (Hg^{2+}) on PON and ARE enzyme activities were determined and IC50 values were calculated as 1700 μM for PON and 4.7 μM for ARE. The IC50 values for nickel (Ni^{+2}) for PON and ARE were found to be 2800 μM and 57 μM, respectively. IC50 values for cadmium (Cd^{2+}) came out to be 6800 μM for PON and 4 μM for ARE. The serum sample used in these experiments was withdrawn from a person whose genotype for the 192QR and 55LM coding region, -107T/C promoter region were determined to be PON1 192QR, 55LM and -107TT.

PP-241
Human serum butyrylcholinesterase interactions with cysplatin and cyclophosphamide

E. Bodur, **A. N. Çokuğraş** and N. Özer
Biochemistry Department, Faculty of Medicine, Hacettepe University, Ankara, Turkey. E-mail: ncokugras@superonline.com

Butyrylcholinesterase (BChE, E. C. 3.1.1.8) found abundantly in serum, has a wide capacity for hydrolyzing ester containing compounds and plays an important role in detoxification. Chemotherapeutics introduced through IV injections interact with BChE before reaching their targets. In this study, the inhibition kinetics of BChE with two chemotherapeutics Cysplatin (CDDP) and cyclophosphamide (CY) often used together in combination therapy are investigated. The time dependent inhibition of BChE with CY was rapid displaying reversible inhibition. In further inhibition kinetics, CY was found to be non-competitive inhibitor with a Ki value of 504 ± 50 μM. On the other hand, in the modification studies with CDDP, inactivation of the enzyme was irreversible and time dependent. The apparent Ki value was found to be 192 ± 71 μM. These results suggest that used in combination therapy, CY and CDDP cause considerable BChE inhibition and thus may aggravate the conditions observed during chemotherapy.

RNA Interference

PP-242
Inhibition of hepatitis D virus small antigen expression by siRNAs

S. Mota and C. Cunha
Molecular Biology, Institute of Hygiene and Tropical Medicine, Lisbon, Portugal. E-mail: ccunha@ihmt.unl.pt

The hepatitis delta virus (HDV) requires the hepatitis B virus surface proteins to form infectious particles. There is still no specific treatment for HBV/HDV patients. The HDV genome consists of a circular, single stranded RNA molecule. The viral RNA has a high degree of intra-molecular base-pairing and can fold to form an unbranched rod-like structure. The HDV produces two forms of the delta antigen (p27 and p24), which are essential for packaging and replication, respectively. In the last years, RNA interference (RNAi) has been shown to effectively inhibit the replication of several viruses and thus could represent an effective approach for the therapeutic treatment for HDV patients. Here, we wanted to investigate if the cellular RNA interference (RNAi) machinery could inhibit the replication of the HDV.
We used web based tool from Clontech to select five different target sequences in the HDV genome for induction of RNAi and used a plasmid-based short interfering RNAs (siRNAs) expression system, BD™ Knockout RNAi. Using this system we were able to select transfected cells using puromycin, which were expressing the siRNAs. RNAi efficiency was analysed by western blot and immunofluorescence. Immunofluorescence analysis revealed that there is a decrease in the number of cells expressing the delta antigens. By western blot we could observe a significant reduction in the amount of delta antigens present in the samples.

Acknowledgment: S. M. is supported by a FCT grant (SFRH/BD/12691/2003).

PP-243
Post-transcriptional regulation of the Brn-3b transcription factor

M. Calissano, J. Diss and D. Latchman
MMBU, Institute of Child Health, London, UK.
E-mail: m.calissano@ich.ucl.ac.uk, j.diss@ich.ucl.ac.uk, d.latchman@ich.ucl.ac.uk

Brn-3b is a transcription factor belonging to the POU IV class of transcription factors and expressed in different parts of the central and peripheral nervous system. The levels of Brn-3b are typically tightly controlled spatio-temporally. For example, in post-mitotic retinal ganglion cells (RGC), the expression of Brn-3b steadily increases from E11.5 to E16.5 and then dramatically falls by P1. Similarly, the levels of Brn-3b are sharply and quickly reduced upon serum starvation-induced differentiation of the ND7 neuroblastoma cell line. While this reduction is likely due to transcriptional repression, Brn-3b levels could also be reduced at a post-transcriptional level via mechanisms acting on the stability of its mRNA.

In this work we wanted to study the role of the 3'UTR of Brn-3b in the stability of its mRNA and identify the mechanism(s) mediating this regulation.

We present evidence that the 3'UTR of Brn-3b does indeed contain regulatory sequences that mediate its degradation upon serum starvation-induced differentiation of ND7 cells. The specific region mediating this effect has been characterized and four putative microRNA identified that could potentially mediate the stability of Brn-3b. In conclusion, we have provided evidence that the tight regulation of Brn-3b expression can occur at the post-transcriptional level and involve mechanisms acting on the stability of its mRNA including specific microRNA(s).

PP-244
Arrest of cancer cells proliferation by dsRNAs

T. O. Kabilova, A. V. Vladimirova, E. L. Chernolovskaya and **V. V. Vlassov**
Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia. E-mail: elena_ch@niboch.nsc.ru

Deregulation of genes encoding components of signaling pathways are considered as a key factor in the development of different types of tumours in humans. We investigated inhibition of MYC genes by dsRNAs in KB-3-1, SC-N-MC and IMR-32 cells. Sequence-specific siRNA (si3ex) targeted to the third exon of c-myc gene was found to decrease the level of c-myc, but not N-myc mRNA. si3ex decreased the rate or even arrested the proliferation of c-myc overexpressing cell lines KB-3-1 and SC-N-MC, but did not affect the proliferation of IMR-32 (which overexpress N-myc). si2ex homological to the conservative region of the second exon of both c- and N-myc was able to downregulate both genes and to reduce proliferation of KB-3-1, SC-N-MC and IMR-32 cells. PKR or/and OAS1 mRNA levels were not effected. Long double stranded RNAs: dsMyc homologous to the 3 exon of c-myc gene, dsGFP homologous to mRNA of EGFP gene and GU-rich siRNA, homologous to the intron sequence of human MDR1 gene were found to inhibit proliferation and to decrease the mRNA level of interferon-sensitive genes: c-myc and beta-actin when delivered into cancer human cells. The data suggest, that double stranded RNAs can serve as antiproliferative agents, acting sequence-specifically or activating innate immunity response.

Acknowledgment: This work was supported by RFBR (grant No. 06-04-49480-à), RAS programs 'Molecular and Cellular Biology' and 'Fundamental science for medicine', Interdisciplinary grant from SB RAS, FCSTP RI-012/001/254.

PP-245
Search of new therapeutic targets in ewing tumour through a EWS-FLI1 shRNAi approach

D. Herrero Martin[1], A. S. Martins[1], D. Wai[2], Y. Braun[3], K. L. Schaefer[3], M. Eisenacher[4], C. Mackintosh[1], M. Campos[1], T. Hernandez[1], C. Poremba[3] and E. de Alava[1]
[1]*Laboratorio de Patología Molecular- Banco de Tumores, Centro de Investigación del Cáncer (USAL-CSIC), Salamanca, Spain,* [2]*Department of Pathology, Children's Hospital Los Angeles, Los Angeles, California, USA,* [3]*Institute of Pathology, Heinrich-Heine-University, Duesseldorf, Germany,* [4]*Gerhard-Domagk-Institute of Pathology, University of Muenster, Muenster, Germany.*
E-mail: dherrero@usal.es

Background: Ewing tumour is a neoplasm of unknown origin that bears chimeric proteins, due to chromosomal translocations, that fuse the EWS gene with an ETS transcription factor, mainly FLI-1. These proteins act as uncontrolled transcription factors but also as potent repressors.

Objective: With the aim of finding new targets of the fusion we have used a stable RNA interference model based on the use of a plasmid vector (pSUPER neo gfp) knocking EWS-FLI1 in the cell line TC71 and analysed the resulting changes.

Results: A batch of 40 clones was collected after electroporation and G418 selection. RNA interference as analysed by Western Blot and qPCR was stable through cellular passages 1 to 7. The stable TC71 RNAi clone chosen showed a 65% increase in the apoptotic rate (FACS-annexin V analysis). Two independent analysis of the obtained Microarray data (HG133A) showed a significant reduction ($P < 0.05$) at least 2-fold of genes involved in Ewing tumorigenesis, such IGF1 or EIF4E as well as potential new targets of the fusion as MARK4 or CDKN2D. MTT and apoptosis assays confirmed the increased sensitivity of the clone to the action of IGF1R inhibitor NVP-AEW541 and specific inhibitors of MEK and PI3-K (PD98059 and LY294002).

Conclusion: We have established a stable model of RNAi in Ewing tumour that make TC71 cells more sensitive to apoptotic signals and to the action of inhibitors of important pathways such IGF1R, PI3-K or MEK/MAPK.

Oxidative Stress

PP-246
Genetic diversity at position –308 in the promoter region of the tumour necrosis factor is implicated in the pathogenesis of oxidative stress and operative outcomes

M. Elahi and **M. Matata**
The Cardiothoracic Centre, Liverpool NHS Trust, Thomas Drive, Liverpool, L14 3PE, UK. E-mail: manzoor_elahi@hotmail.com

Objectives: The genetic polymorphism in inflammatory markers in particular Tumour Necrosis Factor has been well studied and found to be associated with the development of coronary artery disease. However, the bi-allelic polymorphism at position –308 in exon 6 of Tumour Necrosis Factor 2 (TNFA*2) and its association with oxidative stress remains to be elucidated. We aim to determine whether CPB-induced oxidative stress and in turn the perioperative complications in patients undergoing cardiac surgery are related to TNF-α gene promoter polymorphism.
Methods: 100 consecutive adult Caucasian patients with double or triple vessel coronary disease elective for cardiopulmonary bypass (CPB) surgery were genotyped for TNFA*1 and TNFA*2 alleles using polymerase chain reaction. Logistic regression analysis was used to examine the relationship between genotypes and intraoperative complications incorporating other variables also into the model.
Results: Genotype frequency was determined in 95 patients with a mean age of 56.6 ± 11.6 years. Three patients died in the early postoperative period. Nco-I digestion of the amplified PCR product showed homozygosity for the allele TNFA*1 in 63.2% of the patients (60 of 95) versus 36.8% heterozygous patients (35 of 95) for the allele TNFA*2. Significantly higher oxidative stress concentrations were observed in patients heterozygous for the TNFA*2 allele compared with patients homozygous for TNFA*1 (TNFA*1/ TNFA*1 versus TNFA*1/ TNFA*2; $P < 0.001$). Logistic regression analysis revealed the association of TNFA*2 genotype with morbidity in these patients. The TNFA*1/ TNFA*2 genotype was significantly associated with the occurrence of increased oxidative stress at the 30 min after the initiation of CPB persisting up to 24 h leading to higher complication rate in these patients (OR 2.72; $P < 0.0007$).
Conclusions: The results have demonstrated a role for TNF-α gene promoter diversity on CPB-induced perioperative complications in patients with coronary heart disease undergoing cardiac surgery. This suggested that determining a patient's TNF promoter genotype before treatment may permit the selection of a homogeneous high-risk group of patients who could benefit from anti-TNF therapy.

PP-247
Endothelial stress, iNOS activity and oxidative damage

M. Zadorin and V. Shevchenko
Biochemistry, National Bio Science, Kiev, Ukraine.
E-mail: evgeniyzadorin@mail.ru

In endothelial cells, the expression of the inducible nitric oxide synthase (iNOS) and the resulting high-output nitric oxide synthesis have often been assumed as detrimental to endothelial function, but recent publications have demonstrated a protective role resulting from iNOS expression and activity. To address this question, we used antisense-mediated iNOS knockdown during proinflammatory cytokine challenge in primary endothelial cell cultures and studied endothelial function by monitoring the expression of stress defence genes. Under these conditions, cytokine addition results in full iNOS protein expression with minimal nitric oxide formation, concomitant with a significant reduction in stress response gene expression and susceptibility to cell death induced by reactive oxygen species. Taken together, our data suggest that cytokine-induced endogenous iNOS expression and activity have key functions in increasing endothelial survival and maintaining function. Thus suppression of iNOS expression or limited substrate supply, as has been reported to occur in atherosclerosis patients, appears to significantly contribute to endothelial dysfunction and death during oxidative stress.

PP-248
Hydrated c60 fullerenes (c60hyfn) protect liver and brain against oxidative stress induced by ethanol

A. O. Tykhomyrov[1], V. V. Corsa[1], G. V. Andrievsky[2] and V. K. Klochkov[2]
[1]Department of Biophysics and Biochemistry, Dnepropetrovsk National University, Dnepropetrovsk, Ukraine, [2]Institute for Therapy, Kharkov, Ukraine. E-mail: artem_tykhomyrov@ukr.net

Chronic ethanol (EtOH) consumption causes structural and functional changes mediated through oxidative impact and free-radical induced cellular damage. Hydrated C60-fullerenes as well as some of their water-soluble derivates are shown to be free radical scavengers in several biological systems. We report the effect of C60HyFn treatment on lipid peroxidation (LPO) induced by EtOH intake. In order to investigate the effects of C60-fullerene water solution (C60FWS) on the intensity of LPO in liver and brain under EtOH intoxication (5–15% per os) Wistar rats consumed C60FWS in drinking water ad libitum with concentration of 30 nM/l during 4 months. Brain and liver homogenates were analysed for thiobarbituric reactive substances (TRABS) as a markers of LPO. 'Open field' test was used to estimate rat behavioural characteristics. It has been shown a significant elevation in TRABS concentration in liver and brain of alcohol-intoxicated rats. C60HyFn administration significantly reduced LPO level in brain (100%) and liver (42%) of EtOH-consumed animals. C60HyFn intake normalized behavioural indices of alcohol-treated rats due to soft sedative and adaptogenic activities of C60FWS. Present results indicate that C60HyFn manifest neuro- and hepatoprotective action even in super small doses. Taken together these data point to further investigation of C60HyFn effects against radical-related toxicity caused by chemical or metabolic injuries.

PP-249
A simple method for isolation, purification and express-analysis of GroEL-like chaperones

N. Yu. Marchenko[1], V. V. Marchenkov[2], A. L. Kaysheva[3], I. A. Kashparov[4], N. V. Kotova[2], P. A. Kaliman[1] and G. V. Semisotnov[2]
[1]*Department of Biochemistry, V. N. Karazin Kharkiv National University, Kharkiv, Ukraine,* [2]*Laboratory of Protein Physics, Institute of Protein Research RAS, Pushchino, Russia,* [3]*Department of Immunology, Udmurtia State University, Izhevsk, Russia,* [4]*Department of Peptide Chemistry, Institute of Protein Research RAS, Pushchino, Russia. E-mail: nina@vega.protres.ru*

Molecular chaperone GroEL is a member of heat-shock proteins family and binds various non-native proteins to prevent their aggregation. Synthesis of heat-shock proteins in various organisms greatly increases not only with rising of temperature but also under various stresses and diseases. We propose fast and simple method for GroEL purification using affinity chromatography on the base of inert resin with covalently attached non-native protein (reduced lysozyme or pepsin at pH 7.5). It is shown that such affinity carrier can be used for producing of high purity GroEL chaperone not only from *Escherichia coli* cells transformed by a multicopy plasmid containing GroE operon but also from wild-type *E. coli* cells which contain considerably less amount of GroEL. Besides, we show that this technique can be used for fast analysis of GroEL-like chaperones content in liver tissue of oxidative-stressed rats (oxidative stress was induced by cadmium chloride). The results allow us to propose the affinity chromatography on a basis of non-native proteins as a simple and effective method for purification and fast analysis of GroEL-like molecular chaperones in different cells and tissues of healthy, stressed and sick organisms.

Acknowledgment: The work was supported by RFBR 04-04-97293-R, HHMI 55000305, Programme MCB RAS.

PP-250
The effect of combined hypoxia and hyperthermia on the metabolism of alveolar macrophages

H. V. Valakhanovich and L. M. Labanava
Department of Biochemistry, Belarusian State Medical University, Minsk, Belarus. E-mail: Lobanovaem@mail.ru

Acute lung injury may result in reduced oxygen tension around alveolar macrophages (AM) in the affected lung areas. Exposure of AM to hypoxia could perturb their functions and trigger the progression of lung injury. Effect of hypoxia on cells is mediated by the cytokine secretion, which could change after the exposure to heat. So combined effect of hypoxia and high temperature, the most frequent symptom accompanied to hypoxia, on metabolism and functions of AM was studied in this work.

It was shown an almost 2-fold decrease of phagocytic activity of AM exposed to 5% oxygen and 42°C for 2 h in comparison with control cells. Simultaneously was observed significantly increased activity of lactate dehydrogenase and decreased – of succinate dehydrogenase. Sharp disorders in oxygen/antioxygen balance were also revealed in AM after hypoxia/heat influence. It revealed in graded increase of hydrogen peroxide and NO concentration as well as TBARS level and parallel attenuation of catalase and glutathione peroxidase but not superoxide dismutase activity. Finally was found a marked augmentation in number of the apoptotic AM at the low oxygen concentration combined with high temperature in comparison with control value.

All these disturbances occur only slightly when 10^{-5} M N-acetylcysteine was added in the incubation medium before treatment. It suggest that oxidative stress is the early steps of hypoxia/hyperthermia-induced disorders in AM.

PP-251
The role of oxidative stress in zearalenone-mediated toxicity in Hep G2 cells: oxidative DNA damage, gluthatione depletion and stress proteins induction

W. Hassen[1], A. Azquetta Oscoz[2], I. Ayed[1], **M. Ladjimi**[3], A. Lopez De Cerain[2] and H. Bacha[1]
[1]*Laboratory of Research on Biologically Compatible Compounds, Faculty of Dentistry, Rue Avicenne, Monastir, Tunisia,* [2]*Centro de Investigación en Farmacobiología Aplicada (CIFA), University of Navarra, Pamplona, Spain,* [3]*Laboratory of Biochemistry of Cellular and Molecular Regulators Signals, FRE 2621, CNRS, Université Pierre et Marie Curie, Paris, France. E-mail: wafa_hsen@yahoo.fr*

Zearalenone (ZEN) is a fusarial mycotoxin with several adverse effects including mainly estrogenicity and hepatotoxicity. While most ZEN toxic effects are quiet well investigated, little is known regarding its mechanism of toxicity. The aim of this study was to find out whether ZEN-induced oxidative cell damage might be relevant mechanism of toxicity. Using human hepatocytes Hep G2 cells, ZEN-induced stress response was monitored at several levels in these cells. ZEN mediated induction of oxidative DNA damage, modulation of gluthatione, cytotoxicity and the oxidative stress responsive gene Hsp 70 and Hsp 90 were investigated with respect to concentration and time dependency.

Our results clearly showed that Hep G2 cells respond to ZEN exposure by loss of cell viability, induction of oxidative DNA damage, gluthatione depletion and Hsp 70 and Hsp 90 induction already at concentrations, which are not yet cytotoxic in a dose and time dependant manner. Our study demonstrates that oxidative damage is likely to be evoked by a direct ZEN action and not only by an unspecific mechanism related to ZEN-induced cytotoxicity. This oxidative damage may therefore be an initiating event and contribute, at least in part, to the mechanism of ZEN different genotoxic and cytotoxic effects.

PP-252
Influence of selenocystein-containingproteins in the redox processes and antioxidant system

A. Kyriakopoulos
Molecular Trace Elements Research in the Life Sciences Hahn-Meitner-Institut Glienicker Str. 100, Berlin, Germany. E-mail: Kyriakopoulos@hmi.de

Selenium in form of selenocysteine as an integral component of the active site of several selenoenzymes and thus has important biological effects in metabolic processes. The membrane-bound selenium-containing proteins were detected by labelling of rats with Se-75, subcellular fractionation of homogenates, separation of the proteins by electrophoresis and autoradiography of Se-75. In this way more than 17 membrane-bound selenium-containing proteins could be distinguished in the membrane fraction of the liver. Of those, six selenium-containing proteins with molecular masses of about 18, 20, 23-25, 28, 42–44, 58–60 and 72–75 kDa were found in the plasma membrane fraction which was isolated from the homogenate by differential centrifugation and further purification steps with detergents. The Se-labelled bands with molecular masses of 18 and 20 kDa and those in the range of 23–25, 28, 42–44 and 58–60 kDa could be attributed to known selenoproteins. A further Se-labelled 16 kDa protein, which had been detected in an earlier study, was characterized as a

selenocysteine-containing selenoprotein with an isoelectric point of 5.4. The localization of the different selenoproteins in the ER is of great interest with regard to the functions of these cellular organelles.
By combining trace element, analytical, biochemical and molecular biochemical methods information on the distribution and involvement of novel selenoproteins in redox processes and antioxidant system have been obtained.

PP-253
Effects on oxidants and antioxidants equilibrium of lithium used for treatment of bipolar feeling disorders

R. Aliyazicioğlu[1], **S. C. Karahan**[2], M. Çolak[2] and B. Vanizor Kural[2]
[1]*Medical Laboratory, Vocational School of Health Sciences, Karadeniz Technical University, Trabzon, Turkey,* [2]*Biochemistry, Faculty of Medicine, Karadeniz Technical University, Trabzon, Trabzon. E-mail: rezzan@ktu.edu.tr*

Bipolar feeling disorders are the group of recurrent disorders, seen in high frequency and need long time treatment. Lithium is the most used drug in those diseases and treatment may continue year by year, even during all the life. In addition to the effects on treating of manic and depressive periods, on extension of the time between periods and on blocking the formation of new periods, lithium have various and serious side effects on metabolism. In the present study, to investigate various effects of lithium, blood samples of 15 patients with bipolar feeling disorders in periods of pretreatment, stable of mood and follow-up were drawn, the levels of lithium, total antioxidant capacity, plasma malondialdehyde (MDA); erythrocyte malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GR), catalase (CAT) were determined. In the follow-up period, total antioxidant capacity ($P < 0.001$), levels were found significantly higher than in periods of pretreatment and stable of mood. But erythrocyte MDA ($P < 0.001$), plasma MDA ($P < 0.001$), erythrocyte GSH-Px ($P < 0.05$), erythrocyte GR ($P < 0.001$), erythrocyte SOD ($P < 0.01$) and erythrocyte CAT ($P < 0.01$) levels were found significantly lower. According to the results, it was suggested that oxidant-antioxidant balance was changed in favour of antioxidant by using long term treatment with lithium and this change effected lithium on stability of mood and blocking of periods.

PP-254
Tissue specific expression of bradykinin B1 receptor and NADPH oxidase in hypertensive animal models

C. R. A. Bertoncini[1], A. C. Azevedo[1], E. B. Almeida[1], D. V. Schneider[1], M. L. Calio[1], I. S. Andrade[1], A. T. Ferreira[1], M. L. Capurro[2] and O. F. Pavao[1]
[1]*Universidade Federal de São Paulo, Escola Paulista de Medicina, São Paulo, Brazil,* [2]*Departamento de Parasitologia, Universidade de São Paulo, Brazil. E-mail: clelia.cedeme@unifesp.epm.br*

Hypertension has been associated to inactivation or low production of nitric oxide (NO). We selected two animal models of hypertension, stroke prone spontaneously hypertensive rats (SPSHR) and endothelial NO syntase knockout mice (e-NOS-KO), to investigate the expression of some genes possibility involved in adaptive responses to diminished NO availability. m-RNAs of the B1 bradykinin receptors (B1), NADPH oxidase and heat shock protein 90 (HSP90) were determined by RT-PCR assays. The vasodilation mediator, B1 receptor, was induced in aorta, brain, ileum and heart isolated from SPSHR, whereas it is barely detected in the same tissues of Wistar Kyoto controls. Up-regulation of B1 was observed in the aorta from e-NOS-KO, but not in another tissues, which exhibit m-RNA levels of B1 similar to that found in the control C57 Bl mice. The m-RNA of p22phox, the redox subunit of NADPH oxidase responsible for superoxide production, was mitigated in the brain of SPSHR and in the heart of both hypertensive models, compared with their controls. HSP90 m-RNA was ≤10% of control β-actin in all strains studied. These results show that adaptive responses are more extensive for whole body in spontaneously hypertensive SPSHR than in specific gene target generated e-NOS-KO model. Thus, altered m-RNA expression in hypertensive models seem so more amplified and intricate as higher is the complexity of the origin of the disease. Supported by FAPESP.

PP-255
CuZn-superoxide dismutase in hippocampus of rats exposed to acute, chronic or combined stress

D. Filipovic and **M. Radojcic**
Laboratory of Molecular Biology and Endocrinology, VINCA Institute of Nuclear Sciences, Belgrade, Serbia and Montenegro. E-mail: dragana@vin.bg.ac.yu

Exposure to stress alters the normal body homeostasis and leads to the development of various pathologies, which might involve alterations in the antioxidant defence system. In the present study CuZn-superoxide dismutase (CuZnSOD) protein expression was followed in the hippocampus of Wistar male rats exposed to 21 daily isolation or social crowding as chronic stressors, sole or in combination with 2 h acute stress of immobilization or cold (4C). The results showed that CuZnSOD protein expression was significantly increased in hipocampus after all acute and chronic stressors. The chronic crowding and chronic isolation were equally potent stressors, judged by the enzyme expression. In combined stress conditions, the only significant change in CuZnSOD expression was observed when crowding was followed by cold exposure. Our study indicated that both acute and chronic stress most probably generate intracellular imbalance between production and elimination of reactive oxygen species (ROS). Relatively increase levels of CuZnSOD in these conditions are required to remove high level of ROS in order to protect against ROS damage in hippocampus. The presumed stress induced changes in redox equilibrium in hippocampus may be prerequisite in generation and propagation of variety of pathological processes. In that view, special care should be payed to development of antioxidant therapeutics for antagonizing stress induced redox disbalances in neuronal cells.

PP-256
Relationship between Hsp 70 induction and oxidative stress in response to mycotoxins exposition

E. El Golli[1], W. Hassen[1], A. Bouslimi[1], C. Bouaziz[1], M. Ladjimi[2] and H. Bacha[1]
[1]*Laboratory of Research on Biologically Compatible Compounds, Faculty of Dentistry, Rue Avicenne, Monastir, Tunisia,* [2]*Laboratory of Biochemistry of Cellular and Molecular Regulators Signals, FRE 2621, CNRS-University of Pierre et Marie Curie, Paris, France. E-mail: emnagol@yahoo.fr*

In this study, We analyse the toxicity mechanisms of several mycotoxins using Hsp 70 expression, cytoprotection of Vero cells

by sub-Lethal Heat Shock and vitamin E. Our aim was (i) to determine whether citrinin, zearalenone and T2 toxin could induce the expression of Hsp 70, (ii) to check whether or not elevated levels of Hsp could provide cytoprotection from these mycotoxins, and finally (iii) to precise the part of oxydatif stress on global mycotoxin's toxicity.
Our study demonstrated that the three examined mycotoxins induce Hsp 70 expression in a dose dependent manner. A cytoprotective effect of Hsp 70 was obtained when Vero cells were exposed to sub-Lethal Heat Shock followed by a 12-h recovery prior to mycotoxins treatment and evidenced by a reduction of their cytolethality. This cytoprotection suggests that Hsp 70 may constitute an important cellular defence mechanism. A cytoprotective action was also obtained although at lesser extent, when cells were pre-treated with vitamin E before mycotoxins treatment. Finally our data shows that oxidative stress could play a part on mycotoxins toxicity, which is variable from one mycotoxin to another. This toxicity could be responsible for Hsp 70 induction.

PP-257
Endothelin receptor antagonist tezosentan improves mesenteric blood flow in septic shock models

A. Erdem[1], **A. M. Sevgili**[1], F. Akbiyik[2], A. B. Iskit[3], Z. D. Balkanci[1] and M. O. Guc[3]
[1]Physiology, Hacettepe University Faculty of Medicine, Ankara, Turkey, [2]Biochemistry, Hacettepe University Faculty of Medicine, Ankara, Turkey, [3]Pharmacology, Hacettepe University Faculty of Medicine, Ankara, Turkey. E-mail: msevgili@hacettepe.edu.tr

Multiple organ injury due to free radicals and decreased mesenteric blood flow are the consequences of septic shock Since the blockade of endothelin receptors was reported to exert beneficial effects, we investigated the effects of tezosentan (TZ), a novel dual endothelin receptor antagonist, in two different models of septic shock produced either by caecal ligation and puncture (CLP) or by the injection of *E. coli* endotoxin (Lipopolysaccharide: LPS, O55:B5, 20 mg/kg, i.p.). Swiss albino mice received TZ (10 mg/kg, i.p.) or its solvent saline (0.9% NaCl, w/v) twice at 2 h and 22 h after CLP or endotoxin injections. At 24th hour, the animals were anaesthetized and the mesenteric blood flow (MBF) were measured by using perivascular ultrasonic Doppler-flowmeter. After exsanguination, spleen, liver, and kidneys were isolated and thiobarbituric acid-reacting substances (TBARS), glutathione (GSH), and myeloperoxides activities (MPO) were determined. Statistical analysis was performed by using Instat® Software and significance was accepted when $P < 0.05$. MBF values were reduced both in LPS and CLP models and TZ significantly blocked the decrease. In both models, liver glutathione levels were decreased (mmol/mg protein, control: 0.04 ± 0.008; LPS: 0.01 ± 0.001; CLP: 0.01 ± 0.001, n = 7–9) while TBARS have increased only in LPS and MPO activity has increased only in CLP model. However, TZ blocked none of the biochemical parameters. Thus, we conclude that TZ improves the mesenteric blood flow in animal models of septic shock via mechanisms other than the blockade of free radicals.

PP-258
Haptoglobin levels in pregnancies complicated by preeclampsia

A. Atanasova[1], I. Samardziski[1], I. Todorovska[1] and **S. Biljali**[2]
[1]Clinic of Gynecology and Obstetrics, University Snt. 'Cyril & Methodist', Skopje, R. Macedonia, [2]Clinical Chemistry Institute, University Snt. 'Cyril & Methodist', Skopje, R. Macedonia. E-mail: aleksandra1_a@yahoo.ca

Haptoglobin (Hp)is an acute phase protein, and one of its important functions is capturing haemoglobin, preventing iron loss and subsequent oxidative damage generated by free iron in the vascular system of the kidneys. Hp is protective against cell damage by scavenging free radicals, such as the hydroxyl radical, formation that is promoted by presence of free haemoglobin. Hp-haemoglobin complex inhibits the vasodilator effect of nitric oxide. Hp itself was identified as a serum angiogenic factor and plays a role in proliferation and differentiation of vascular endothelium. Preeclampsia, which is characterised by pregnancy-induced hypertension and proteinuria, can be complicated by the haemolysis, elevated liver enzymes and low platelets. Aim of our study was to determinate levels of haptoglobin in pregnant woman who had developed signs of preeclampsia and correlation between haptoglobin and haemoglobin levels before and after delivery. Serum haptoglobin was assayed turbidimetric using polyclonal Rabbit Anti-Human-Hp. Haptoglobin levels decreased significantly ($P < 0.001$)before delivery. Correlation of haptoglobin and haemoglobin was $r = 0.13$ ($P < 0.05$).Pathogenesis of preeclampsia and cardiovascular related diseases, essential hypertension and coronary artery disease, share common characteristics including thrombophilia, endothelial damage, and oxidative stress. Our studies suggested that decreased levels of haptoglobin are in association with endothelial damage and oxidative stress of vascular system in woman with preeclampsia.

PP-259
The effect of carnosine on ethanol-induced damage in rat kidney

U. Ozel Turkcu[1], O. Mertoglu Caglar[1], A. Bilgihan[1], G. Biberoglu[2], I. Seven[1] and G. Take[3]
[1]Department of Medical Biochemistry, Gazi University Medical School, Ankara, Turkey, [2]Department of Paediatrics, Division of Paediatric Metabolism and Nutrition, Gazi University Medical School, Ankara, Turkey, [3]Department of Histology, Gazi University Medical School, Ankara, Turkey. E-mail: ozelu_ozelu@yahoo.com

Chronic ethanol consumption also causes toxic effects in kidney as well as liver. Myeloperoxidase (MPO) is one of the enzymes that constitute the defence system of immune cells. Carnosine is an endogenously synthesized dipeptide with antioxidant role. We investigated the protective role of exogen carnosine against ethanol-induced oxidative damage in rat kidney. Male Wistar rats (250–300 g) were divided into four groups of ten each and maintained for 13 days as follows: Control (0.9% saline, i.p.), Ethanol (2 g/kg/day, i.p.), Carnosine (1 mg/kg/day, orally) and Carnosine + Ethanol. On the 14th day, the rats were sacrificed. Kidney tissue was taken for the determination of malondialdehyde (MDA), MPO activity, advanced oxidation protein products (AOPP), and histopathologic analysis. Data were analysed by SPSS for windows version 10.0 using Kruskal Wallis Variance Analysis and Mann-Whitney U test. The highest levels of MDA increase was observed in the ethanol group ($P < 0.001$). and a significant decrease was observed in the ethanol + carnosine group compared with the ethanol group ($P < 0.001$). MPO activity in the ethanol group were decreased significantly

compared with the control and carnosine groups. Histological analysis of the kidney slices showed severe degeneration of the ethanol group. Less degeneration was observed in the carnosine + ethanol group compared with ethanol group but the observations were almost the same with control group.

PP-260
L-carnitine effects on erythrocyte membrane enzyme activities in basketball players

T. Parthimos[1], S. Tsakiris[1], P. Angelogianni[1], K. H. Schulpis[2], N. Parthimos[1] and **C. Tsopanakis**[1]
[1]*Department of Experimental Physiology, Medical School of Athens, University of Athens, Athens, Greece,* [2]*Institute of Child Health, Research Center, 'Aghia Sophia' Children's Hospital, Athens, Greece. E-mail: ctsop@cc.uoa.gr*

Background/Aims: To investigate whether the activities of erythrocyte membrane acetylcholinesterase (AChE), (Na^+, K^+)-ATPase and Mg^{2+}-ATPase are modulated in basketball players with or without L-Carnitine (L-C) supplementation, after the end of a forced basketball training.
Subjects and Methods: Blood was obtained from 10 male players without L-C before (group A) and at the end of the game (group B) and after 1 month L-C supplementation (2 g/24 h orally) before (group C) and at the end of the training (group D). Lactate, pyruvate and total antioxidant status (TAS), were measured with commercial kits and the enzyme activities spectrophotometrically.
Results: Lactate, Pyruvate, AChE, (Na^+, K^+)-ATPase were increased ($P < 0.001$) and TAS was decreased ($P < 0.001$) in group B. In contrast, TAS remained unaltered and the above enzyme activities were reduced ($P < 0.001$) in group D at the same time of study. Mg^{2+}-ATPase activity remained unchanged in the group B. *In vitro* incubation of the reduced AChE and Na^+, K^+-ATPase with L-C (25 μM) from group D resulted in a complete restoration of their activities.
Conclusions: Stimulation of AChE and Na^+, K^+-ATPase activities may be due to the rise of catecholamines and/or serotonin in group B, whereas carnitine utilization by the muscles during the training may inhibit the enzyme activities during the training. The latter is supported by the recovery of the enzyme activities after incubation of the membranes from group D with L-C.

PP-261
Differential vulnerability to oxidative stress in cancer cell lines of various tissue origin

K. Kania[1], G. Bartosz[2] and L. Pulaski[1]
[1]*Laboratory of Transcriptional Regulation, Centre for Medical Biology PAS, Lodz, Poland,* [2]*Department of Molecular Biophysics, University of Lodz, Lodz, Poland. E-mail: kkania@cbm.pan.pl*

Immortal cancer cell lines in culture give a unique opportunity of testing the behaviour and response of living human cells in various environmental stress conditions, serving as models of their tissues of origin *in vivo*. The tightly regulated balance between oxidising and reducing chemical species in a living cell, known as cellular redox homeostasis, is crucial for proper metabolism and cell survival. In order to compare the level of endogenous antioxidant defences in cells derived from various organs and tissues of the human body, we tested the comparative resistance to various reactive oxidant-related insults for a panel of 12 human cancer cell lines. The investigated cell lines represented sites of the human body known differ both in their vulnerability to physiological oxidative stress and in the activity of antioxidant mechanisms: they originated from the liver, respiratory system, gastrointestinal system, reproductive system and haemopoietic lineage. Cell viability was measured after treatment with reactive oxidants with various chemical mechanism of action (peroxides, quinones, compounds decreasing endogenous antioxidant level). Statistical analysis of obtained results shows that the tissue of origin is significantly correlated with the resistance level, which suggests that our data may be used to draw conclusions relevant to the *in vivo* situation. Specifically, the liver and respiratory system seem to be very efficiently protected against exogenous oxidative stress.

PP-262
Components of glutathione and thioredoxin antioxidant systems as determinants of redox homeostasis

L. Pulaski[1], K. Kania[1], J. Drzewinska[2], A. Pekowska[2], M. Ratajewski[1] and G. Bartosz[2]
[1]*Laboratory of Transcriptional Regulation, Centre of Medical Biology PAS, Lodz, Poland,* [2]*Department of Molecular Biophysics, University of Lodz, Lodz, Poland. E-mail: lpulaski@who.net*

In mammalian cells, electron transfer mechanisms centred on thiol peptides are the frontline defence against oxidative insult. It remains contentious, however, which of these antioxidant systems (composed of enzymes dedicated to the redox metabolism of glutathione - GSH and thioredoxin – TRX, respectively) is more versatile and useful *in vivo*. We undertook a screening study on the two thiol-based cellular antioxidant systems in a panel of 12 human cancer cell lines, differing in tissue origin, growth properties etc. We first characterised their redox homeostasis phenotype: resistance against exogenous oxidative stress (viability after exposure to peroxides) and endogenous production of reactive oxidants (oxidation of fluorescent dye precursors). Then, expression of genes encoding major ubiquitous components of both antioxidant systems (GSH system: GCLC, GSR, GPX1 and GLRX; TRX system: TXN, TXNRD1, PRDX1 and SESN1) was measured by real-time PCR, and enzymatic activity of GSH and TRX reductases and GSH and TRX peroxidases was determined. Phenotypic and molecular parameters were correlated by non-parametric and PCA statistical tests. Results show clearly that both thiol-based antioxidant electron transfer chains are important for maintenance of cellular redox homeostasis, since higher resistance to oxidative stress was usually paralleled by increased expression and activity of proteins from both groups, raising questions on mechanisms of co-ordinate regulation of these genes.

PP-263
Modulation of NO-ergic system in the lymnaea's CNS results in antioxidant enzyme activity changes

A. Sidorov and G. Maslova
Physiology, Belarusian State University, Minsk, Belarus. E-mail: sidorov@bsu.by

The radical gaseous nitric oxide (NO) is a versatile neuromodulatory molecule widely distributed across the animal kingdom. In the CNS of the freshwater snail, Lymnaea stagnalis the presence of NO synthase (NOS) activity was demonstrated in central nervous system. Here, we compare the action of NO precursor L-arginine (L-Arg) and NOS inhibitor NG-Nitro-L-arginine (L-NNA), on the activity of key antioxidant enzyme – superoxide dismutase (SOD) and reduced glutathione (GSH) level in the Lymnaea's CNS. We also investigate if this drugs can determine programmed cell death of neurons. Drugs were added in final concentration of 0.1 mM M for L-Arg and 0.01 mM for

L-NNA. SOD and GSH were detected within 4, and DNA assay 10 days after treatment. In control, SOD activity and GSH level were measured as 0.62 ± 0.075 U/mg of soluble protein and 39.9 ± 4.63 nmoles/mg wet mass. Both L-Arg and L-NNA decrease SOD activity: 0.36 ± 0.043 and 0.32 ± 0.058 U/mg of soluble protein ($P < 0.05$, *t*-test). There were no statistically significant changes in GSH level during L-NNA and L-Arg treatment. Nevertheless antioxidant defences in this conditions were lower we were unable to determine any apoptotic DNA damage during its electrophoresis in 1.5% agarose gel. Thus, modulation of NO-ergic system resulted in decrease of SOD activity, but was not effective to change GSH level. Changes in antioxidant defence enzymes activity can not influent on DNA damage processes.

Acknowledgment: Supported by BRFFR (grant B05K-113).

PP-264
Endoplasmic reticulum stress and apoptosis in the liver of scorbutic guinea pigs

E. Margittai, G. Banhegyi, G. Nagy, J. Mandl and M. Csala
Department of Medical Chemistry, Semmelweis University, Budapest, Hungary. E-mail: meva@puskin.sote.hu

Insufficient ascorbate intake causes scurvy in certain species. Beyond its known functions, it has been suggested that ascorbate participates in oxidative protein folding in the endoplasmic reticulum (ER). Because redox imbalance in this organelle might cause ER stress and apoptosis, this might contribute to the pathology of scurvy. Guinea pigs were divided into seven groups: the control group was fed a commercial guinea pig food for 4 weeks, five groups consumed an ascorbate-free food for 0, 1, 2, 3, or 4 weeks and one group was fed this scorbutic diet for 2 weeks and then the commercial food for 2 weeks. TBARS generation and the expression of some ER chaperones were determined in hepatic microsomes. The apoptotic index was assessed in histological sections. Although ascorbate, measured by HPLC, was undetectable in the livers of the guinea pigs after they had consumed the scorbutic diet for 2 weeks, microsomal TBARS level was elevated relative to the initial value only at week 4. Western blot revealed the induction of GRP78, GRP94, and protein disulfide isomerase at week 3 and 4. Apoptosis was greater than in the control, beginning at week 3. None of the alterations occurred in the groups fed the commercial guinea pig food or ascorbate-free food followed by ascorbate supplementation. Therefore, persistent ascorbate deficiency leads to ER stress, unfolded protein response, and apoptosis in the liver, suggesting that insufficient protein processing participates in the pathology of scurvy.

PP-265
Calcium-dependent protein kinases in oxidative stress-induced mitochondrial permeability transition

L. M. Pérez, J. E. Ochoa, E. J. Sánchez Pozzi and **M. G. Roma**
Faculty of Biochemical and Pharmaceutical Sciences, Institute of Experimental Physiology (IFISE), CONICET, National University of Rosario, Rosario, Argentina.
E-mail: mroma@fbioyf.unr.edu.ar

Mitochondrial permeability transition (MPT) is a key event in oxidative stress-induced hepatocellular death. Although Ca^{2+} plays a crucial role, its mechanisms are largely unknown. We examined here whether activation of Ca^{2+}-dependent protein kinases (PK) are involved in lipid peroxidation and MPT induced by *tert*-butylhydroperoxide (tBOOH) in isolated hepatocytes. tBOOH (500 μM) increased cytosolic Ca^{2+} and lipid peroxidation by 2623% and 723%, respectively. Ca^{2+} chelation with BAPTA, calmodulin (CM) inhibition with W7 or trifluoperazine, and CM-dependent-PKII inhibition with KN-62 reduced lipid peroxidation to a similar extent to CsA, without additive effect; this suggests PKII-dependency of MPT. In line with this, tBOOH-induced mitochondrial membrane depolarisation, a surrogate MPT marker, was counteracted by all these compounds to a similar extent to CsA (ca. –30%, $P < 0.025$). Neither PKC inhibition with staurosporine nor PKC activation with phorbol myristate acetate had any effect, despite tBOOH increased thrice Ca^{2+}-dependent PKCα in membrane. Involvement of calcineurin, a CsA-sensitive, CM-dependent protein with MPT-pore-opening properties, was also discarded, as its specific inhibitor, FK506, had no effect. We concluded that Ca^{2+} facilitates tBOOH oxidative damage *via* MPT, by a mechanism involving CM formation and further PKII activation.

PP-266
The neuropeptides influence on the mitochondrial membranes of the white rats in stress conditions

M. Melkonyan, G. Hoveyan, G. Zakaryan and **L. Ayvazyan**
Department of General and Bioorganic Chemistry, Yerevan Medical State University, Yerevan, Armenia.
E-mail: mag_melk@freenet.am

The results of previous investigations confirm, that the leading role of the destructive action of the noise on the whole organism, was due to the changes of the content of phospholipid (PL) components of cellular biomembranes, the prime target for lipid peroxidation (LP). The goal of this investigation was to study the influence of some biologically active neuropeptides in acute acoustic stress conditions as well as the role of their antioxidative activity in observed effects. The experiments were carried out on white male rats. Acoustic stress was produced by noise influence (91 dBA) within maximal energy of average and high frequency during 2 and 16 h. The synthetic peptide(SP, 2 mkg/kg), analogous of part of immunophyline and delta-sleep peptide(DSIP, 120 mkg/kg) were injected intraperitoneally before the noise action.

The data obtained particularly revealed significant increase after noise action of brain mitochondrial membranes lisophosphatidylcholins content well known mainly due to their membranolythic properties, which correlated with the intensity of LP and lipid-depending enzymes, ATPases, activity changes. The results of investigation of preliminary injection of neuropeptides revealed more expressed regulatory influence of DSIP, compared with SP, on the studied parameters, particularly on the LP intensity, content of individual representatives of PL and ATPases activities, in acoustic stress conditions.

PP-267
Free iron ions altered the surface electric charge of the human erythrocytes

Z. Shopova[1], V. Doltchinkova[1], N. Hristova[1] and J. Dimitrov[2]
[1]*Department of Biophysics and Radiobiology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria,* [2]*Department of Immunology, Stefan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria.*
E-mail: zlatina_shopova@yahoo.com

The exposure of cellular membranes to excess of iron supply has a key role in initiation of free radical-mediated damage, which results in change in the net surface charge of the cell. Further, these processes could influence the protein-membrane

interactions. We used lectines and immunoglobulin G (IgG) to clarify the role of iron in the binding of these molecules to membrane surfaces. The effect of phytohemagglutinins (PHA M, PHA S), concanavalin A, peanut agglutinin and wheat germ agglutinin binding on the surface of erythrocyte membrane are thus explored using cell micro-electrophoresis. Lectin binding cause specific changes in the electrophoretic mobility (EPM) of erythrocytes. We observed that the exposure to ferrous ions (1 mM) resulted in a recovery of the net negative surface charge of the PHA M-pretreated erythrocytes, but not in the presence of the other lectins.
We have also studied the role of iron ions in the IgG-erythrocyte interactions by using the electrokinetic approach. The presence of immunoglobulin G (IgG) molecules induced a large decrease in EPM of erythrocytes. This value is again recovered in the presence of Fe(II) ions in IgG exposed erythrocytes. These results provide an explanation for the aggregation and EPM of erythrocytes after IgG exposure, as well as for specific iron-mediated interactions contributing to IgG-erythrocyte binding in physiology and pathology.

PP-268
On the interventional role of eNOS in hydrogen peroxide-induced HARP/pleiotrophin up-regulation in endothelial cells

C. Polytarchou, M. Hatziapostolou and E. Papadimitriou
Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, Greece. E-mail: cpolyt@upatras.gr, hatziap@upatras.gr, epapad@upatras.gr

We have recently shown that hydrogen peroxide (HP) increased human endothelial cell (EC) proliferation and migration *in vitro* through regulation of endothelial Nitric Oxide Synthase (eNOS) activity. Heparin Affin Regulatory Peptide (HARP) seems to be an important mediator of HP stimulatory effects on cancer cells. In the present work we studied whether HARP mediates the stimulatory effects of HP in ECs and explored the possible involvement of eNOS. HP significantly increased HARP protein amounts in a concentration-dependent manner, an effect that was abrogated by eNOS inhibitors. In the same line, eNOS inhibition abrogated HP-induced luciferase activity of the 5'-flanking region of the HARP gene introduced in a reporter gene vector. Mitogen Activated Protein Kinases (MAPKs) are involved in HP signalling and U0126 eliminated HP effects. eNOS seems to act upstream of MAPKs, since inhibition of eNOS activity abolished the HP-induced Extracellular signal Regulated Kinases 1/2 phosphorylation. Activator Protein-1 (AP-1) is involved in HP-stimulatory effects on Human Umbilical Vein ECs (HUVEC), as revealed using curcumin. The effect of HP seems to be due to binding of Fra-1, JunD and phospho-c-Jun to the HARP promoter, an effect that was blocked by eNOS inhibition. These results support the notion that HARP is important for human EC functions and establish for the first time the intervention of eNOS in the up-regulation of HARP expression by low concentrations of HP.

PP-269
L-arginine effect on free radical production in brain neurotoxicity

J. Nikolic, D. Sokolovic and J. Basic
Department of Biochemistry, School of Medicine Nis. E-mail: nikdan@bankerinter.net

Arginase is enzyme of L-Arginine metabolism and with regulatory functions in the brain. Uptake and metabolism of L-arginine may be altered in some conditions such as inflammation, sepsis, and toxic effects of various drugs. Mercury exerts a variety of toxic effects in the body. Lipid peroxidation, DNA damage and depletion of reduced glutathione suggest an oxidative stress-like mechanism for $HgCl_2$ induced toxicity.
The aim of this study was investigation the effect of L-arginine against $HgCl_2$-induced oxidative damage.
Male Sprague Dawley rats weighing about 200 g. were used in the experiment. Rats were divided in four groups: (a) Control group of animals was treated with 0.9% NaCl; (b) rats injected with mercury chloride in a dose of 3 mg/kg; (c) group of animals, treated with L-Arginine (250 mg/kg) and (d) L-Arginine injected (250 mg/kg) 1 hour before mercury chloride. The animals were killed 24 hours after $HgCl_2$ administration.
Brain tissue samples were taken for determination of arginase activity and malondialdehyde (MDA) level. The results show that $HgCl_2$ induces oxidative tissue damage, as evidenced by increases in MDA level ($P < 0.05$) and decreases arginase activity ($P < 0.05$). L-arginine pretreatment decreases lipid peroxidation level.
Conclusion: $HgCl_2$-induced brain toxicity alters metabolic pathway of arginine through arginase reaction, which is followed by increased lipid peroxidation level. L-Arginine may be helpful to improve brain function to intoxicated rats.

PP-270
Reactive oxygen species (ROS) augment B-cell activating factor (BAFF) expression

E. Y. Moon, S. K. Ryu and S.Y. Oh
KRIBB. E-mail: eunyimoon@yahoo.com

B-cell activating factor (BAFF) plays a role for mature B cell generation and maintenance. Lipopolysaccharide (LPS) activates toll-like receptor 4 (TLR4)-dependent signal transduction and induces ROS production. Here, we investigated BAFF production regulated by reactive oxygen species (ROS). BAFF expression was augmented by LPS-stimulation and by serum deprivation that also induced ROS production. BAFF expression was inhibited by the treatment with various antioxidants including N-acetyl-L-cystein (NAC). We also investigated BAFF expression *in vivo* using peroxiredoxin II (Prx II)-deficient mouse spleen cells. Prx II is a member of antioxidant enzyme family that protects cells from oxidative damage. Constitutive production of endogenous ROS was detected in spleen cells lacking Prx II. Serum BAFF protein level and BAFF transcript expression in splenocytes were significantly higher in Prx II–/– mice than wild type mice. Higher BAFF level is consistent with the higher total number of splenocytes and B220+ cells. Results were supported by NF-kB activation as judged by reduced IkB-alpha degradation and increased nuclear translocation of p65/RelA with LPS-stimulation, serum deprivation and Prx II-deletion. Data suggest that TLR4-mediated BAFF expression was increased by ROS and it was inhibited by Prx II controlling ROS production.

PP-271
Iron homeostasis and methionine-centered redox cycle in aging of head and neck organs

V. Vinokur[1], E. Berenshtein[1], M. Gross[2], A.Z. Reznick[3], M. Chevion[1] and R. Eliashar[2]
[1]Department of Cellular Biochemistry and Human Genetics, Hebrew University, Jerusalem, Israel, [2]Department of Otolaryngology/Head & Neck Surgery, The Hadassah-Hebrew University Hospital, Jerusalem, Israel, [3]Department of Anatomy and Cell Biology, Bruce Rappaport Faculty of Medicine, Technion, Israel Institute of Technology, Haifa, Israel. E-mail: vladiv@md.huji.ac.il

Age-related changes in four rat head and neck organs were investigated. The levels of tissue ferritin and its saturation with iron,

as well as the levels and activities of methionine sulfoxide reductase (Msr) and its MsrA and MsrB isoforms, thioredoxin (Trx), and thioredoxin reductase (TrxR) were evaluated.
A significant age-related increase in ferritin concentration was observed in both sternohyoid muscle and tongue, while its saturation with iron remained unchanged. Ferritin level in the esophagus was lower in old rats, whereas its iron saturation increased significantly. The level of TrxR has increased significantly with age in the muscular organs and insignificantly in the larynx, while an age-related decrease was observed in the esophagus. TrxR activity in the tongue, sternohyoid muscle and larynx matched the TrxR levels in these organs. Trx level did not change with aging in the muscular organs, while a dramatic decrease was observed in the other tissues, being a possible reason for age-related disorders. Marked changes in Msr activity were observed in all organs, with more prominent changes in the MsrB, selenium-containing isoform.
Our results are consistent with the notion that the esophagus is considered to be the most vulnerable among the above head and neck organs to age-related pathologies. The correlation between TrxR and ferritin levels may hint that a common regulatory pathway may exist.

PP-272
The effect of different dialysis membranes on lipid and protein oxidation in hemodialysis patients

E. Dursun[1], M. Timur[1], H. I. Varan[2], G. Suleymanlar[2] and T. Ozben[1]
[1]*Department of Biochemistry, Akdeniz University, Medical Faculty, Antalya, Turkey,* [2]*Department of Nephrology, Akdeniz University, Medical Faculty, Antalya, Turkey.*
E-mail: evrimdursun@akdeniz.edu.tr

Oxidative stress has been defined as a loss of counterbalance between free radicals and antioxidant systems, with negative effects on carbohydrates, lipids, and proteins. An increase in oxidative stress may contribute to lipid and protein oxidation. Bioincompatibility of the membranes used in hemodialysis is a potential factor causing increased oxidative stress. In this study, the acute effects of cuprophane and polysulphone membranes on lipid and protein oxidation were measured and compared in hemodialysis patients. We investigated oxidative modification of plasma proteins by measuring protein carbonyl content and lipids by thiobarbituric acid-reactive substances(TBARS). Reduced glutathione levels(GSH) were determined to reveal the protective antioxidant effect of thiol groups.
Fifteen hemodialysis patients(M/F:9/6) and 15 healthy subjects were included in the study. In the beginning of the study, dialysis was performed using cuprophan membrane. After 2 weeks of wash-out period, dialysis was performed using polysulphone membrane in the same patients. Protein carbonyl content, TBARS and GSH were measured in blood samples obtained from healthy subjects and patients before and after dialysis. Cuprophane membranes enhanced lipid peroxidation and protein oxidation and decreased reduced glutathione in comparison to polysulphone membranes. We conclude that biocompatible membranes like polysulphone are more effective in struggling with oxidative stress produced during hemodialysis.

PP-273
Melatonin prevents UV-induced signalling that leads to photoaging in dermal fibroblasts *in vitro*

M. Yuksel Egrilmez[1], G. Oktay[2], H. Resmi[1], S. Aktan[3], S. Ozkan[3], G. Guner[1] and S. Kocturk[2]
[1]*Research Laboratory, Dokuz Eylul University School of Medicine, Inciralti, Izmir, Turkey,* [2]*Department of Biochemistry, Dokuz Eylul University School of Medicine, Inciralti, Izmir, Turkey,* [3]*Department of Dermatology, Dokuz Eylul University School of Medicine, Inciralti, Izmir, Turkey.*
E-mail: mehtap.yuksel@deu.edu.tr

Human skin is exposed to solar ultraviolet (UV) radiation, which results in photoaging. It was previously reported that UV causes photoaging by activation of growth factor and cytokine receptors in dermal cells. They lead to downstream signal transduction through activation of mitogen-activated protein kinase (MAPK) pathways. These pathways induce matrix metalloproteinases (MMPs) that degrade skin connective tissue. Generation of reactive oxygen species by UV is also critical in triggering MAPK pathways. Melatonin, the principal secretory product of the pineal gland, is the most effective free radical scavenger. We examined whether melatonin ameliorates UV-A/UV-B-induced responses *in vitro*. Dermal fibroblasts were isolated from punch biopsies of healthy individuals. Fibroblasts were pretreated with melatonin (10–6 M) for 1 h and then exposed to UV-A/UV-B. c-Jun N-terminal kinase (JNK) activation was analysed by Western blotting. Epidermal growth factor receptor (EGFR) activation, MMP-1 activity, levels of nitrotyrosine and tissue inhibitor of MMP (TIMP-1) was measured by ELISA. Procollagen type I C-peptide (PIPC) levels was determined by EIA. Pretreatment with melatonin inhibited UV-A and UV-B-induced JNK and EGFR activation, increases in nitrotyrosine levels and MMP-1 activity. Pretreatment with melatonin increased UV-A and UV-B-induced increases in levels of TIMP-1 and PIPC. These data indicate that melatonin with antioxidant activities may prevent photoaging.

PP-274
Exposure of human diploid fibroblasts to hypoxia delays significantly replicative senescence

E. Poulios, I. P. Trougakos and E. S. Gonos
Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, Athens, Greece.
E-mail: epoulios@eie.gr

Hypoxia is a condition of low oxygen concentration found in many pathological conditions. In this study, we investigated the effects of hypoxia, induced either by low oxygen concentration (1.5% O2) or by transition metals (CoCl2), on protein expression, proliferative capacity and cell viability of normal and immortalized human diploid fibroblasts (HDFs). In either cell line both hypoxia and CoCl2 induced the main regulator of transcriptional responses to reduced oxygen tension, namely the Hypoxia Inducible Factor-1alpha (HIF-1alpha). However, the extent and the kinetics of HIF-1alpha induction differ between these cell types. Moreover, cell exposure to hypoxia results in the induction of proteins being associated with senescence and/or apoptosis. To determine whether oxygen concentration affects cellular lifespan, we investigated the effects of hypoxia (1.5% O2) on normal HDFs proliferative capacity and we found a significant extension of the cellular lifespan, as compared to standard culture conditions, which include 20% oxygen. Next, we determined whether

cells grown under hypoxic conditions are more resistant to stress, as compared to those cultured at 20% oxygen. We found that hypoxia sensitizes human cells to all cytotoxic agents tested. These findings clearly identify oxygen concentration as a critical parameter in the cellular ability to cope with various cytotoxic insults and highlight its crucial role in regulating cellular lifespan of normal human cells.

PP-275
The effect of melatonin on protein oxidation in the brain tissue of hypoxic neonatal rats

S. Eskiocak[1], F. Tutunculer[2], U. N. Basaran[3] and E. Cakir[1]
[1]*Department of Biochemistry, School of Medicine, Trakya University, Edirne, Turkey,* [2]*Department of Paediatry, School of Medicine, Trakya University, Edirne, Turkey,* [3]*Department of Pediatric Surgery, School of Medicine, Trakya University, Edirne, Turkey. E-mail: drseskiocak@yahoo.co.uk*

It was determined that free reactive oxygen species were generated under hypoxic conditions in the brain. Free radicals are also known to cause modifications of proteins and alteration in their functions. Melatonin is a potent antioxidant agent that can scavenge radicals. In this study, we have investigated the effect of melatonin on protein oxidation during hypoxia. Seven days old Sprague Dawley newborn rats were divided into three groups. Hypoxic ($n = 9$) and melatonin ($n = 11$) groups were subjected to 2 h of hypoxic exposure (a humidity mixture of nitrogen 92% and oxygen 8% gases). Melatonin (10 mg/kg) was administrated 30 min before the onset hypoxia and then at 24th and 48th hours after the end of the hypoxic exposure. The tissue concentration of advanced oxidation protein products (AOPP) and protein thiol (P-SH) was used as an index of protein oxidation.
We have found that, while AOPP increased significantly, the levels of P-SH decreased in the hypoxic group. The level of AOPP was decreased by melatonin treatment However, perturbed thiol status could not be recovered by melatonin treatment.
These results indicate that exogenous melatonin partially reduces protein oxidation. Melatonin could protect newborn rats against hypoxia-induced brain damage. However, melatonin alone was observed to be an incomplete treatment to prevent protein oxidation.

PP-276
Upregulation of bradykinin B1 receptor by H_2O_2 in mouse endothelial cells

A. C. Azevedo[1], T. V. Gualberto[1], O. F. Pavao[1], M. L. Capurro[2] and C. R. A. Bertoncini[1]
[1]*Universidade Federal de São Paulo, São Paulo, Brazil,* [2]*Departamento de Parasitologia, Universidade de São Paulo, SP, Brazil. E-mail: aline.cedeme@epm.br*

The bradykinin B1 receptor mediates vasodilation and its expression has been pointed as a worsen factor of several diseases. We asked if B1 receptors could be induced by pro-oxidant conditions. Cultures of endothelial cells from mouse lymph nodes were unstimulated or treated with 0.1–1.5 mM H_2O_2 or 10 mg/ml LPS and kept for further 5 h in culture medium. RT-PCR analyses shown H_2O_2 dose dependent expression of the B1 receptor, with maximum at 1mM pulse. No significant changes were found in NADPH oxidase, a source of superoxide. However, increasing H_2O_2 concentrations lead to progressively less expression of HSP90, an enzyme required by endothelial nitric oxide (NO) syntase so that its absence induces HSP90 to produce superoxide instead NO. The functionality of the B1 receptors in 1 mM H_2O_2–treated-cells was similar to that induced by LPS, as determined by extracellular acidification in response to B1 agonist des-arg^9-bradykinin. Overall the results suggest that the B1 receptor induction by oxidative stress could be a benefit adaptive response to harmful effects of oxidant species, particularly if superoxide or its derivate H_2O_2 contributes to vasoconstriction and endothelial dysfunction.

PP-277
Adriamycin-induced alterations in cytochromes biosynthesis and superoxide dismutase (SOD) activity in *Salmonella typhimurium*

S. Khavari-nejad[1], E. Keyhani[1] and D. Minai-Tehrani[2]
[1]*Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran,* [2]*Biochemisty Laboratory, Biology Department, Faculty of Sciences, Shahid Beheshti University, Tehran, Iran. E-mail: khavari_nejad@ibb.ut.ac.ir*

The use of adriamycin (AD), an anthracycline antibiotic highly effective against solid and hematologic malignancies, is limited by acute and chronic cardiotoxicity. No detailed understanding of the mechanism of the drug activity has been reached yet. In this work, cytochromes biosynthesis and SOD activity in *S. typhimurium* grown in the presence of AD, were investigated.
S. typhimurium cells cultured for 24 h with 1, 5, 20, 50, 100, 150 or 300 μg/ml AD, were harvested and washed twice in phosphate buffer 0.1 M, pH 7.4. The cytochromes content was evaluated by reduced-minus-oxidized difference spectra of cell suspensions. Cytochrome d content was unchanged in up to 150 μg/ml AD; cytochrome b595 doubled in AD 1 to 100 μg/ml. Cytochrome b560 content, unchanged in up to 5 μg/ml AD, decreased progressively to one-fifth at 50 μg/ml AD; it could not be detected in cells grown in higher AD concentrations because of interference by the drug's own absorption spectrum. SOD activity, determined in lysozyme-treated bacteria, decreased as AD concentration increased. It was 5.8 μ/mg prot in the control and 1.6 u/mg prot in cells grown with 300 μg/ml AD. Data showed that cytochrome b560 was the haemoprotein most sensitive to AD in *S. typhimurium* respiratory chain. The steady decrease in SOD activity, first enzyme in anti-oxidative stress defence, suggested a decrease in the cell's fighting ability as one of the elements in AD metabolic toxicity.

PP-278
The delayed effect of ischaemic preconditioning on hepatic ischemia/reperfusion injury in rats

E. Ofluoglu[1], M. Kerem[2], H. Pasaoglu[1], N. Turkozkan[1], I. Seven[1], T. U. Yilmaz[2] and A. Bedirli[2]
[1]*Department of Medical Biochemistry, Gazi University Medical School, Ankara, Turkey,* [2]*Department of General Surgery, Gazi University Medical School, Ankara, Turkey. E-mail: ebruofluoglu@yahoo.com*

The aim of this study was to elucidate the effect of ischaemic preconditioning (IPC) on the energy charge at the late phase of hepatic ischemia/reperfusion (IR) together with the level of hepatocellular damage and cellular mechanisms. Thirty Wistar rats were randomly divided into sham, IR and IPC groups. The rats were subjected to 60 min hepatic ischemia, pretreated by IPC (10/15 min) or not. The model of partial hepatic IR was used. After 24 h of reperfusion serum alanine transferase(ALT), liver tissue arginase, nitrite and nitrate, malondialdehide (MDA),adenosine triphosphate (ATP), adenosine diphosphate (ADP),adenosine monophosphate (AMP)and energy charge(EC) were measured. The levels of tissue arginase and serum ALT

were reduced by IPC. In rats pretreated with IPC, tissue nitrite and nitrate levels were significantly higher than IR group ($P < 0.001$). Tissue levels of MDA in IPC group were found to be significantly lower than in IR group ($P = 0.001$). After the 24 h of hepatic ischemia, ATP and EC levels were higher in pretreated rats with IPC than in the IR group ($P = 0.001$, $P = 0.002$, respectively). The IPC procedure significantly reduced the hepatic necrosis ($P < 0.001$). The results of this study demonstrate that pretreatment with IPC improve tissue ATP, energy charge, and hepatic necrosis at late stages of ischemia reperfusion injury of the liver. Reduced arginase activity, increased nitric oxide and reduced MDA seem to play regulating role in this protective effect.

PP-279
Oxidative stress, reduced glutathione levels and catalase activities in leprosy patients

G. Ozan[1], S. Yılmaz[2], M. Erişir[2], İ. Erden[3] and S. Temizer Ozan[2]
[1]*Faculty of Medicine, Department of Biochemistry, Fırat University, Elazıg, Turkey,* [2]*Faculty of Veterinary Medicine, Department of Biochemistry, Fırat University, Elazıg, Turkey,* [3]*Elazığ Dermatologia and Leprosy Hospital, Elazıg, Turkey. E-mail: goncaozan@yahoo.com, sevyilars@yahoo.com, mineerisir@yahoo.com, ilkererden@mynet.com*

Leprosy, an infection caused by *Mycobacterium leprae*, primarily affects superficial tissues, especially the skin and peripheral nerves. The purpose of this study was to investigate the plasma malondialdehyde (MDA) levels, blood reduced glutathione (GSH) levels and catalase activities in inactive lepromatous leprosy patients.

The subjects for this study were healthy human volunteers (HVs, $n = 20$) and inactive lepromatous leprosy patients released from treatment (LPs, $n = 34$). The levels of MDA (HVs; 6.21 ± 0.22, LPs; 9.73 ± 0.46) increased significantly ($P < 0.001$) in inactive lepromatous leprosy patients. Also, the levels of GSH (HVs; 5.15 ± 0.35, LPs; 7.07 ± 0.33) and catalase activities (HVs; 10.97 ±0.84, LPs; 28.26 ± 2.82) increased significantly ($P < 0.01$, $P < 0.001$, respectively), in inactive lepromatous leprosy patients in comparison with control group. High MDA levels and antioxidant status observed in leprosy patients indicated that there is an oxidative stress in leprosy. In early stage of the leprosy, a suitable antioxidant therapy can be suggested in addition to antibacterial therapy to prevent possible tissue injury.

PP-280
Peroxisomal Lon protease and pexophagy play key roles in cellular housekeeping and vitality

E. Bener Aksam, A. Koek, J. A. K. W. Kiel, S. Jourdan, M. Veenhuis and I. J. van der Klei
Groningen Biomolecular Sciences and Biotechnology Institute. E-mail: e.bener@rug.nl

Peroxisomes are subcellular organelles that are comprised of a protein rich matrix surrounded by a single lipid membrane. By definition, peroxisomes contain hydrogen peroxide producing oxidases and catalase for detoxification. The generation and removal of ROS (Reactive Oxygen Species) should be precisely balanced in order to prevent ROS induced damage inside these organelles and the cell. Evidence is now accumulating that damaged peroxisomes may cause release of enhanced ROS levels thereby contributing to cell ageing. In our study, we analysed two aspects of peroxisomal housekeeping in *Hansenula polymorpha*. First, we showed that a peroxisomal Lon protease, Pln, plays a role in degradation of unfolded and non-assembled peroxisomal matrix proteins. Deletion of the *PLN* gene resulted in accumulation of protein aggregates in peroxisomes, enhanced levels of intracellular ROS and a decrease in cell viability. Secondly, we demonstrate that in wild type cells peroxisomes are constitutively degraded, a process that is prevented upon deletion of Hp*ATG1*, a gene required for pexophagy. Like *H. polymorpha pln* cells, *atg1* cells also showed enhanced intracellular ROS levels and decreased viability. Highest intracellular ROS levels and lowest cell viability were observed in a *pln.atg1* double deletion strain. Our data imply that Pln and pexophagy are important in cellular housekeeping and contribute to the viability of *H. polymorpha* cells.

PP-281
Apoptosis-inducing factor is a functional component of the electron transport chain

N. Apostolova[1], A. M. Cervera[1], V. M. Victor[1], S. Cadenas[2], A. Sanjuan-Pla[1], A. Alvarez-Barrientos[2], J. V. Esplugues[3] and K. J. McCreath[1]
[1]*Unidad Mixta de Investigación, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Universitat de Valencia, Valencia, Spain,* [2]*Centro Nacional de Investigaciones Cardiovasculares (CNIC), Melchor Fernández Almagro, Madrid, Spain,* [3]*Departamento de Farmacologia, Facultad de Medicina, Valencia, Spain. E-mail: napostolova@cnic.es*

The apoptosis function of Apoptosis-Inducing Factor (AIF) has been well documented in the literature, but its physiological role in the mitochondrion is less certain. Using small interfering RNA (siRNA) strategy, we studied whether modulation of AIF in cultured cells influenced the production of reactive oxygen species (ROS). We found that siAIF-transfected cells had reduced AIF protein levels and this was paralleled by an approximate two-fold increase in ROS. The increased ROS were mitochondrial in origin as a similar silencing strategy in cells devoid of a functioning electron transport chain (ETC) did not result in ROS-increases. Increased ROS were sufficient to activate HIF-1α, a ROS-sensitive transcription factor. Examination of oxygen consumption revealed that AIF-depleted cells had a major impairment of respiration, at Complex I in the ETC. Western blot analysis also showed a loss of Complex I protein subunits. Studies using both a broad-range antioxidant (N-acetyl cysteine) and a novel mitochondrial-targeted antioxidant (MitoQ), revealed that respiratory competence could be regained in AIF-silenced cells. We are currently overexpressing natural antioxidant proteins in our model to test the generality of this response. Our results lead to the conclusion that the defect in respiration is downstream of Complex I protein loss and is presumably due to ROS-mediated damage to the ETC. This suggests an integral role for AIF, in the mitochondrion, as a redox modifier.

PP-282
Serum advanced oxidation protein products, myeloperoxidase and ascorbic acid in pre-eclampsia

T. Noyan[1], A. Güler[2], M Sekeroglu[1] and M. Kamacı[2]
[1]*Medical Faculty, Department of Clinical Biochemistry, Yuzuncu Yil University, Van, Türkiye,* [2]*Medical Faculty, Department of Gynecology and Obstetrics, Yuzuncu Yil University, Van, Türkiye. E-mail: tevfiknoyan@hotmail.com*

Pre-eclampsia and eclampsia are the medical complication of pregnancy and aetiologies of these diseases are still unclear. It is aimed to determine the levels of both oxidant and antioxidant

status in pre-eclampsia and eclampsia in the present study. Twenty-one pregnant women with pre-eclampsia, 11 pregnant women with eclampsia and 19 healthy pregnant women were included in the study. Serum concentrations of MDA, AA and CAT activity were not significantly different in pre-eclampsia as compared with the eclampsia. There were no significant differences in serum concentrations of AOPP among the groups. Serum concentrations of MDA were significantly higher in pre-eclampsia and eclampsia as compared with normal pregnancy ($P < 0.05$). Serum concentration of MPO was significantly higher in eclampsia as compared to pre-eclampsia and normal pregnancy ($P < 0.05$), whereas there was no significant difference between the pre-eclampsia and normal pregnancy. Serum concentration of AA was significantly higher in pre-eclampsia as compared to normal pregnancy ($P < 0.05$). Serum CAT activity were significantly higher in pre-eclampsia and eclampsia as compared with normal pregnancy ($P < 0.05$). According to these results it was suggested that increased oxidative stress might be contribute to the pathophysiological mechanisms of pre-eclampsia and eclampsia as well as AA and CAT may have a protective role via free radical-scavenging properties.

PP-283
A targeted antioxidant reveals the importance of mitochondrial ROS in the hypoxic signalling of HIF-1

A. Sanjuán-Pla, A. M. Cervera, N. Apostolova and K. J. McCreath

Unidad Mixta, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Universitat de València, Valéncia, Spain. E-mail: asanjuan@cnic.es, asanpla13@hotmail.com

Exposure to limiting oxygen in cells and tissues induce the stabilization and transcriptional activation of the hypoxia-inducible factor 1 alpha (HIF-1α) protein, a key regulator of the hypoxic response. Reactive oxygen species (ROS) generation has been implicated in the stabilization of HIF-1α during this response, but this is still a matter of some debate. In this study we utilized a novel mitochondria-targeted antioxidant, MitoQ, and examined its effects on the hypoxic stabilization of HIF-1α. Our results show that under conditions of reduced oxygen tension (3% O2), MitoQ ablated the hypoxic induction of mitochondrial ROS production and also reduced the levels HIF-1α via de-stabilization of the protein. This in turn led to an abrogation of HIF-1 transcriptional activity. Oxygen consumption analysis demonstrated that MitoQ did not interfere with the electron transport chain, and studies with a broad-range Nitric Oxide synthase inhibitor ruled out the participation of this molecule in our model. Pharmacological-induced normoxic stabilization of HIF-1α was unchanged in the presence of MitoQ suggesting that ROS were not involved in these processes. This study strongly suggests that mitochondrial ROS contribute to the hypoxic stabilization of HIF-1α. We are currently utilizing MitoQ to address the contributions of Nitric Oxide to HIF-1α de-stabilization in different model systems during hypoxia.

PP-284
Cold stress induced oxidative modifications in brain

E. Sahin and S. Gumuslu

Department of Biochemistry, Faculty of Medicine, Akdeniz University, Antalya, Turkey. E-mail: esahin@akdeniz.edu.tr

It is well known that exposure to low temperatures results in compensatory changes in the antioxidant defence system. The aim of this study was to investigate the effect of cold stress on the antioxidant enzyme activities (copper, zinc-superoxide dismutase (Cu, Zn-SOD), catalase (CAT) and selenium-dependent glutathione peroxidase (Se-GSH-Px)), reduced glutathione (GSH), protein oxidation and lipid peroxidation in the brain. For this reason, sixteen male Wistar rats (3-months-old) weighing 220 ± 20 g were used. Rats were divided into two groups as the control group (C) and cold stress group (CS). Cold stress was applied to the animals by placing in a cold room (ambient temperature 5°C) for 15 min/day for 15 days. At the end of the experimental periods, corticosterone levels were measured in plasma, while antioxidant enzymes, GSH, protein carbonyl (PC) and lipid peroxidation products (conjugated diene (CD) and thiobarbituric acid-reactive substances (TBARS)) were measured in the brain. Corticosterone level of CS group (751.00 ± 7.92 ng/ml) was found to be increased ($P < 0.001$) according to the C group (274.50 ± 10.39 ng/ml). Cu, Zn-SOD, CAT and Se-GSH-Px activities, and PC, CD and TBARS levels were found to be increased while GSH levels were decreased in brain of CS group. These results lead us to conclude that cold stress can disrupt the balance in an oxidant/antioxidant system and cause oxidative damage to brain by altering antioxidant status, protein oxidation and lipid peroxidation.

PP-285
Effect of melatonin against oxidative stress in rat exposed to alcoholic rat heart tissue

A. Karabulut[1], G. Söğütlü[2], N. Karadağ[3], A. Cıkım[4] and T Kıran[5]

[1]Department of Biochemistry, Medical Faculty, İnonu University, Malatya, Turkey, [2]Department of General Surgery, Medical Faculty, İnonu University, Malatya, Turkey, [3]Department of Pathology, Medical Faculty, İnonu University, Malatya, Turkey, [4]Department of Endocrinology and metabolism, Medical Faculty, İnonu University, Malatya, Turkey, [5]Department of Biochemistry, Medical Faculty, İnonu University, Malatya, Turkey. E-mail: abkarabulut@inonu.edu.tr

Ethanol consumption causes renal toxicity by means of oxidative stress. Free radical formation and lipid peroxidation due to ethanol causes cardiovascular damage. Melatonin, a potent antioxidant, is proven to be effective against biochemical and histopathological changes of ethanol toxicity in many tissues. We investigated the same protective effect of melatonin in rat both biochemically and histologically.

Two groups of rats (10 subjects each) received ethanol and ethanol plus melatonin respectively for 4 weeks. Another 10 rats were sham operated. Nitric oxide (NO) Malondialdehyde (MDA), reduced glutathion (GSH), levels were determined in hearts of all groups. In all groups, cytological examination was also performed and correlation with biochemical findings was investigated. Mann Whitney-U Test for inter-group comparisons and Spearman Test for correlations were used.

Ethanol-only group revealed higher levels of MDA, NO and lower levels of GSH ($P < 0.05$) were found compared to melatonin administered rat heart. Ethanol only group higher levels of MDA, NO and lower levels of GSH ($P < 0.05$) compared to control. Differences of NO levels were insignificant in ethanol only group compared to melatonin group.

In conclusion, Melatonin helps preventing the oxidative stress of ethanol on rat heart. This may be helpful for patients with alcohol consumption and detoriated cardiovascular function and further studies on human may supply important data.

PP-286
Immobilization stress causes oxidant-antioxidant imbalance in different tissues of rats

S. Gumuslu and E. Sahin
Department of Biochemistry, Faculty of Medicine, Akdeniz University, Antalya, Turkey. E-mail: sgumuslu@akdeniz.edu.tr

The aim of this study was to determine the effects of immobilization stress on antioxidant status, protein oxidation and lipid peroxidation in the brain, liver, kidney, heart and stomach. Sixteen male Wistar rats (3 months old) were used and the rats were divided into two groups as control group (C) and immobilization stress group (IS). Rats were immobilized for 180 min/day for 15 days in IS group. Blood samples were taken for measuring plasma corticosterone levels. Tissues were obtained for measuring the antioxidant enzyme activities (copper, zinc-superoxide dismutase, catalase and selenium-dependent glutathione peroxidase), reduced glutathione, protein oxidation and lipid peroxidation. Corticosterone levels were increased in IS group. Copper, zinc-superoxide dismutase activities were increased in the brain, liver and kidney, whereas it decreased in the heart and stomach. Catalase activities were increased in the brain, kidney and heart, whereas it decreased in the liver and stomach. Selenium-dependent glutathione peroxidase activities were decreased in the brain and kidney, whereas it increased in the heart and stomach. Reduced glutathione levels were decreased, while protein carbonyl, conjugated diene and thiobarbituric acid-reactive substances levels were increased in all tissues. Our results supported the idea that the response of tissues to immobilization stress is different from each other with regard to the antioxidant status, protein oxidation and lipid peroxidation.

PP-287
Interaction of triarylmethane dyes with redox system enzymes

E. Topaloglu and G. Ucar
Department of Biochemistry, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey. E-mail: gulberk@hacettepe.edu.tr

In the present study, the interactions of triarylmethane (TAM) dyes with catalase and glutathione S-transferase (GST) were investigated. Malachite green (MG) and crystal violet (CV) appeared to inhibit bovine liver catalase reversibly in a pure non-competitive manner with the apparent Ki value of 27.17 ± 2.88 μM. Although LMG and CV appeared to inhibit the enzyme, the apparent Ki values couldn't be calculated since the data suggested that these dyes seem to interact with the enzyme through at least two binding sites. Interaction of the corresponding dyes with equine liver GST was investigated using CDNB and GSH as substrates. MG and LMG were found to inhibit the enzyme noncompetitively with the apparent Ki values of 23.07 ± 7.26 and 30.72 ± 2.60 μM, respectively, with respect of CDNB as substrate. MG and LMG appeared to inhibit liver GST in a linear mixed type manner with the apparent Ki values of 210.00 ± 15.23 and 59.56 ± 3.70 μM, respectively, when GSH was used as substrate. It was concluded that these dyes may bind to an independent binding side besides the substrate binding site on the enzyme and may cause some conformational changes on the enzyme molecule which may further lead the enzyme inactivation. CV had no inhibitory activity on liver GST. These results revealed that MG, LMG and CV appeared as potent inhibitors of catalase and GST, which may be resulted in a significant increase in free radical production and cell damage in humans.

PP-288
Effects of PDTC on colonic anastomoses healing in the cecal ligation and puncture model of sepsis

Z. Teke[1], F. Aytekin[1], C. Yenisey[2], C. Aydin[1], B. Kabay[1], **N. Genc Simsek**[2], S. Sacar[3], K. Tekin[1] and K. Tekin[1]
[1]General Surgery, Pamukkale University, Denizli, Turkey, [2]Biochemistry, Adnan Menderes University, Aydin, Turkey, [3]Clinical Microbiology and Infection Diseases, Pamukkale University, Denizli, Turkey. E-mail: ngencsimsek@adu.edu.tr

Pyrrolidine dithiocarbamate (PDTC) is thiol antioxidant and potent inhibitor of nuclear factor-kappaB activation. We aimed to investigate the effects of PDTC on healing of colonic anastomoses in the presence of intraperitoneal sepsis. Anastomosis of the left colon was performed on the following day of cecal ligation and puncture (CLP) in 30 rats that were divided into three groups (n = 10): sham-operated control (laparotomy and cecal mobilization, I); cecal ligation and puncture (CLP)(II); PDTC-treated group (100 mg/kg i.v. before the construction of colonic anastomosis, III). On postoperative day 6, all animals were sacrificed and anastomotic bursting pressures measured *in vivo*. In tissue samples, anastomotic hydroxyproline (HYP) content, perianastomotic myeloperoxidase (MPO), malondialdehyde (MDA) and glutathione (GSH) levels were determined. There was a statistically significant increase in activity of MPO and MDA levels in the CLP group(II), along with a decrease in GSH levels, anastomotic HYP content and bursting pressure values when compared to controls(I). All of the investigated parameters were normalized in PDTC-treated animals(III). In conclusion, PDTC treatment significantly prevented the delaying effect of CLP-induced i.p. sepsis on anastomotic healing in the colon. Further clinical studies are needed to clarify whether PDTC may be a useful therapeutic agent to increase the safety of the anastomosis during particular operations where sepsis-induced injury occurs.

PP-289
The defensive effects of synthetic organoselenium compounds on the antioxidative system of DMBA induced rat brain

Z. Selamoglu Talas[1], I. Yılmaz[2], I. Ozdemir[2], Y. Gok[2], I. Orun[3] and **B. Cetinkaya**[4]
[1]Biology, Inonu University, Malatya, Turkey, [2]Chemistry, Inonu University, Malatya, Turkey, [3]Biology, Nigde University, Aksaray, Turkey, [4]Chemistry, Ege University, Izmir, Turkey. E-mail: ztalas@inonu.edu.tr

DMBA [7,12-dimethyl (a) antracene] is among the very strong polycyclic aromatic hydrocarbon (PAH), which has the ability to induce the tissue damage and the main cause of tumour formation in rats. It is known that this compound produces the type of reactive oxygen species and increases the oxidative stress in the cell. The reduced form of oxygen, produced by a part of metobolitical period in aerobic organism and also by a normal physiological pathway, is toxic form which oxidizes many biomolecules. Selenium is an element, which has non-enzymatic antioxidative properties physiologically. Synthetic organo-selenium compounds prevent cell damage in the targeted cells by the attack of radical attack and also inhibit the DMBA-DNA adducts formation so that carcinogenesis was inhibited at the first starting period.
In this study, DMBA induced mature Wistar type female albino rats was used to determine the oxidative damage caused by DMBA in rats and the preventative effect of organo selenium

compounds that was prepared in our laboratory and coded as Se I and Se II in order to achieve this phenomena glutathione peroxidase (GSH-Px), superoxide dimutase (SOD), catalase (CAT) glutathione reductase (GR) enzymatic activity beside total glutathione level and MDA level change were closely scrutinized. and as a result, the results found herein were found to be the most promising because, novel organoselenium compounds have effect of decrease in oxidative stress made by DMBA induction.

PP-290
The effects of selenium on rainbow trout liver treated with heavy metals

Z. Selamoglu Talas[1], I. Orun[2], A. Alkan[1], I. Ozdemir[3], K. Erdogan[1] and **I. Yılmaz**[3]
[1]Biology, Inonu University, Malatya, Turkey, [2]Biology, Nigde University, Nigde, Turkey, [3]Chemistry, Inonu University, Malatya, Turkey. E-mail: ztalas@inonu.edu.tr

It was the aim of this work to study the effect of Cr, Cd and Se metals on biochemical parameters in liver tissue of Oncorhynchus mykiss. Then the effect of such metals was researched to determine the effect of various heavy metals for human health in fish forming a ring of biologic circle and consumed as a protein source.
By rainbow trout were exposed to heavy metal stress (Cd, Cr) at 2 ppm dosage, this study was undertaken to determine the protective effect of selenium treatment at the same dosage (2 ppm) on some biochemical parameters. The activity of catalase (CAT), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and the changes in levels of malondialdehyde (MDA) from biochemical parameters were determined in liver tissue of the groups exposed to heavy metals and a mixture of heavy metal and selenium applied groups. In the result of this study, while the activities of CAT, GSH-Px, SOD in the tissues of fish exposed to the stress of Cr and Cd were significantly lower than the control groups ($P < 0.05$), the closer values to the control groups were found in selenium added groups (Cr + Se, Cd + Se). For the level of MDA, the last production of lipid peroxidation, while the statistically significant increases ($P < 0.05$) were found in the groups exposed to the metal stress, the falls were obtained in selenium applied groups.
The results showed that the negative effects occurred in the biochemical parameters of the applied groups exposed to the toxicity of heavy metal, for statistically, were significantly eliminated ($P < 0.05$) as a result of selenium treatment.

PP-291
Pyrrolidine dithiocarbamate reduces lung injury in mesenteric ischemia-reperfusion

B. Kabay[1], Z. Teke[1], A. Ozden[1], **C. Yenisey**[2], F. Bir[3], T. Bicakci[2] and E. Erdem[1]
[1]General Surgery, Pamukkale University, Denizli, Turkey, [2]Biochemistry, Adnan Menderes University, Aydin, Turkey, [3]Pathology, Pamukkale University, Denizli, Turkey. E-mail: cyen2002@hotmail.com

Objective: To study the effect of anti-oxidant pyrrolidine dithiocarbamate (PTDC) on lung injury induced by mesenteric ischemia.
Methods: Male Wistar albino rats randomized into three groups: sham operated control ($n = 12$), 60 min of mesenteric ischemia by superior mesenteric occlusion and 2 h of reperfusion ($n = 12$) and PDTC (100 mg/kg intravenously) treated group after 30 min of reperfusion ($n = 12$). We assessed the degree of lung tissue destruction biochemically by measuring myeloperoxidase (MPO), malondiealdehyde (MDA), glutathione (GSH) and nitric oxide (NO) activities. Histological assessments were based on neutrophil infiltration and on acute organ injury score in the lung tissue. Pulmonary oedema was evaluated by Evans blue extravasation and lung tissue wet/dry ratio.
Results: PTDC treatment significantly reduced lung damage, which was biochemically associated at the tissue level with reduced MDA and NO levels and, increased GSH levels. Lung neutrophil sequestration was not affected by PDTC treatment, but pulmonary edema and histopathological severity of the lung injury were significantly attenuated by the treatment.
Conclusion: PDTC treatment attenuates lung injury caused by intestinal ischemia-reperfusion.

PP-292
Antioxidant capacity in the brain tissues of Alzheimer's disease experimental rat model

A. Sapcanin[1], I. Tahirovic[1], E. Sofic[1], M. Salkovic-Petrisic[2], Z. Lackovic[2] and P. Riederer[3]
[1]Department of Chemistry, Faculty of Science, University of Sarajevo, Bosnia and Herzegovina, [2]Department of Pharmacology, School of Medicine, University of Zagreb, Croatia, [3]Clinical Neurochemistry, Clinic and Policlinic for Psychiatry, University of Wuerzburg, Germany. E-mail: ida@bih.net.ba

Streptozotocin (STZ) and alloxan (AL) intracerebroventricular (icv) administration produces long-term and progressive deficits in learning, memory and cognitive behaviour in rats, as well as deficits in cerebral glucose and energy metabolism. These changes resemble those found in the brain of patients with the Alzheimer's disease (AD), therefore streptozotocin-icv treated rats have been proposed as an experimental model of sporadic AD. The objective of this study was to evaluate the effects of STZ and AL on the antioxidant capacities of rat brain, frontal cortex (CC) and brainstem-cerebellum (BS-CB) by using the ORAC assay with two different FR radical generators. The initial samples consisted of thirty five male Wistar rats treated with STZ, and AL and controls (C) were used in this study. The protein content in the brain BS-CB tended to increase during the one month treatment with STZ ($P < 0.001$) and during the 3-month treatment with AL ($P < 0.003$) in comparison with brain CC. The antioxidant capacity in the brain BS-CB decreased significantly in comparison with brain CC during the one month treatment with STZ ($P < 0.003$) and during the 3-month treatment with AL ($P < 0.003$). No STZ and AL effect was observed in the antioxidant capacity in the brain CC and in the brain BS-CB in comparison with C.

PP-293
Oxidative stress parameters in patients with slow coronary flow

Y. Enli[1], H. Evrengul[2], M. Turk[1], R. Akbay[1], O. Kuru[2], H. Tanriverdi[2], D. Seleci[2] and A. Kaftan[2]
[1]Department of Biochemistry, Faculty of Medicine, Pamukkale University, Denizli, Turkey, [2]Department of Cardiology, Faculty of Medicine, Pamukkale University, Denizli, Turkey. E-mail: enli20@yahoo.com

Slow coronary flow (SCF) is a phenomenon characterized by delayed opacification of coronary arteries in the absence of epicardial occlusive disease, in which many etiological factors such as microvascular and endothelial dysfunction, and small vessel disease have been implicated. We aimed to investigate the free radical damage in SCF by using oxidative stress parameters. Forty-three patients with angiographically proven SCF (mean age 54.1 ± 10.7 years; 16 females, 27 males) and 44 cases with

normal coronary flow (NCF) pattern (mean age 55.4 ± 10.7 years; 24 females, 20 males) with similar risk profile were enrolled in this study. We measured erythrocyte superoxide dismutase (SOD), erythrocyte reduced glutathione (GSH), serum malondialdehyde (MDA), serum catalase (CAT) and serum myeloperoxidase (MPO) levels. There were statistically significant differences between groups according to the level of SOD, GSH and MDA. MDA (2.09 ± 2.13 versus 1.19 ± 1.19 nmol/ml, $P = 0.018$) and SOD levels (4716 ± 2795 versus 2142 ± 1468 U/g Hb, $P = 0.000$) were found increased in SCF group when compared to NCF group. The level of GSH (7.14 ± 1.94 versus 8.97 ± 3.83 μmol/g Hb, $P = 0.006$) was lower in patients with SCF than in NCF. There were no differences between two groups in CAT (1120 ± 791 versus 1113 ± 685 kU/L, $P = 0.962$) and MPO levels (198 ± 243 versus 220 ± 210 U/L, $P = 0.662$).

These results indicate that free radical damage may play a role in the pathogenesis of SCF.

PP-294
Elevated iron uptake by heart mitochondria by diazoxide and ruthenium red

M. Kim, S. Yoon and E. Song
Department of Life Science, College of Science, Sookmyung Women's University, Seoul, Korea. E-mail: erate@sookmyung.ac.kr

Heart is highly dependent on the mitochondria because continuous generation of ATP by mitochondrial OXPHOS is essential for mechanical function in myocytes. As excessive intracellular iron levels make radicals through Fenton reaction to result in diseases and aging, iron homeostasis has to be tightly controlled for the heart tissue.

We are trying to identify the factors by which iron is actively transported to mitochondria. In our system with isolated heart mitochondria, iron was transported to up to 70% when mitochondria were incubated in the presence of 5 μM $FeSO_4$. The amount of mitochondrial iron was increased by 21%, 37% and 38%, in the presence of ADP, diazoxide and ruthenium red, respectively. Neither ATP nor methylene ADP showed the stimulating effect of ADP. Similarly, atractyloside nullified the ADP effect. In the presence of calcium, iron was hardly taken up by mitochondria, which was consistent with the result of ruthenium red. Likewise, glybenclamide showed the antagonistic action against diazoxide. However, ADP enhanced the stimulating effect of diazoxide, though not additive. These results were dependent on the incubation temperature, pH, phosphate concentration and membrane potential. Similar results were obtained with the primary cardiac myocytes. Accordingly, we suggest that adenine nucleotide translocator, Ca^{2+} uniporter and mitochondrial K_{ATP} channel are involved in mitochondrial iron uptake.

PP-295
Experimental model for the study of effect of ionizing radiations on membrane proteins using a fluorescence method

M. Radu[1], M. Nae[2], D. Gazdaru[2] and A. Acasandrei[1]
[1]Department of Health and Environmental Physics, Horia Hulubei National Institute of Physics and Nuclear Engineering, Bucharest, Romania, [2]Department of Biophysics, Faculty of Physics, University of Bucharest, Bucharest, Romania.
E-mail: mradu@nipne.ro

Results related to the effects of ionizing radiations (gamma rays) on the structure of a membrane model (liposomes doped with gramicidin A) are reported. The liposomes were qualitatively characterized by turbidimetric measurements. The presence of gramicidin A molecule in the lipid bilayer of liposomes was analysed by means of UV and fluorescence spectrometry. The exponentially decrease of the tryptophan emission intensity with respect to increasing doses of irradiation proves the partially damaging of the tryptophan residues from the peptide. The removal of the molecular oxygen and the presence of ethanol prevent at least partially, the decay of tryptophan fluorescence emission, proving the important role of the indirect action of the radiation (water radiolysis). The decrease of the irradiation effect with increasing of the dose-rate (at a constant dose) is a good evidence for the major role of the peroxidation processes in the inactivation of the gramicidin A molecules.

PP-296
Antioxidant capacity in brain tissues of rat model of cerebral diabetes

I. Tahirovic[1], A. Sapcanin[1], I. Gavrankapetanovic[2], E. Sofic[1], M. Salkovic-Petrisic[3], Z. Lackovic[3] and P. Riederer[4]
[1]Faculty of Science, University of Sarajevo, Bosnia and Herzegovina, [2]Faculty of Medicine, University of Sarajevo, Bosnia and Herzegovina, [3]Department of Pharmacology, Faculty of Medicine, University of Zagreb, Croatia, [4]Clinical Neurochemistry, University of Wuerzburg, Germany. E-mail: itah@pmf.unsa.ba

Objectives: Peripheral application of high doses of betacytotoxic drugs e.g. streptozotocin (STZ) produces experimental diabetes mellitus, which is also associated with cognitive deficits and alterations of brain glucose metabolism and could be a disorder termed cerebral diabetes. The objective of this study was to evaluate the effects of STZ and thyoglucose (TG) on the antioxidant capacities of rat brain cerebellum (CB) and brain stem (BS).

Methods: The initial samples consisted of 35 rats treated with STZ, TG and controls (C) were used. Antioxidant capacity in the supernatants was measured using oxygen radical absorbance assay (ORAC) on a Perkin Elmer spectrometer LS 55 with a fluorescent filters (Ex: 485 nm; Em: 520 nm). Hydroxyl (OH) and peroxyl (ROO·) radical generators were used. Fluoresceine was used as a target of free radical attack.

Results: The results showed that ORAC-OH· in HPC of treated rats for 3 months with TG, and TG + STZ decreased significantly in comparison with C. Furthermore, ORAC-ROO·in HPC of treated rats with TG decreased significantly in comparison with C. We found, also that ORAC-OH· in CB and BS of treated rats for 12 weeks with TG, and TG + STZ decreased significantly in comparison with C.

Conclusion: The upregulation of antioxidant systems in the various brain regions (HPC, CB and BS) of treated rats with TG and TG + STZ were found.

PP-297
Endogenous and induced lipid peroxide levels in tissues of taurine-depleted rats

H. Parıldar-Karpuzoğlu[1], J. Balkan-Karabaşoğlu[2], S. Doğru-Abbasoğlu[1], G. Aykaç-Toker[1] and M. Uysal[1]
[1]Department of Biochemistry, Faculty of Medicine, İstanbul University, İstanbul, Turkey, [2]Department of Biochemistry, Medical Faculty, Yedi Tepe University, İstanbul, Turkey.
E-mail: handekarpuzoglu@yahoo.com

We aimed to investigate the effect of decreased taurine levels on endogenous and induced lipid peroxide levels in liver, brain, heart and erythrocytes as well as pro-oxidant and antioxidant balance in the liver of rats administered b-alanine (3%, w/v) in

drinking water for 1 month to decrease taurine levels of tissues. This treatment caused significant decreases in taurine levels of liver (86%), brain (36%) and heart (15%). We found that endogenous and ascorbic acid-, NADPH- and cumene hydroperoxide-induced malondialdehyde (MDA) levels did not change in the liver, brain and heart homogenates following b-alanine treatment. Also, H2O2-induced MDA levels remained unchanged in erythrocytes. In addition, we did not observe any changes in levels of MDA, diene conjugate, glutathione, a-tocopherol, ascorbic acid and the activities of superoxide dismutase, glutathione peroxidase and glutathione transferase in the liver. According to this, buffering or sequestering capacity of tissues to exogenous stimuli was not influenced by reduced taurine levels in tissues of rats.

PP-298
The effect of zinc sulphate on ethanol–induced damage in rat liver

O. Mertoglu Caglar[1], A. Bilgihan[1], U. Ozel Turkcu[1], G. Biberoglu[2] and G. Take[3]
[1]*Department of Medical Biochemistry, Gazi University, Ankara, Turkey,* [2]*Department of Paediatrics, Division of Pediatric Metabolism and Nutrition, Gazi University, Ankara, Turkey,* [3]*Department of Histology, Medical School, Gazi University, Ankara, Turkey. E-mail: caglaroznur@gmail.com*

Liver is more vulnerable to ethanol (E)-induced damage than any other organ in the body, because E is metabolized mainly in the liver. Zinc (Zn) is an essential trace element with antioxidant properties. The aim of this study was to investigate the protective role of Zn against E induced oxidative damage in the rat livers. Male Wistar rats, weighing 250–300 g, were divided into four groups and maintained for 13 days as follows: control group ($n = 10$) was injected intraperitoneally (i.p.) with 0.9% saline; E group, ($n = 10$) with 2 g/kg/day E; Zn group ($n = 10$) received 7 mg/kg/day oral zinc sulphate; and E-Zn group ($n = 9$) received Zn (oral) and E (i.p.). On the 14th day, rats were sacrificed. Liver tissues were taken for determination of malondialdehyde (MDA), advanced oxidation protein products (AOPP), Zn levels, glutathione peroxidase (GSH-Px) activities and histopathological analysis. E caused statistically significant increases in the levels of MDA, GSH-Px and a decrease in AOPP levels when compared with the control group ($P < 0.005$, $P < 0.001$, $P < 0.005$, respectively). In the E-Zn group MDA levels and GSH-Px activities decreased with respect to the E group ($P < 0.05$, $P < 0.001$, respectively). Hepatic Zn levels increased only in the Zn group. Rats, which received Zn, had increased levels of MDA when compared with the control group ($P < 0.005$). Histological findings also revealed that zinc had a protective effect on E induced oxidative damage in the rat livers.

PP-299
Fluctuation of zinc-enzymes in rat brain and salivary glands in relation to zinc status

S. Hayashi
Department of Biochemistry, Kyushu Dental College, Kitakyushu, Japan. E-mail: haya-sue@mail.kyu-dent.ac.jp

D-aspartate-oxidase (D-AspO) catalyses oxidative deamination of D-aspartate to oxaloacetate, hydrogen peroxide and ammonia. D-AspO has been found in many mammal tissues, showing that D-AspO differs from D-amino-acid-oxidase (DAO) and is located in the peroxisomes of mammal livers and kidneys. We aimed to produce and eliminate hydrogen peroxide. Besides zinc (Zn) is an essential element for the normal growth of animals on the mechanisms whereby Zn is transported. Rats were given a diet containing different levels of Zn. We examined copper-zinc-superoxide dismutase (Cu/Zn-SOD), D-AspO and catalase to assess Zn nutrient in the brain and salivary glands. The results indicated that Cu/Zn-SOD in the salivary glands was higher than those in other tissues, and the highest in the sublingual gland. These results suggest that Cu/Zn-SOD was sensitive to Zn supplementation and deprivation in the sublingual and parotid glands. Implications of the present results for hydrogen peroxide metabolism were discussed. In brain stem, Cu/Zn-SOD slightly increased, in high Zn and Zn deficient diet. D-AspO increased in sublingual and parotid glands in high Zn and decreased in submandibular and sublingual glands in deficient Zn diet. In peroxisomes, Cu/Zn-SOD produced active oxygen to be converted to hydrogen peroxide. D-AspO, DAO and urate oxidase also produced hydrogen peroxide to be degraded by catalase. Aging results in the decreased ability to erase active oxygen from the peroxisomes, which can be fatal.

PP-300
P53 gene exon 4 RFLP polymorphism and relation with severity of disease in patients with coronary artery disease

D. U. Cakir[1], B. Yokus[2], S. Tekes[3], K. Iltimur[4], N. Mete[5] and E. Uysal[6]
[1]*Department of Biochemistry, Public Hospital of Diyarbakır, Diyarbakır, Turkey,* [2]*Department of Biochemistry, Faculty of Veterinary, Dicle University, Diyarbakir, Turkey,* [3]*Department of Genetics, School of Medicine, Dicle University, Diyarbakir, Turkey,* [4]*Department of Cardiology, School of Medicine, Dicle University, Diaybakir, Turkey,* [5]*Department of Biochemistry School of Medicine, Dicle University, Diyarbakir, Turkey,* [6]*Department of Biostatistics, High school of Diyarbakir, Dicle University, Diyarbakir, Turkey. E-mail: ducakir@yahoo.com*

It is possible, while major P53 mutations may lead to the development of malignant changes, some DNA variations at the P53 gene may also be associated with atherogenesis. Our goal was to study the connection between P53 gene exon 4 RFLP polymorphism and severity of disease ın patients with CAD.
The 51 patients was seperated into: (MI) (n : 20), ,(UAP) (n : 16), (SAP) (n : 15), and Control (n : 14). The P53 genotype was determined using PCR and RFLP method. P53 genotypes were 11 Arg/Arg, 25 Arg/Pro, and 15 Pro/Pro. In the CAD(+) group, Arg/Pro and Pro/Pro genotypes was observed as in higher rate In SAP, Arg/Pro and Pro/Pro genotypes was determined as equal and in higher rate than Arg/Arg genotype. When working groups compared with respect whether Arg/Arg, Arg/Pro versus Pro/Pro genotype carrierness statistical difference was not observed ($P > 0.05$).
Our results suggest that P53 exon4 polymorphism is not related statistically to the severity of the CAD. We also demonstrated that Arg/Arg genotype in SAP has less of a effect than Arg/Pro versus Pro/Pro genotypes and P53Pro variant may be more efficient than P53Arg at SAP pathology. Increasing Arg/Pro and Pro/Pro genotypes on the patients with SAP has been thought that SAP may be the disease with hereditary origin. The concerning whether P53 gene polymorphisms can be considered as a risk factor, more definite judgements can be set forth in the

involvement of extensive groups for cases and with controlled prospective studies.

PP-301
The role of nitric oxide synthase (eNOS) gene polymorphism in coronary artery diseases and relation with severity of disease

S. Tekes[1], D. U. Cakir[2], B. Yokus[3], N. Mete[4] and K. Iltimur[5]
[1]*Department of Genetic School of Medicine, Dicle University, Diyarbakir, Turkey,* [2]*Department of Biochemistry, Public Hospital of Diyarbakır, Turkey,* [3]*Department of Biochemistry, Faculty of Veterinary, Dicle University, Diyarbakır, Turkey,* [4]*Department of Biochemistry, School of Medicine, Dicle University, Diyarbakir, Turkey,* [5]*Department of Cardiology, School of Medicine, Dicle University, Diyarbakir, Turkey. E-mail: stekes@sur.dicle.edu.tr*

Polymorphisms on eNOS gene are one of the genetic risk factors considered as having important role in formation of coronary artery disease (CAD). Determination of genotype distribution relating to in 4 intron 27 bp polymorphism on eNOS gene in patients with CAD and allel frequencies was investigated.
The 74 patients, diagnosed as having CAD(+) group was seperated as: Myocard infarction (MI) (n:20), Unstable Angina Pectoris (UAP) (n : 18), Stable Angina Pectoris (SAP) (n : 36), and Control (n : 20). When genotypes in eNOS gene 4 intron 27 bp zone in cases examined, bb, ab ve aa genotype frequencies were found as 65%, 35% and 0% in MI, 66.67%, 29.17% and 4.17% in UAP, 52.78%, 47.22% and 0% in SAP, 70%, 25% and 2% in control group respectively. In CAD (+) and CAD(-) cases, it wasn't determined a difference between aa genotype frequencies .In all cases, generally bb genotype was determined as in higher rate independently from sex. When working groups compared with respect whether aa or bb genotype carrierness statistical difference was not observed ($P > 0.05$). In the CAD(+) group, aa + ab genotypes was observed as in higher rate significantly ($P < 0.05$).
In conclusion, increasing ab genotype on the patients with SAP has been thought SAP may be the disease with hereditary origin. eNOS gene 4 intron 27 bp zone b allele carrierness may be genetic marker on the CAD. More definite judgements can be set forth in the involvement of extensive groups for cases and with controlled prospective studies.

PP-302
Evaluation of lung toxicity, oxidant/antioxidant status and erdosteine treatment in rats kept in coal mine ambience

F. Armutcu[1], B. Dogan Gun[2], A. Gurel[1] and R. Altın[3]
[1]*Department of Biochemistry, Zonguldak Karaelmas University School of Medicine, Zonguldak, Turkey,* [2]*Department of Pathology, Zonguldak Karaelmas University School of Medicine, Zonguldak, Turkey,* [3]*Department of Pulmonology, Zonguldak Karaelmas University School of Medicine, Zonguldak, Turkey. E-mail: drferah@yahoo.com*

Occupational exposure to coal dust causes pneumoconiosis and other diseases. Reactive oxygen species have been implicated in the pathogenesis of coal dust-induced lung toxicity. In this experimental study, we investigated the oxidant/antioxidant status, nitric oxide and hydroxyproline levels, and inducible nitric oxide synthase formation in lungs of rats exposed to coal dust in mine ambience. In addition, we also investigated the attenuating effects of erdosteine. At the end of the experiment processes, tissue levels of HP, TBARS and NO, as well as the activities of superoxide dismutase, glutathione peroxidase, catalase, xanthine oxidase myeloperoxidase were evaluated spectrophotometrically, and formation of iNOS by immunohistochemical methods in the lung tissues of rats. Exposure to coal dust resulted in a significant increase in the oxidant parameters and HP level, as compared to the controls. A decrease in activities of antioxidant enzymes, and an increase in MPO activity were found in the study group, compared to the controls. A strong staining for iNOS antibody in lung tissue and increased levels of lung NO was found in the study group that were significantly reduced by erdosteine. Our studies provide evidence that supports the hypothesis for ROS-induced pneumoconiosis. ROS can also be generated by phagocytic cells stimulated by coal dust. Erdosteine may be beneficial in the coal dust-induced lung toxicity via antioxidant and free radical scavenger properties.

PP-303
Effects of carbohydrate-rich drink on gastric juice MDA levels and pH in elective surgery patients

G. Yilmaz[1], F. M. Yilmaz[1], V. Fidancı[1], H. Yıldız[2], S. Günal[2] and **D. Yücel**[1]
[1]*Biochemistry, Ministry of Health Ankara Hospital, Ankara, Turkey,* [2]*Anaesthesia Department, Ministry of Health Ankara Hospital, Ankara, Turkey. E-mail: vildanfidanci@gmail.com*

The approach to the elective surgery patients for intake of clear fluids before the operation has been changed over the last decade. Intake of fluids like water, coffee, tea or fruit juice have been shown to decrease subjective complaints like hunger, weakness, tiredness and preoperative thirst etc. Different preoperative oral fluid protocols have been developed for preoperative use and there are reports indicating the positive effects of these fluid protocols to insulin resistance besides decreasing subjective complaints without effecting the gastric volume and pH. In this study, we aimed to investigate the effects of oral carbohydrate-rich fluid (Nutricia®) to gastric pH and gastric juice MDA levels. The patients (CHG) were scheduled to take 800 ml on the evening before and 400 ml on the morning of the surgery and compared with overnight fasting (FG). A total of 45 patients (29 FG, 16 CHG) were included in the study. Gastric fluid pH values were 1.81 ± 0.30 and 2.08 ± 0.37; MDA concentrations were 0.62 ± 0.18 μmol/l and 0.81 ± 0.59 μmol/l in the FG and CHG, respectively. There was no statistical difference between MDA levels of the groups ($P > 0.05$). Gastric juice pH values were significantly higher in CHG than FG ($P < 0.05$). In conclusion, the increase in pH may be protective for the development of stress ulceration associated with surgery but no significant effect for the lipid peroxidation was observed in this study.

PP-304
The relationship between the enzyme activity, lipid peroxidation and red blood cells deformability in hemizygous and heterozygous glucose-6-phosphate dehydrogenase deficient individuals

N. Gurbuz[1], O. Yalcin[2], T. A. Aksu[1] and O. K. Baskurt[2]
[1]*Department of Biochemistry, Akdeniz University, Antalya, Turkey,* [2]*Department of Physiology, Faculty of Medicine, Akdeniz University, Antalya, Turkey. E-mail: ngurbuz@akdeniz.edu.tr*

Abstract Glucose-6-phosphate dehydrogenase (G6PD) activity, red blood cell (RBC) lipid peroxidation and deformability were investigated in hemizygous and heterozygous G6PD deficient

subjects and compared with normal individuals. None of the subjects were in acute haemolytic crises. G6PD activity was assessed based on the spectrophotometric determination of generated NADPH. Lipid peroxidation was measured as thiobarbutiric acid reactive substance(TBARS). RBC deformability was analysed by ektacytometry. RBC lipid peroxidation was found to be significantly higher in hemizygous subjects compared to control and heterozygous subjects, while RBC deformability was found to be significantly impaired. However, although lipid peroxidation was higher than control, RBC deformability was not significantly different from control in heterozygous individuals, characterized by significantly lower RBC G6PD activity. There were no significant correlations between these three parameters when the three groups were analysed separately, but a significant negative correlation was found to exist between G6PD activity and TBARS when the pooled data from the three groups were used for the analysis. This was also true for the relationship between RBC deformability and G6PD activity. It has been concluded that G6PD activity is not a good predictor of oxidative damage resulting in mechanical impairment in heterozygous individuals.

PP-305
Impact of Cu and Fe concentrations on oxidative damage in male infertility

B. Aydemir[1], İ. Onaran[2], A. R. Kızıler[1], B. Alıcı[3] and M. C. Akyolcu[1]
[1]*Departments of Biophysics, Istanbul University, Istanbul, Turkey,* [2]*Medical Biology, Istanbul University, Istanbul, Turkey*
[3]*Cerrahpasa Faculty of Medicine, Urology, Istanbul University, Istanbul, Turkey. E-mail: birsenay2001@yahoo.com*

Oxidative stress in the reproductive system is thought to have an effect on the fertilizing ability of sperm. The purpose of this study was to assess the interaction of iron (Fe) and copper (Cu) ions in suspected subfertile and fertile male groups and to find out the relations of semen parameters (sperm count, motility and abnormal morphology), glutathione (GSH), malondialdehyde (MDA) and reactive oxygen species (ROS) with these variables. Semen and blood obtained from 60 men of subfertile and from 40 fertile volunteers were examined. The sperm count and motility in subfertile male group were found lower than those in fertile male group ($P < 0.001$). Cu levels in serum and seminal plasma in subfertile male group were significantly higher than those in fertile male group ($P < 0.001$, $P < 0.05$, respectively). There was also a significant increase in Fe level of seminal plasma in subfertile male group ($P < 0.001$). However, there was no significant difference in Fe level of serum in subfertile male group. In conclusion, these findings suggest that Cu and Fe may be mediator of the effects of oxidative damage, play an essential role in spermatogenesis and male infertility and the determination of Fe and Cu levels in serum and seminal plasma during infertility investigation is recommended.

PP-306
Oxidant/antioxidant balance in patients with thyroid cancer

B. Çetin[1], F. Kosova[2], M. Akıncı[1], A. Sepici[2], N. Altan[2], S. Aslan[1] and A. Çetin[1]
[1]*Department of General Surgery, Ankara Oncology Hospital, Ankara, Turkey,* [2]*Department of Medical Biochemistry, School of Medicine, Gazi University, Ankara, Turkey.*
E-mail: fundakosova@gmail.com

Reactive oxygen species have been known to play an important role in the initiation and promotion of multistage carcinogenesis. The cellular antioxidant defence plays a crucial role in neoplastic disease. The aim of this study was to compare the antioxidant enzyme activities such as SOD, GSH-Px and MDA in blood samples of thyroid cancer patients compared to healthy controls. The patients presented with multinodular goiter in whose fine needle aspiration revealed malignant cytology. Forty-three patients with thyroid cancer and 43 control subjects were included into this study. Before thyroidectomy SOD activities were not significantly different ($P > 0.05$) but GSH-Px activities were lower and MDA levels($P < 0.05$) were higher than the control group. In post- thyroidectomy, GSH-Px activity($P < 0.05$) was increased and MDA activity($P < 0.05$) was decreased but SOD activity ($P > 0.05$) was not significantly changed compared to prethyroidectomy levels. However, post-thyroidectomy, SOD activity($P > 0.05$) was not significantly different, GSH-Px ($P < 0.05$) levels were significantly higher than the control group. Although MDA levels were significantly decreased, they were still higher than the control group ($P < 0.05$). In conclusion increased antioxidant GSH-Px activity and unchanged SOD activity together with decreased lipid peroxidation product MDA after thyroidectomy may explain one part of pathological processes in the thyroid cancer however, this assumption, require further studies with more patients.

PP-307
Changes of the plasma levels of thromboxane A2 (TXA2) in patients with different stage of renal failure

I. Karamouzis[1], A. Sioulis[2], M. Karamouzis[1], A. Triantos[1], K. Kalaras[3], A. Dimitriadou[1] and D. Grekas[2]
[1]*Biochemical Laboratory, School of Medicine, University of Thessaloniki, Greece,* [2]*First Department of Medicine, University of Thessaloniki, Greece,* [3]*Physiology Laboratory, School of Medicine, University of Thessaloniki, Greece. E-mail: mkaram@med.auth.gr*

Thromboxane A2 (TXA2) is a catabolic product of Arachidonic Acid (A.A.). A.A. released from the cell wall by phospholipase A2, is converted to prostaglandin endoperoxides on the effect of cyclo-oxygenase (endoperoxide synthase). Endoperoxides are then converted to prostaglandins (PGs), prostacyclin (PGI2) and thromboxane A2 (TXA2). Being a potent platelet aggregating and very efficient vasoconstrictors agent, TXA2 is an antagonistic to prostacyclin. It is believed that physiological balance between the two components plays an important regulatory role in the maintenance of normal vascular tone and in pathogenesis of various cardiovascular disorder. Since TXA2 is rapidly converted to thromboxane B2 (TXB2), a chemically stable but biological inactive hydration product, thromboxane synthesis of biological tissues has been monitored by measuring TXB2. The purpose of this study was to evaluate the plasma levels of TXB2 in patients with different degree of Chronic Renal Failure (CRF).

Results: Our measurements are shown that the plasma levels of CRF patients are gradually increase as creatinine clearance is reduced. The maximum levels of TXB2 were in the haemodialysed patient before treatment, (seven times that normal), while after haemodialysis were statistical significantly lower than before.

Conclusion: Plasma levels of TXB2 are a good biochemical index of the morbidity and severity of the patients with CRF.

PP-308
Bioactivation of mitomycin C responsible for formation of 'ROS' and DNA scission by lung cytochrome p450 reductase

H. Çelik and E. Arinç
Biochemistry Graduate Programme, Middle East Technical University, Ankara, Turkey. E-mail: hcelik@metu.edu.tr

Bioreductive anticancer prodrugs are a class of chemical agents that have received considerable interest as anticancer drugs. Mitomycin c is the most widely used bioreductive antitumour agent. It has been used clinically for the treatment of the several different types of cancer including stomach, pancreas, colon, breast, lung and oesophagus. In this study, NADPH-cytochrome P450 reductase purified from sheep lung microsomes in our laboratory was used to determine the ability of mitomycin C to induce single strand breaks in pBR322 plasmid DNA following its reductive activation in the presence of cofactor NADPH. A cell-free agarose gel electrophoresis method was employed to determine the effects of varying concentrations of cytochrome P450 reductase, mitomycin C and incubation time on DNA damage. When only drug and DNA were incubated together, no scission of DNA was observed. Addition of reductase was necessary to accomplish DNA breaks. The extend of single-strand breaks in DNA was found to increase with increasing drug or enzyme concentration as well as with incubation time. DNA damage was quantified by using 'Scion Image' software. The DNA damage was effectively protected by hydroxyl radical scavengers such as DMSO, thiourea and antioxidant enzymes, catalase and SOD. These results show that reactive oxygen species formed via redox cycling in the aerobic environment during reduction of anticancer prodrug mitomycin C by lung cytochrome P450 reductase promote DNA damage.

PP-309
Oxidative stress and susceptibility of LDL to oxidation in renal transplant recipients

F. Bakar[1], A. C. Akbasli[1], F. Aktan[1], K. Keven[2], S. Erturk[2], A. Tuzuner[3], B. Erbay[2] and S. Nebioglu[1]
[1]*Biochemistry, Faculty of Pharmacy, Ankara University, Ankara, Turkey,* [2]*Nephrology, Faculty of Medicine, Ankara University, Ankara, Turkey,* [3]*Surgery, Faculty of Medicine, Ankara University, Ankara, Turkey.*
E-mail: bakar@pharmacy.ankara.edu.tr

Transplantation is a widely used alternative in patients with end stage of renal failure. In short term, renal transplantation may cause more deleterious effect on oxidative stress (OS) due to ischemia reperfusion injury and calcineurin inhibitors (CI). Tacrolimus (FK506)and Cyclosporine (CsA)increase reactive oxygen species and lipid peroxidation; however, there has been no long term detailed data of CI regarding their effect on OS in transplantation. In this study, we aimed to evaluate OS and oxidative susceptibility of LDL in renal transplant(RT) recipients receiving CsA and FK506. The increase of susceptibility of LDL to oxidation supported the increased risk of development of atherosclerosis at the post transplantation term. Hence, 17 RT patients were evaluated. LDL oxidation was initiated with Cu^{2+} solution and oxidation was measured by the change of the absorbance at 234 nm. Lag phase was indicated from the time course graphic. The lag phase reduced 28% at the second month after the renal transplantation. The shorter lag phase shows that LDL is more susceptible to oxidation. LDL oxidizability is related to unsaturated fatty acid composition. So, the fatty acid composition of LDL fraction was measured. It was found that at the second month, the unsaturated fatty acid levels were higher than the pre-transplant term's results.

Acknowledgment: The study was supported by Ankara University Research Foundation (2001-08-03-028), Ankara University Biotechnology Institute (35) and TUBITAK SBAG-AYD-438.

PP-310
Melatonin – an effective antioxidant in patients with chronic renal failure

T. Tchervenkov[1], **B. Galunska-Kaltcheva**[1], D. Paskalev[2], D. Ivanova[1], D. Gerova[1], K. Nenov[2], R. Zorcheva[2] and T. Yankova[1]
[1]*Department Preclinical, Clinical Pharmacology and Biochemistry, Medical University of Varna, Varna, Bulgaria,* [2]*Clinics of Nephrology and Dialysis, Medical University of Varna, Varna, Bulgaria. E-mail: bistra.galunska@gmail.com*

Free radical processes participate in the pathogenesis of chronic renal failure (CRF), which demands a supplementation therapy with antioxidants. Recently preferences are given to physiological antioxidants, such as melatonin (Mel).
The effect of Mel on markers of oxidative processes and antioxidant defence was evaluated in a prospective study of 25 patients with CRF on haemodialysis (HD). All patients received supplementary therapy for 3 months with 'Melatonin Adipharm' (pills, 2 1 mg/d), a single dose before night sleep.
Biochemical parameters were measured monthly before the HD procedure. Plasma total thiols (TT) are often found to be decreased in HD-patients. An elevation of TT level was measured a month after the administration of Mel (from 308 mM to 365 mM) and was persistent during treatment. Mel reduced significantly ($P < 0.001$) in a time-dependent mode the serum malonedialdehyde levels indicating the reduction of lipid peroxidation processes: from 5.47 mM before treatment to 2.79 mM at the 90th day. Hydroperoxide content was measured to be at the upper reference limit (250–300 Carr U) indicating increased oxidative load in those patients. No effect of Mel on total antioxidant capacity (TAC) values was found. In conclusion, Mel could be considered an effective antioxidant in patients with CRF.

PP-311
Protective effect of lycopene on adriamycin-induced cardiotoxicity and nephrotoxicity

S. Yilmaz[1], A. Atessahin[2], E. Sahna[3] and I. Karahan[2]
[1]*Department of Biochemistry, Faculty of Veterinary, Firat University, Elazığ, Türkiye,* [2]*Department of Pharmacology and Toxicology, Faculty of Veterinary, Firat University, Elazığ, Türkiye,* [3]*Department of Pharmacology, Faculty of Medicine, Firat University, Elazığ, Türkiye.*
E-mail: sevyilars@yahoo.com, aatessahin@hotmail.com, esahna@firat.edu.tr, izzetkarahan@hotmail.com

The aim of this study was to investigate the possible protective role of lycopene on adriamycin (ADR)-induced heart and kidney toxicity. Rats were randomly divided into four groups. The first group received no medication and was regarded as the control group; the second group was injected with a single dose of ADR; the third group was treated with lycopene for 10 days before ADR injection and the last group was treated with lycopene for 2 days before and for 3 days after the administration of a single dose of ADR. ADR (10 mg/kg) was intraperitoneally injected as a single dose and lycopene (4 mg/kg) was administered in corn oil by gavage.
The levels of malondialdehyde (MDA) and reduced glutathione (GSH) in both the heart and kidney were higher in the group treated with ADR alone than in the control group, and were lower in the groups administered with lycopene than in the ADR alone group. Although the activity of catalase (CAT) in the heart was higher in the ADR alone group than in the control group, it

was lower in the kidneys. In particular, treatment with lycopene post-injection normalized both cardiac and kidney CAT activities. In heart and kidney tissues, glutathione peroxidase (GSH-Px) activities were not significantly different between all groups. In conclusion, this study clearly indicated that ADR treatment markedly impaired cardiac and renal function and that treatment with lycopene might prevent this toxicity in rats.

PP-312
The effect of methionine supplementation on hepatotoxicity and prooxidant-antioxidant status in the liver of chronically ethanol treated rats

S. Yalçınkaya, Y. Ünlüçerçi and M. Uysal
İstanbul Faculty of Medicine, Department of Biochemistry, İstanbul University, İstanbul, Turkey.
E-mail: syalcinkaya74@yahoo.com

The purpose of this study was to investigate whether high methionine diet may contribute to the development ethanol-induced hepatotoxicity and prooxidant-antioxidant balance in the liver. Rats were divided into four groups; (a) Control group (C): Rats were fed a normal commercial diet; (b) Ethanol group (E): Rats were received the drinking water containing 20% ethanol (v/v); (c) High methionine group (HM): Rats were fed a normal commercial diet supplemented with 2% L-methionine (w/w) and (d) Ethanol plus high methionine-treated group (E + HM): Rats received drinking water containing ethanol (20% v/v) and methionine supplemented diet (2%; w/w). These treatments lasted for 75 days. Although prooxidant-antioxidant balance did not change in the liver of rats in HM group, E + HM diet caused further increases in plasma aspartate transaminase activities and hepatic protein carbonyl levels as compared to E group. Plasma homocysteine levels were found to be increased in HM and E groups, but not in E + HM group as compared to C group. Although glutathione levels remained unchanged in the liver, superoxide dismutase, glutathione peroxidase and glutathione transferase activities were observed to decrease in the E + HM groups as compared to E group. In conclusion, our results show that HM diet may augment hepatotoxicity and oxidative stress in the liver of chronically ethanol treated rats.

PP-313
Total antioxidant capacity of patients during coronary artery bypass surgery

Y. Atay[1], A. Atay[2], F. Isleten[2], M. H. Koseoglu[2], C. Engin[1] and T. Yagdi[1]
[1]Department of Cardiovascular Surgery, Ege University Medical Faculty, Bornova, Izmir, Turkey, [2]Department of Biochemistry and Clinical Biochemistry, Ataturk Training and Research Hospital, Basın Sitesi, Izmir, Turkey. E-mail: yuksel.atay@ege.edu.tr

Objective: Production of reactive oxygen species has a deleterious effect on the endogenous antioxidant defence system. Cardiac surgery may lead to generate formation of oxidation products during ischemia and reperfusion. The changes of total antioxidant capacity (TAC) following coronary artery bypass grafting (CABG) in correlations to uric acid, bilirubin and albumin levels of blood plasma were investigated in this study.
Methods: We studied 40 patients undergoing CABG, using the cardiopulmonary bypass (CPB) procedure. Blood samples were withdrawn from patients at four time intervals to measure TAC concentrations of plasma. The total antioxidant status of the plasma was measured using a colorimetric method developed by Erel.
Results: TAC levels were decreased significantly ($P = 0.0001$) at first hour after the CPB. We have seen an increase of TAC at 24 h and a decrease at 72 h after the operation. TAC changes were positively related to the albumin and bilirubin levels in blood samples collected at 24 h after the operation ($r = 0.404$, $p = 0.01$ and $r = 0.541$, $p = 0.0001$ respectively).
Conclusions: CPB induces systemic inflammatory response associated with severe oxidative stress. This response may be related with TAC and plasma antioxidants such as albumin and bilirubin. Albumin and bilirubin levels, which were correlated with the TAC levels may be taken into account when measuring TAC.

PP-314
Total antioxidant status of patients after on-pump and off-pump congenital heart surgery

Y. Atay[1], A. Atay[2], F. Isleten[2], M. H. Koseoglu[2], O. Oguz[1] and E. A. Alayunt[1]
[1]Department of Cardiovascular Surgery, Ege University Medical Faculty, Bornova, Izmir, Turkey, [2]Department of Biochemistry and Clinical Biochemistry, Ataturk Training and Research Hospital, Basın Sitesi, Izmir, Turkey. E-mail: yuksel.atay@ege.edu.tr

Objective: Congenital heart surgery may activate generation of free radical products during both on-pump and off-pump surgery. Oxidative stress occurs when free radical generation exceeds the human antioxidant defence mechanisms. Total antioxidant capacity (TAC) were investigated in patients treated with cardiac surgery using both on-pump and off-pump techniques.
Methods: Thirty-five patients with congenital heart defects were divided in two groups: Group I ($n = 20$)operated with cardiopulmonary bypass (CPB, on-pump), group II ($n = 15$)without CPB (off-pump). Blood samples were withdrawn before operation and 1, 24 and 72 h after surgery. Measured biochemical parameters were plasma levels of TAC, albumin, bilirubin, uric acid. TAC was determined using the method developed by Erel.
Results: There was a significant decrease($P = 0.04$) in TAC levels at first hour after surgery in group I. TAC levels were markedly increased (more than preoperative level) at 24 h and decreased back to the preoperative level at 72 h after the operation. In contrast to group I, TAC levels were significantly increased ($P = 0.009$) at first hour and remained high at 24 and 72 h after surgery in group II. TAC levels were positively related to the albumin levels at 24 h ($r = 0.67$, $P = 0.01$) in group I.
Conclusions: Antioxidant response markedly changed in both groups of patients. Antioxidant capacity may be suppressed by on-pump surgery at first hour. In contrast to on-pump surgery TAC levels increased at all times in the patients operated without CPB.

PP-315
Neuronal glutamate transporter functions as cysteine transporter in primary cortical cultured neurons

T. Toshiyuki[1] and M. Masamichi[2]
[1]Faculty of Pharmacy, Department of Toxicology and Pharamacology, Musashino University, Tokyo, Japan [2]Trauma and Critical Care Center, School of Medicine, Teikyo University, Tokyo, Japan. E-mail: himicsi@tmd.ac.jp

Glutathione, a tripeptide composed of glutamate, cysteine and glycine, is one of the most important anitoxidant and, plays an essential role in cellular antioxidative defence. The biosynthesis of glutathione is late-limited by L-cysteine uptake. Here we show that neuronal glutamate transporter EAAT3 plays an essential role in L-cysteine uptake in cultured neuronal cells. We prepared nearly

pure neuronal cell culture and pure astrocyte culture. In these cultures neurons expressed EAAT3 and not EAAT1 or EAAT2. Astrocytes expressed EAAT1 and EAAT2 but did not express EAAT3. In mixed cell cultures, glutamate transporter inhibitors inhibited not only glutamate uptake but also L-cysteine uptake, though these inhibitors did not influence L-cysteine uptake in the pure astrocyte cultures. Increased level of extracellular cysteine enhanced the L-cysteine uptake and intracellular glutathione level, and inhibitors of glutamate transporters inhibited this effect of extracellular cysteine. Antisense oligonucleotide for EAAT3 decreased cysteine uptake and glutatione level in the neurons, and increased neuronal vulnerability against oxidative stress. These facts indicate that neuronal glutamate transporter EAAT3 plays an important role in neuronal cysteine uptake and glutathione synthesis, and that sodium dependent and independent conventional cysteine uptake systems are less active in neuronal cells.

PP-316
Lipid peroxidation and cell injury by peroxisome proliferators in rat hepatocytes

H. Kawano, **M. Kanazawa** and M. Masaharu
Pharmaceutical Sciences, Kobe Gakuin University, Kobe, Japan.
E-mail: kawano@pharm.kobegakuin.ac.jp

Objective: Enhancement of peroxisomal fatty acid beta-oxidation (Px-ox) by peroxisome proliferators (Pxps) accompanied by increase of H2O2 production, which causes DNA damage, followed by tumour formation in rodent liver. Since an increase rate of Px-ox activity is always higher than that of catalase activity by Pxps administration to rats, it was hypothesized that an excess of H2O2 production is a cause of the hepatic injury. In this study redox imbalance system was employed and the relation between cell toxicity and scavenger potency by Pxps was examined in rat hepatocytes.
Methods and results: Hepatocytes from male Wistar rats of 7-week-age were cultured in DMEM with 5% CS. After the culture periods, PBS-washed cells were harvested in a proper solvent according to each biological assay and homogenized. Cells were treated with Nafenopin (Nf), and aminotriazol (AT) for catalase inhibition, buthionine sulfoximine (BSO) for GSH inhibition. Px-ox, cellular TBA, LDH in medium was determined, and DNA damage was judged by microscopically counting the cells stained with Hoechst 33342-PI method. LDH releasing was not much different in each treatment. TBARS were increased to 1.3, 1.3, 1.8 fold in Nf-cell, AT-cell, BSO-cell, respectively. The number of DNA damaged cells was not significantly different between control and Nf-cells. It was suggested that intracellular membrane lipid moieties but not nuclei were injured by Nf, and GSH might contribute to reduce H2O2.

PP-317
Effect of ^{60}Co gamma irradiation on the lipid peroxidation and ultrastructure of rat gastrocnemius muscle

S. Yalin[1], N. Yilmaz[2], S. Aslantas[4], U. Comelekoglu[3], M. Berkoz[1], V. Guler[1], P. Eroglu[1], S. Oztuna[2], B. Coskun[2] and I. Oguz[2]
[1]*Pharmacy Faculty, Biochemistry Department, Mersin University, Mersin, Turkey,* [2]*Embryology-Histology Department, Mersin University, Mersin, Turkey,* [3]*Medical Faculty, Biophysics Department, Mersin University, Mersin, Turkey,* [4]*Radiology Department, Numune Hospital, Adana, Turkey.*
E-mail: syalin01@hotmail.com

In this study, the effect of ^{60}Co gamma irradiation has been investigated on the lipid peroxidation and ultrastructure of the rat gastrocnemius muscle. Seventeen male Wistar albino rats were divided into two groups as control ($n = 7$) and study ($n = 10$) groups. Study group rats were exposed to a single 10 Gy dose of ^{60}Co gamma rays. The irradiation was performed by the ^{60}Co radiotherapy system. Ten days after irradiation, the animals were sacrificed and gastrocnemius muscle was isolated to examine the biochemical and electron microscopic changes. Level of malondialdehyde (MDA) and catalase activity in the gastrocnemius muscle of rats was measured by using the biochemical methods. MDA concentration and catalase activity in irradiated group were significantly higher ($P < 0.05$) than control group. Ultrastructurally, we observed dilatation in the sarcoplasmic reticulum of myocites. It was shown that strong gamma irradiation with a dose of 10 Gy lead to disturbance of structure and function of sarcoplazmic reticulum.

PP-318
Ligamentum flavum hypertrophy in lomber spinal stenozis and TGF-beta1

D. Gokpinar[1], S. Yaycioglu[1], C. Yenisey[2] and M. Yilmaz[2]
[1]*Neurosurgery, Adnan Menderes University, Aydin, Turkey,* [2]*Biochemistry, Adnan Menderes University, Aydin, Turkey.*
E-mail: drmustafa09@yahoo.com

The seemingly pivotal role of TGF-beta1 in various conditions in hyperthrophy of the ligamentum flavum (LF) of the lumbar spinal stenosis might be driven by the elevated of this potent cytokine at the sites of stenosis. In order to prove the validity of this hypothesis, TGF-beta1 has been measured in LF taken from patients with lumbar disk herniation ($n = 18$), lumbar spinal canal stenosis ($n = 21$) and with lumbar spinal spondylolisthesis surgery ($n = 7$). Also, thickness of LF has been determined by using the averages of lumbar MRI and tissue thickness measurement. Ligamentum flavum thickness taken from each three groups results have been found as in HNP 3.46 ± 1 mm, in spondylolysthesis 4.67 ± 1.29 mm and in lumbar spinal stenosis 5.63 ± 1.35 mm. Statistically, the differences are significant ($P = 0.000$) and this differences have been caused by HNP with lumbar spinal stenosis case groups. TGF-beta1 group averages with standard deviations are (1676.47 ± 642 pg/g wet tissue) in HNP, (1661.45 ± 1004.54 pg/g wet tissue) in spondylolystesis, and (6816.68 ± 5147.57 pg/g wet tissue) in lumbar spinal stenosis. The differences were statistically significant ($P = 0.000$) and it has been found that these differences have caused of spinal stenosis. Consequently, we have demonstrated that in lumbar spinal stenosis TGF-beta1, has an effect on ligamentum flavum hypertrophy on the other hand mechanic stress and instability hasn't got any efficient effect on ligamentum flavum hypertrophy.

PP-319
The antioxidant status in rheumatoid arthritis and osteoarthritis

A. Alturfan[1], E. Uslu[1], E. Kokoglu[1], G. Hatemi[2] and I. Fresko[2]
[1]*Cerrahpasa Medical Faculty, Department of Biochemistry, Istanbul, Turkey,* [2]*Department of Internal Medicine, Division of Rheumatology, Istanbul, Turkey. E-mail: aaalturfan@ttnet.net.tr*

Elevated protein oxidation in inflamed joints and impaired antioxidant system have been shown in Rheumatoid arthritis (RA) and Osteoarthritis (OA). In this study, we aim to elucidate the plasma protein oxidation/antioxidant status in patients with OA and RA. Protein oxidation was assessed by the levels of protein carbonyls (PC) and total thiol (TT) levels where as the antioxidant status in plasma was determined by evaluating the levels of superoxide dismutase (SOD), reduced glutathione (rGSH) and

total antioxidant status (TAS). Furthermore, serum sialic acid (SA) concentrations which is potentionally useful but non- specific disease marker has also been assessed. In OA, plasma TAS, SOD ($P < 0.05$) significantly decreased where as (PC) ($P < 0.05$) significantly increased, furthermore serum SA, plasma SH and rGSH ($P > 0.05$) levels insignificantly decreased, conversely total protein levels ($P > 0.05$) insignificantly increased when compared with the controls. However in RA, plasma SOD ($P < 0.05$) levels significantly decreased, in contrast PC, TP, SA and TT levels ($P > 0.05$) insignificantly increased. Consequently, our findings demonstrate that oxidation of proteins and depletion in antioxidant status in plasma is common in OA and RA patients. Furthermore, we suppose that therapeutic use of antioxidants in some diseases may be beneficial in this regard.

PP-320
Effects of magnetic field to lipid peroxides and antioxidant activity in diabetic rats

E. D. Yenilmez[1], A. Pelit[2], D. Duzgunce[1], E. Ozkarali[1], A. Tuli[1] and I. Günay[2]
[1]*Biochemistry Department,* [2]*Department of Biophysics, Cukurova University Faculty of Medicine, Adana, Turkey.*
E-mail: edundar@cu.edu.tr

Diabetes is one of the leading causes of morbidity and mortality all over the world. The disease especially changes the lipid peroxidations and makes differences in lipid profiles. An increased oxidative stress has been observed in diabetic patients as indicated by high free radical production. Magnetic Field (MF) is one of the most common environmental factors that influence living systems and alters free radical production of cells. MF may affect biological systems by increasing life span of free radicals. To prove this hypothesis, we have investigated whether 50 Hz MF induce lipid peroxidative stress in Wistar rat skeletal muscle or not. Rats were divided into two groups as diabetic and control. Diabetic and control groups were also exposed to 50 Hz MF at 1.5 mT, 30 min per day for 30 days. The levels of malondialdehyde (MDA), marker for lipid peroxidative stress and superoxide dismutase (SOD), major antioxidant enzyme were examined in all of these groups. In conclusion, the muscle MDA levels increased significantly in diabetic group with exposure to MF (0.012 mmol/l) than the diabetic group which is not exposed (0.005 mmol/l) and the control groups were also the similar ($P < 0.05$). The SOD levels of control group that exposed to MF were higher than those were not exposed. In diabetic rats, SOD levels were 15 U/mg protein and 3.49 U/mg protein in exposed and nonexposed groups, respectively. These results were found to be statistically significant ($P < 0.05$).

PP-321
Effects of exogenous stress on UCP2 level and apoptosis in neonatal rat cardiomyocytes

M. Modriansky[1], J. Psotova[1], K. Smolkova[2], **J. Santorova**[2] and P. Jezek[2]
[1]*Medical chemistry and biochemistry, Palacky University, Olomouc, Czech Republic,* [2]*Institute of physiology, Czech academy of science, Prague, Czech Republic. E-mail: oregon23@email.cz*

Mitochondrial uncoupling proteins, especially UCP2, are considered necessary components of ischemia tolerance and cellular antioxidant defence program. UCP2 expression increases in mitochondria from liver, lung, and kidney following lipopolysaccharide (LPS) challenge in adult rats. We observed no change in UCP2 mRNA and protein in adult rat hearts after LPS treatment (UCP3 was increasing). To study this phenomenon we exposed primary cell cultures of neonatal rat cardiomyocytes to TNFα, superoxide, H2O2, valinomycin, CCCP, and A23187. We assessed UCP2 protein levels, mitochondrial membrane potential, and cell viability using *in situ* fluorescent staining and caspase activity. While all stressful stimuli caused variable levels of apoptotic markers, none of them resulted in down-regulation of UCP2. On the contrary, TNFα and surprisingly A23187 induced increase in UCP2 expression by approximately two-fold. Based on these data we conclude that UCP2 is constitutively expressed during early postnatal development irrespective of exogenous stress and its expression can be enhanced on certain stimuli to provide higher antioxidant defence.

Acknowledgment: This research is supported by grants GACR 301/05/0221 and MSM 6198959216.

PP-322
Oxidative stress in cows with prolapsus uteri, caesarean section, and retained placenta

M. Erişir[1], Y. Akar[2], S. Yaralioğlu Gürgöze[3], M. Yüksel[2] and Ö. **Kaynar**[4]
[1]*Department of Biochemistry, Faculty of Veterinary Medicine, University of Firat, Elazig, Turkey,* [2]*Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Firat University, Elazig, Turkey,* [3]*Department of Biochemistry, Faculty of Veterinary Medicine, University of Harran, Urfa, Turkey,* [4]*Department of Biochemistry, Faculty of Veterinary Medicine, University of Atatürk, Erzurum, Turkey.*
E-mail: mineerisir@yahoo.com

The periparturient period is especially critical for health and subsequent performance of dairy cows. The aim of the study was to detect the occurrence of an oxidative stress by the determination of the plasma malondialdehyde (MDA) concentration and the erythrocyte glutathione peroxidase (GSH-Px) and catalase (CAT) activities in cows with prolapsus uteri, caesarean section, and retained placenta. A total of 46 Holstein cows (15 cows with normal parturition, 15 cows with retained placenta, eight cows with prolapsus uteri, eight cows with caesarean section) of the Research and Practice Farm of Firat University were used in this study. CAT activity did not significantly vary in diseased animals ($P > 0.05$), while in cows with prolapsus uteri and caesarean section, plasma MDA concentrations significantly increased ($P < 0.001$), and GSH-Px activity was significantly lowered compared to the control group or to cows with RP ($P < 0.01$). In retained placenta cows, plasma MDA concentrations and GSH-Px activity were not significantly altered although the enzyme activity tended to increase. These results suggest that the antioxidant systems would be impaired and peroxidation reactions accelerated in cows with prolapsus uteri and caesarean section.

PP-323
Protein oxidation and antioxidant status in methimazole induced hypothyroid rats

N. Dariyerli[1], A. Alturfan[2], K. Gumustas[2], E. Aytac[3], E. Zengin[2], M. Aslan[4] and E. Kokoglu[2]
[1]*Department of Physiology, Cerrahpasa Medical Faculty, Istanbul, Turkey,* [2]*Department of Biochemistry, Cerrahpasa Medical Faculty, Istanbul, Turkey,* [3]*Cerrahpasa Medical Faculty, Istanbul, Turkey,* [4]*Department of Microbiology and Clinical Microbiology, Cerrahpasa Medical Faculty, Istanbul, Turkey.*
E-mail: dariyerli@yahoo.com

Recent studies have suggested that hypothyroidism protects rats against oxidative damage. These findings led us to explore the peroxynitrite mediated damage and antioxidant enzyme activities

in methimazole induced rat plasma. In this study, we measured the content of nitrotyrosine (NT), the antioxidant enzyme superoxide dismutase (SOD), the levels of reduced glutathione (rGSH), copper (Cu) and Zinc (Zn) in rat plasma. NT was performed by ELISA technique, the level of SOD and sialic acid were determined spectrophotometrically. Finally, Cu and Zn contents were detected using Atomic Absorption Spectrophotometry. Our results indicate that, the levels of NT, rGSH, Cu, Zn ($P < 0.0001$) diminished significantly where as SOD ($P > 0.0001$) insignificantly in rats with hypothyroidism when compared with the controls. Briefly, our results demonstrate that levels of NT which reflects peroxynitrite mediated oxidative damage in human diseases was apparently diminished. This diminution might root from a defect in the thyroid gland as a consequence of administering methimazole. Thus, low level of secreted thyroxin may reflect low level of nitrated tyrosine residues.

PP-324
Dynamic changes of nitric oxide and oxidative stress in rat heart during ischaemic preconditioning

V. C. Dang, J. B. Youm, N. Kim, M. Warda, W. S. Park, S. Kang, H. Kim, T. Kim and J. Han
Mitochondrial Signalling Laboratory, Department of Physiology and Biophysics, College of Medicine, Cardiovascular and Metabolic Diseases Research Center, Biohealth Products Research Center, Inje University, Busan, Korea. E-mail: phyhanj@inje.ac.kr

The present study attempted to determine the changes of NO and superoxide (SOX) in rat heart during ischaemic preconditioning. Isolated rat heart were perfused by Langendorff system and subjected to 30 min of ischaemic solution then 60 min of reperfusion by normoxic solution, or preconditioned two times 5 min interval with ischaemic solution, 300 μM S-nitroso-N-acetylpenicillamine (SNAP), 0.1 mM S-Methylisothiourea prior to ischemia/reperfusion episodes. eNOS, iNOS expression in whole heart and in epicardium, midcardium, endocardium were detected by Western blotting and immunofluorescence assay. NO and SOX were monitored by mean of fluorescent specific dyes visualized under confocal microscope. Infarction sizes were observed by TTC staining. Nucleus and mitochondrial DNA oxidative damage were detected by comet assay and DNA fragmentation methods. Ischaemic preconditioning and SNAP significantly reduced infarction size. Both eNOS and iNOS were highly expressed in ischaemic preconditioning group but showed high intensity of iNOS in endocardium area. This is the first evidence showed the expression of eNOS, iNOS in epi-mid-endocardium areas and dynamic changes of NO during anoxic preconditioning. The results demonstrated that NO play important role to reduce infraction size induced by ischemia/reperfusion and iNOS may effect the distribution of injury from epicardium to endocardium during ischaemic preconditioning.

PP-325
Redox state of human serum albumin in liver disease

K. Oettl[1], J. Greilberger[1], V. Stadlbauer[2], C. Putz-Bankuti[2], C. Lackner[3] and R Stauber[3]
[1]Institute for Physiological Chemistry, [2]Division of Gastroenterology and Hepatology, [3]Institute of Pathology. E-mail: karl.oettl@meduni-graz.at

Human serum albumin has one cysteine residue, which is not involved in a disulfide bond and comprises the major thiol pool in plasma. There are three major fractions of albumin: Mercaptalbumin (HMA) with a free thiol group, nonmercaptalbumin1 (HNA1) with cysteine or glutathione bound by a disulfide bond and nonmercaptalbumin 2 (HNA2) with cysteine oxidized to sulfenic, sulfinic or sulfonic acid. Functional disturbances and changes of the fractions of albumin (fHMA, fHNA1, fHNA2) have been described in patients with advanced liver disease.
We have analysed albumin fractions by HPLC in patients with acute-on-chronic liver failure (ACLF) and liver cirrhosis without liver failure (CIR) in comparison to a group of healthy subjects. Patients with ACLF were followed during albumin dialysis by the Molecular Adsorbent Recirculating System (MARS).
While fHNA2 was essentially the same in control and CIR subjects ($3.1 \pm 0.5\%$ and $5.1 \pm 3.3\%$, respectively) it was dramatically increased in ACLF patients ($17.2 \pm 5.4\%$; $P > 0.0001$). fHNA1 was significantly increased in both patient groups compared to controls. During a total of 43 albumin dialysis treatments, fHNA1 was found to be slightly decreased ($P = 0.015$). There is a dramatic and significant shift in serum albumin towards the more oxidized state in liver diseases. Albumin dialysis treatment may only slightly improve this disturbed redox state.

PP-326
Effects of melatonin and vitamin E on oxidative-antioxidative status in rats exposed to irradiation

E. Yilmaz[1] and **S. Yilmaz**[2]
[1]Department of Orthopaedics and Traumatology, College of Medicine, Firat University, Elazığ, Türkiye, [2]Department of Biochemistry, Faculty of Veterinary, Firat University, Elazığ, Türkiye. E-mail: yilmazerh@yahoo.com

In this study, the effects of treatment with vitamin E and melatonin and irradiation-induced lipid peroxidation and its association with antioxidant enzymes in the total bone (bone and bone marrow) and skeletal muscle of rats subjected to total body irradiation was investigated. Rats were i.p. treated with 100 mg/kg vitamin E or melatonin before exposure to 720 cGy irradiation. Control, irradiation, vitamin E plus irradiation, melatonin plus irradiation groups were sacrificed on the 10th day after irradiation exposure. Application of total body irradiation elevated malondialdehyde (MDA) levels in rat skeletal muscle ($P < 0.001$), but glutathione peroxidase (GSH-Px) and catalase (CAT) activities remained unchanged. Application of vitamin E with irradiation or melatonin decreased the MDA levels in skeletal muscle ($P < 0.01$), but did not affect the GSH-Px and CAT activity. MDA levels were found elevated in total bone, GSH-Px activity decreased ($P < 0.001$) and CAT activity remained unchanged in the group treated with irradiation. Application of vitamin E with irradiation increased the GSH-Px activity in total bone ($P < 0.01$), but the activity of MDA and CAT remained unchanged. Treatment of the animals with melatonin concurrent with irradiation reduced MDA levels and elevation in antioxidant enzymes in total bone ($P < 0.01$). We conclude that melatonin may protect the total bone from the damaging effects of irradiation exposure, and its actions protect total bone from oxidative stress.

PP-327
Possible role of paraoxonase in exercise induced oxidative stress in kidney tissues of pregnant rats

N. Karagenç[1], S. Turgut[2] and G Turgut[2]
[1]*Department of Medical Biology and Genetics, School of Medicine, Pamukkale University, Denizli, Turkey,* [2]*Department of Physiology, School of Medicine, Pamukkale University, Denizli, Turkey. E-mail: nkaragenc@hotmail.com*

Effects of exercise on tissue paraoxonase activity and oxidation status investigated in pregnant rats. Paraoxonase (PON) consists of three isoenzymes named PON1, PON2, and PON3. PON1 and PON3 are synthesized in liver and transported to serum and they participate in HDL structure whereas PON2 remains in cytoplasm and expressed throughout body and exerts antioxidant activity. Fourteen female rats, eight control and six threadmill exercised, used in this study. In liver and kidney tissue homogenates, malonedialdehyde (MDA) levels, PON activity and protein levels were studied. Daily exercise caused a slight increase in kidney MDA levels (from 1.36 ± 0.27 to 1.8 ± 0.57 nmol MDA) Liver MDA levels contraversially reduced following daily exercise (from 0.63 ± 0.32 to 0.37 ± 0.09 nmol MDA). A significant decrease was observed in kidney paraoxonase(from 0.93 ± 0.29 to 0.38 ± 0.1 U) and arylesterase activity (from 0.94 ± 0.43 to 0.52 ± 0.21 U). In liver only arylesterase activity reduced following daily exercise while no changes was observed in paraoxonase activity of the enzyme. Results suggest that, lower PON activity may be responsible for increased oxidation while unchanged PON activity in liver may have protective effects against oxidation. Therefore, isoenzymes in different tissues might exert specific functions in regulation of oxidative stress.

PP-328
Effect of hydrogen peroxide on nonenzymatic glycation of haemoglobin

M. Can[1], S. Acikgoz[1], G. Mungan[1], E. Kocak[2], M. Ataymen[1], E. Ugurbas[1] and S. Demirtas[3]
[1]*Department of Biochemistry, Faculty of Medicine, Karaelmas University, Zonguldak, Turkey,* [2]*Department of Internal Medicine, Faculty of Medicine, Karaelmas University, Zonguldak, Turkey,* [3]*Department of Biochemistry, Faculty of Medicine, Ufuk University, Ankara, Turkey. E-mail: drcanmurat@yahoo.com*

Complications in patients with type 2 diabetes have been linked to oxidative stress and impaired antioxidant defence. Therefore, we wanted to investigate the alterations in glycated haemoglobin levels (HbA1c) in the erythrocytes incubated *in vitro* with hydrogen peroxide in patients with type 2 diabetes.

Red blood cells collected from diabetic patients (n = 10) were treated with 50 mmol/l glucose with or without 50 and 250 mmol/l hydrogen peroxide at 37°C for two days. At the end of the incubation HbA1c percentages were measured from haemolyzed blood samples by a latex enhanced turbidimetric immunoassay on a Roche Integra 800 Analyser.

There was an increase in all HbA1c values treated with glucose alone. A low HbA1c value was obtained in erythrocyte treated with both 50 and 250 mmol/l hydrogen peroxide with glucose when compared with erythrocyte treated with glucose alone. A significant difference was obtained between HbA1c values in erythrocyte treated with 50 and 250 mmol/l at 50 mmol/l glucose concentrations.

In our study we have shown that hydrogen peroxide decreased HbA1c formation in erythrocyte isolated from blood of diabetic patients. These finding suggest that hydrogen peroxide induced structural and functional modifications of haemoglobin may thus have important effects on nonenzymatic glycation of haemoglobin. However further studies are necessary to define how hydrogen peroxide induced modifications can occur in haemoglobin.

PP-329
Valuation of serum purine catabolism enzymes and nitric oxide level in the patients with recurrent aphthous ulceration

A. Gurel[1], C. Altinyazar[2], F. Armutcu[1], M. Unalacak[3], R. Koca[2]
[1]*Department of Biochemistry, Zonguldak Karaelmas University School of Medicine, Zonguldak, Turkey,* [2]*Department of Dermatology, Zonguldak Karaelmas University School of Medicine, Zonguldak, Turkey,* [3]*Department of Family Medicine, Zonguldak Karaelmas University School of Medicine, Zonguldak, Turkey. E-mail: dragurel@yahoo.com*

Recurrent aphthous ulceration (RAU) is the most common oral mucosal disorder. There are three clinical subtypes on the basis of ulcer properties (minor, major, and herpetiform). Although the exact aetiology of RAU remains unknown, local and systemic conditions, and genetic, immunologic, and infectious microbial factors all have been identified as potential aetiopathogenic agents. Xanthine oxidase (XO) and adenosine deaminase (AD) are the most important two enzymes of purine catabolism. While XO catalyses the last reactions of the pathway that causes production of reactive oxygen species, AD plays important role in the maturation and function of T lymphocytes. Nitric oxide helps to maintain blood pressure by dilating blood vessels, assists the immune system, and is the most common oxidant agent in organism. The aim of our study was to evaluate the change of XO and AD activities and NO level in plasma of RAU patients. A total of 26 patients with minor RAU that recurred at least four times a year for at least 1 year were included in the study. Twenty-two healthy voluntaries were selected to form the control group. There was a remarkable increase in XO and AD activity in patients with RAU compared to control subjects. Statistically significant increase in level of plasma NO was noted in RAU group. As a result, increased XO and AD activities were thought to take role I the pathogenesis of RAU. We suggest to use XO inhibitors, as a new approach, in the treatment of RAU.

PP-330
Oxidative stress parameters in the yeast *Schizosaccharomyces pombe*

M. Pekmez, N. Arda, I. Hamad, C. Kig and G. Temizkan
Molecular Biology and Genetics, Faculty of Science, Istanbul University, Istanbul, Turkey. E-mail: muratpekmez@yahoo.com

The fission yeast *Schizosaccharomyces pombe* is a model organism for studies of eukaryotic cells. In this study, some of the reliable indicators of oxidative stress in *S. pombe* were described. Oxidative stress was evaluated by exposure of the yeast cells to hydrogen peroxide. Hydrogen peroxide was found to induce damages to lipids, lower the reduced glutathione (GSH) level and increase the intracellular oxidation as well as catalase activity. *S. pombe* has also been considered to represent a suitable model system for the elucidation of molecular mechanisms of oxidation/reduction reactions. Data obtained here is expected to constitute a basis for the further studies on redox balance and related processes in eukaryotic cells.

PP-331
Reduced glutathione levels affect the culmination and cell-fate decision in *Dictyostelium discoideum*

C-H. Choi, B-J. Kim, S-Y. Jeong, C-H. Lee, J-S. Kim, S-J. Park, H-S. Yim and **S-O. Kang**
School of Biological Sciences and Institute of Microbiology, Seoul National University, Seoul, South Korea.
E-mail: chchoi93@snu.ac.kr

Glutaredoxins have been known to be glutathione (GSH)-dependent oxidoreductases that participate in the redox regulation of various cellular processes. To understand GSH-dependent redox regulation of development, we examined the role of glutaredoxin 1 (Grx1) in *Dictyostelium discoideum*. Its mRNA was highly accumulated at the mound and the culmination stages. When Grx1-overexpressing cells were developed, they showed the delayed culmination and the reduced expression of prespore and spore marker genes. Interestingly, they had about 1.5-fold higher reduced GSH levels than parental cells and their prolonged migration was repressed by the external oxidants. To confirm the effect of the reduced GSH levels on the culmination, glutathione reductase (Gsr) was also overexpressed. The reduced GSH levels and the phenotype of Gsr-overexpressing cells were similar to those of Grx1-overexpressing cells. In contrast, the knockdown of Gsr by RNA interference resulted in nearly 50% decrease in the reduced GSH levels and the acceleration of culmination. Taken together, these data suggested that the culmination of *Dictyostelium* is controlled by the reduced GSH levels. In addition, the cells having higher reduced GSH levels showed a prestalk tendency in the chimeric slugs with control cells, indicating that a difference in the reduced GSH levels may determine cell fate.

PP-332
Protective effects of Trolox in the yeast *Schizosaccharomyces pombe*

I. Hamad, N. Arda, M. Pekmez, S. Karaer and G. Temizkan
Molecular Biology and Genetics, Faculty of Science, Istanbul University, Istanbul, Turkey. E-mail: ismailhamad@yahoo.com

Reactive oxygen species cause damage to proteins, lipids and nucleic acids, and thereby compromise cell viability. The protective effects of Trolox, a vitamin E analogue, against oxidative damages during normal cellular metabolism in the fission yeast *Schizosaccharomyces pombe* were examined. Trolox treatment was found to enhance the relative viability, decrease intracellular oxidation level, increase catalase activity and effectively suppress the protein carbonyl groups generated during normal cellular metabolism.
This study suggests that Trolox treatment protects *S. pombe* cells from reactive oxygen species produced by normal cellular metabolism. Additionally, results indicate that *S. pombe* is a good model organism for studying intracellular oxidation and oxidative stress in eukaryotic cells.

PP-333
The susceptibility of serum and apo-B containing lipoproteins to copper-induced lipid peroxidation increase in aged rats

B. Depboylu, S. Doğru-Abbasoğlu, G. Aykaç-Toker and M. Uysal
Department of Biochemistry, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey. E-mail: drbilge78@yahoo.com

Increased atherosclerosis is one of the major causes of morbidity and mortality during aging. There is increasing evidence that lipoprotein oxidation is an important primary event in atherogenesis. However, there is inadequate knowledge in the literature about lipoprotein oxidation in aging. Thus, we wanted to investigate baseline and copper-induced lipid peroxidation in apo B-containing lipoproteins [low density lipoproteins plus very low density lipoproteins (LDL + VLDL)] and serum of young (6 months) and old (22 months) rats. For this reason, LDL + VLDL were precipitated from EDTA-plasma by dextrane sulfate and MgCl2. Baseline diene conjugate (DC) and copper-induced oxidation kinetic of LDL + VLDL fraction were estimated. Lag phase and the rate of DC formation were calculated. The susceptibility of serum to copper-induced lipid peroxidation was also determined in young and old rats. Our results clearly indicate that the susceptibility of both LDL + VLDL fraction and whole serum to copper-induced lipid proxidation increased in aged rats.

PP-334
Fibronectin and AOPP in women with pre-eclampsia

H. Ozturk[1], C. Coskun[1], H. Buyukasik[2], F. Basinoglu[1], A. Kural[1], H. Gorgen[2], H. Seval[1], C. Bati[1] and Y. Doventas[1]
[1]Department of Biochemistry, Haseki Education and Research Hospital, Istanbul, Turkey, [2]Department of Gynaecology and Obstrectics, Haseki Education and Research Hospital, Istanbul, Turkey. E-mail: hmyoz@hotmail.com

Objective: Pre-eclampsia is characterized by placental malfunction. The pathological processes are associated with the release of specific molecules. In this study, we measured the maternal plasma fibronectin and AOPP concentrations at 19–25 gestational weeks on fetal pre-eclampsia.
Methods: Both maternal plasma fibronectin concentrations were measured by a nephelometric procedure and AOPP were measured by a spectrophotometric procedure in 70 pregnant women at 19–25 gestational weeks. In addition, we measured AOPP in 23 healthy non pregnant women at the same ages with pregnant women.
Results: (a) In a cohort of 70 pregnant women 19 cases of pre-eclampsia developed and 51 cases were without complication; (b) The plasma fibronectin levels in women with pre-eclampsia were significantly higher than the control group ($P < 0.01$). The plasma AOPP levels had no significant difference between normal and pre-eclamptic pregnant women ($P > 0.05$). According to the comparison of the AOPP levels in non-pregnant women and pregnant women a significant increase had been detected ($P < 0.05$).
Conclusion: The maternal plasma fibronectin may be used as an earlier predictor for screening of pre-eclampsia. We found out that there is a significant difference in AOPP levels between pregnant and non pregnant women. However, there was no difference in AOPP levels between pre-eclamptic and normal pregnant women. Therefore, it may not necessarily important to use AOPP as an earlier predictor for screening of pre-eclampsia.

PP-335
The effect of organosulfur compounds on peroxynitrite-induced cytotoxicity: attenuation or potentiation?

N. Trakranrungsie[1], P. Yatmark[2], K. Kirtikara[3] and **Y. Maneerat**[4]
[1]Faculty of Veterinary Science, Mahidol University, Thailand, [2]Faculty of Pharmaceutical Science, Chulalongkorn University, Thailand, [3]National Center for Genetic Engineering and Biotechnology, Thailand, [4]Faculty of Tropical Medicine, Mahidol University, Thailand. E-mail: vsntk@mahidol.ac.th

The present study was aimed to evaluate the effect of naturally occurring organosulfur compounds namely allyl isothiocyanate

(AITC) and benzyl isothiocyanate (BITC) on peroxynitrite-induced cytotoxicity in milk-derived polymorphonuclear neutrophils (PMNs), utilizing the tetrazolium salt XTT assay to measure cell survival. 3-Morpholinsydnonimine (SIN-1), a peroxynitrite donor, induced the loss of PMNs viability in a concentration-dependent manner with estimated EC50 value of 375 μM. Pre-treatment with AITC or BITC further enhanced cytotoxicity in SIN-1-treated cells; however, AITC or BITC alone at concentration ≤3 μM did not cause the cytotoxic effect as compared to untreated control cells. On the contrary, glutathione (GSH), melatonin and uric acid markedly and concentration dependently attenuated the cytotoxic effect of SIN-1. These data suggest that these organosulfur compounds could render cells more susceptible to injury and death mediated by oxidants.

Acknowledgment: This work was supported by the Commission of Higher Education, Ministry of Education and the Thailand Research Fund.

PP-336
DNA fragmentation, semen quality and seminal plasma nitrite/nitrate levels in oligoasthenoteratozoospermia

M. Yanik[1], T. Irez[2], M. Pekmez[3] and **N. Arda**[3]
[1]*In Vitro* Fertilization Unit, Suleymaniye Maternity Hospital, Eminonu, Istanbul, Turkey, [2]The Cerrahpasa Faculty of Medicine, *In Vitro* Fertilization Unit, Department of Gynecology and Obstetrics, Istanbul University, Istanbul, Turkey, [3]Faculty of Science, Department of Molecular Biology and Genetics, Istanbul, Turkey. E-mail: narda@istanbul.edu.tr

This study aimed to evaluate DNA fragmentation in the spermatozoa of patients with oligoasthenoteratospermia and to correlate it with semen parameters and seminal plasma nitric oxide (NO) concentration. Semen samples from fifty men attending *in vitro* fertilization (IVF) unit were collected. A ¼ part of each sample on volume basis was liquefied, following which an aliquot was removed in order to construct a conventional semen profile. The remaining (3/4) part of each sample was centrifuged and DNA fragmentation rate (%) in pelletted spermatozoa was detected by fluorescence based terminal nick-end labelling (TUNEL) assay. Seminal plasma NO concentration was measured as nitrite/nitrate (nmol/ml) levels. Semen qualities were very poor in oligoasthenoteratospermic individuals, and sperm DNA fragmentation rates as well as seminal plasma nitrite/nitrate levels were found to be significantly higher then those healthy donors. DNA fragmentation rates negatively correlated with sperm concentration and motility, and positively correlated with seminal plasma nitrite/nitrate levels. Multiple regression analysis indicated that motility was also related with these two parameters. We concluded that seminal plasma nitrite/nitrate level could represent sperm DNA fragmentation rate in oligoasthenoteratospermic patients. Thus, nitrite/nitrate assay, which is an easier and cheaper test then the TUNEL test, could be performed routinely in order to predict sperm DNA fragmentation rate.

PP-337
Total antioxidant response and oxidative stress index and relation with severity of disease in coronary artery disease

N. Mete[1], D. U. Cakir[2], B. Yokus[3] and K. Iltimur[4]
[1]*Department of Biochemistry, School of Medicine, Dicle University, Diyarbakir, Turkey,* [2]*Department of Biochemistry, Public Hospital of Diyarbakır, Turkey,* [3]*Department of Biochemistry, Faculty of Veterinary, Dicle University, Diyarbakir, Turkey,* [4]*Department of Cardiology, School of Medicine, Dicle University, Diaybakir, Turkey. E-mail: nuriyemete@dicle.edu.tr*

Since oxidative stress is thought to be causally related to chronic and acute events in atherosclerosis and coronary artery disease, it was aimed in this study to determine the oxidative and antioxidative status in comparison with the disease severity in Coronary Artery Disease (CAD). Unstable angina pectoris (UAP) often leads to acute myocardial infarction (MI); and the reason why UAP is so much more prone to complications than stable angina pectoris (SAP) is presently a subject of intensive research. The study included 55 patients with MI, 20 with UAP, and 18 with SAP. The following parameters were determined: total peroxide (TP), oxidative stress index (OSI) and total antioxidant response (TAR). Patients in SAP group had significantly higher TAR levels than those in MI, $P = 0.010$. No differences were found in TP and OSI values in all three groups. We have concluded that increased TAR levels in the SAP group might have prevented the occurrence of more severe forms of the disease; unstable place formation; thus diminished TAR can be evidenced in patients with MI.

PP-338
Mitochondrial antioxidant enzyme activities in the rat brain and eye tissues under circadian rhythm alterations

M. B. Yerer[1], M. P. Alcolea Delgado[2], F. J. Garcia-Palmer[2], P. R. Salom[2] and S. Aydogan[1]
[1]*Department of Physiology, Faculty of Medicine, University of Erciyes, Kayseri,* [2]*Department of Biochemistry and Molecular Biology, University of Balearic Islands, Mallorca, Spain. E-mail: eczbetul@yahoo.com*

During the normal metabolism, the electron transport chain (ETC) is the main source of reactive oxygen species within the cell, which may lead to decrease in the activities of mitochondrial complexes and the ATP production that at least in part results in the cell death by necrosis or apoptosis. In the last decade a new effect of melatonin on the mitochondrial homeostasis has been discovered although the exact mechanism for this effect remains unknown. The aim of this study is to investigate the role of the melatonin levels via different circadian rhythms in the mitochondrial respiratory chain antioxidant defence system in brain and eye tissues. Fifty Sprague Dawley male rats weighing 200–250 g were used in five groups of different circadian rhythms. The control group was 12/12 h of Light/Dark (L/D) cycle. and different circadian rhythms of 24/0 h L/D, 0/24 h L/D, 16/8 h L/D and 8/16 h L/D cycles were applied to the groups for one week, respectively, in special cages where the duration of the light and the climate can be adjusted. The GSH-Px and SOD activities in the mitochondria of brain and eye homogenates were determined by spectrophotometric micro methods. The plasma melatonin levels were measured by the ELISA kit (IBL, Turkey). The SOD activity in the brain was found significantly lower in the 24/0 h L/D group compared to the control where it has been found to increase significantly by the increase in the dark period

($P < 0.05$) where as the SOD activity was found to be slightly affected in the eye mitochondria. The GSH-Px activity was significantly higher in the 24/0 h L/D group ($P < 0.05$) as well as all the other groups, however the increase in the 0/24 h L/D group more significantly ($P < 0.001$) where the melatonin levels were found to be significantly increased ($P < 0.05$) in plasma. These results were found exactly the similar in the eye mitochondria ($P < 0.05$).

As a result, in the dark cycle where the melatonin levels are found to be the highest in the plasma, both in the brain and eye mitochondria the activity of the GSH-Px were increased revealing even the physiological level changes via circadian rhythms are effective on the mitochondrial antioxidant defence system. Furthermore, these results indicate that melatonin maintains a GSH homeostasis in the isolated mitochondria in the brain and eye homogenates, which clearly identifies the protective effects of the melatonin in the mitochondria against the oxidative damage leading to the aging and cell death by apoptosis.

Acknowledgment: This study was granted by NATO Science Fellowships A2 Programme of TUBITAK.

PP-339
The role of monocyte inflammation and oxidant stress in the aetiopathogenesis of cystic fibrosis

G. Unluguzel[1], S. Unsalan[2], Z. S. Uyan[3], G. Haklar[1] and E. Daglı[3]

[1]Department of Biochemistry, Faculty of Medicine, Marmara University, İstanbul, Turkey, [2]Department of pharmaceutical chemistry, Faculty of Pharmacy, Marmara University, İstanbul, Turkey, [3]Departments of Internal Medicine, Subdepartment of Pulmonary Medicine, Faculty of Medicine, Marmara University, İstanbul, Turkey. E-mail: mdgoksenin@yahoo.com

Cystic fibrosis (CF) is the most common recessively inherited lethal disease of Caucasians. Although the organs affected in CF also include the pancreas, gut, liver and reproductive tract, the clinical picture is dominated by pulmonary involvement, with recurrent cycles of infection leading to inflammation, bronchiectasis etc. The lung disease of cystic fibrosis is associated with a chronic inflammatory reaction and an over abundance of oxidants relative to antioxidants. The aim of this study is to investigate the intensity of oxidative stress and free radical damage that our patients were exposed to and to identify any relationship between inflammatory status and oxidative damage. To evaluate oxidative damage intensity, we measured plasma concentrations of malondialdehyde (MDA) and to evaluate inflammatory status we measured hs-CRP levels and oxidative burst of monocytes of cases. Prepubertal cystic fibrosis cases ($n = 26$, median age 10.7 ± 2.7 years,) and eight bronhciectasis controls (median age 12.6 ± 2.08 years,) were enrolled. Serum MDA measurements were done with HPLC and hs-CRP immunoturbidometrically. Respiratory burst of monocytes were measured by luminol-enhanced chemiluminescence before and after phorbol-myristate acetate (PMA) induction. Monocyte respiratory burst (PMA induction-basal) activations were significantly increased in CF group compared to the bronchectasis cases (5.29 ± 0.84 versus 4.6 ± 0.83 log AUC/ml, $P < 0.01$). Plasma MDA concentrations were also significantly elevated in CF cases. But, hs-CRP measurements were not found out to be different between groups. Monocyte respiratory-based induced oxidant damage is evident in CF and should be attributed to systemic nature of the disease rather than pulmonary pathology.

PP-340
Role of endoplasmic reticulum in acetaminophen-induced liver injury

G. Nagy[1], L. Wunderlich[1], A. Szarka[2], G. Banhegyi[1] and J. Mandl[1]

[1]Endoplasmic Reticulum Research Group, Hungarian Academy of Sciences, Budapest, Hungary, [2]Department of Biochemistry and Food Technology, Budapest University of Technology and Economics, Budapest, Hungary. E-mail: gnagyi@yahoo.com

It is well known that overdosage of acetaminophen (AAP) leads to acute hepatocellular injury. Glutathione depletion and oxidative challenge both have pivotal roles in the pathomechanism. The endoplasmic reticulum (ER) via its phase I and II biotransformational enzymes plays a direct role in metabolizing AAP. This together with the fact that ER functions are sensitive to redox imbalance led us to the presumption that ER-dependent non-mitochondrial apoptotic pathways are initiated upon AAP overdose. We investigated alterations in ER redox status and important markers of ER stress in the early phase of AAP-induced liver injury in mice. Microsomal free reduced glutathione decreased by 90% after AAP administration. AMS labelling of thiol-groups of ERp72, a PDI family oxidoreductase, showed that ERp72 is present only in its oxidized form after AAP-treatment. GADD153 and the ER-associated procaspase-12 were found to be robustly induced 3 h after the treatment. Another ER stress-responsive protein, the ATF6, was activated upon AAP-treatment, as was shown by immunoblot detection of its 50 kDa cleaved fragment. On the contrary, we found no induction in the expression of ER-resident chaperones (protein disulfide isomerase, ERp72, GRP78, GRP94) upon AAP-treatment. Our *in vivo* data suggest that AAP treatment causes redox imbalance in the ER lumen. Certain elements of the ER stress response are then initiated with the dominance of pro-apoptotic factors.

PP-341
Probable antioxidant effects of tryptophan in streptozotocin-diabetic rats

A. Z. Karabay and Z. Buyukbingol

Biochemistry, Ankara University Faculty of Pharmacy, Ankara, Turkey. E-mail: zeynepkarabay@yahoo.com

Oxidative stress, described as a disturbance in the pro-oxidant-antioxidant balance in favour of the pro-oxidant, has been suggested to play an important role in complications of diabetes. In the present study, probable antioxidant effects of tryptophan (Trp) on the oxidative- antioxidative status of liver and kidney tissues affected by diabetes were examined in diabetic rats. Streptozotocin (STZ) was used as diabetogenic agent, diabetes was induced 72 h after intravenous (i.v.) injection of a single 40-mg/kg dose of STZ. Trp treatment was started after 8 weeks of diabetes and continued for 4 weeks. Rats were divided into four groups: control, diabetic, Trp treated control and Trp treated diabetic. Treated rats received 100 mg/kg body weight/per day Trp for 4 weeks. TBARS (thiobarbituric acid reactive substances) and GSH (reduced glutathione) levels and SOD (superoxide dismutase) and CAT (catalase) activities were examined. TBARS levels and SOD and CAT activities in kidney and liver tissues were increased in diabetic rats compared to control rats. Elevated SOD and CAT activities in diabetic liver and kidney tissues were reduced but did not reach to control levels by Trp administration. Trp administration did not reduce the elevated TBARS levels in diabetic tissues to control levels and also did not show significant effect on decreased kidney and liver GSH levels in diabetes. Our results suggest that Trp can partially affect the impaired antioxidant defence system in diabetes.

PP-342
Reactive oxygen species in reflux esophagitis of childhood

G. Haklar[1], G. Unluguzel[1], E. Tutar[2], D. Ertem[2] and E. Pehlivanoglu[2]
[1]*Department of Biochemistry, School of Medicine, Marmara University, Istanbul, Turkey,* [2]*Department Pediatric Gastroenterology, Hepatology and Nutrition, School of Medicine, Marmara University, Istanbul, Turkey.*
E-mail: goncahaklar@marmara.edu.tr

Gastroesophageal reflux(GER)symptoms are diverse during childhood. It has been shown that reactive oxygen species (ROS) are increased during early phases of esophagitis. The aim of this study was to assess the correlation between GER symptoms, histopathologic findings and the levels of ROS in oesophageal mucosa.
In the study, children (n = 50, mean 9.8 ± 3.9 years) who underwent upper gastrointestinal endoscopy were enrolled. These patients were divided into two groups according to the presence of symptoms of GER (27 with and 23 without symptoms). Biopsies were obtained for histopathological examination and for measurement of ROS by chemiluminescence method.
Sixteen children in symptom positive, and 10 children in symptom negative group had endoscopic esophagitis. There was no statistically significant relation between GERD symptom scores and endoscopic esophagitis. Histological esophagitis was found in 37.5% positive group and 34.8% of symptom free group, and no correlation was found between symptoms scores and histopathologic esophagitis. The levels of ROS were not different in symptom positive and negative groups statistically. Similarly, there was no correlation between the levels of ROS and the presence of endoscopic and histopathologic esophagitis in our study group. No correlation was found between GERD symptom scores in children with or without esophagitis. Likewise, no correlation was found between the levels of ROS and histopathologic esophagitis in children.

PP-343
Ischemia-modified albumin and troponin I levels in isoproterenol-induced myocardial infarction in rats

C. Kazezoglu[1], S. Eskiocak[1], S. Süer Gökmen[1], U. Usta[2] and E. Çakir[1]
[1]*Departments of Biochemistry, Trakya University School of Medicine, Edirne, Turkey,* [2]*Departments of Pathology, Trakya University School of Medicine, Edirne, Turkey.*
E-mail: ckazez@hotmail.com

Alteration of human serum albumin by ischemia has recently been evaluated as a serum biomarker of cardiac ischemia. In the literature, we could not encounter any studies, which include determination of ischaemi-modified albumin (IMA) in isoproterenol (ISO)-induced myocardial infarction. The aim of this study is to investigate IMA and Troponin I (TnI) levels in ISO-induced myocardial infarction. The rats were given ISO (150 mg/kg daily, i.p.) for two days. Blood samples were taken before the first dose and end of the second day. The rats were sacrificed at 24 h after the second dose of ISO. Heart tissues were excised and processed immediately for histopathological studies. IMA was measured albumin cobalt binding test. Albumin and TnI were measured by automatically with commercial kit. Wilcoxon two sample test was used to analyse the results. IMA and TnI levels were significantly elevated at 24 h post-infarction ($P < 0.001$). As a result, we can report that serum IMA levels are elevated also in ISO-induced myocardial infarction in rats similar to humans.

PP-344
Protective role of 27 bp deletion polymorphism in intron 4 of eNOS gene in cerebral small-vessel disease

M. Yemisçi[1], **İ. Sinici**[2], H. A. Özkara[2], M. Hayran[3], H. Ay[1], B. Çeltikçi[2], E. Önder[2] and T. Dalkara[1]
[1]*Department of Neurology, Faculty of Medicine, Institute of Neurological Sciences and Psychiatry, Hacettepe University, Ankara, Turkey,* [2]*Department of Biochemistry, Faculty of Medicine, Hacettepe University, Ankara, Turkey,* [3]*Department of Preventive Oncology, Faculty of Medicine, Hacettepe University, Ankara, Turkey. E-mail: isinici@hacettepe.edu.tr*

* M.Y and İ.S. equally contrıbuted to the study.
In cerebral small-vessel disease (SVD) genetic factors may influence endothelial function, such as the polymorphisms of the endothelial nitric oxide synthase (eNOS) gene. By the catalytic function of eNOS, nitric oxide, which is a key mediator of endothelial function is synthesized from L-arginine. We investigated whether the three potential polymorphisms in the eNOS gene (T786C in the promoter region, G894T in exon 7, 27 bp repeat in intron 4) were associated with an increased risk of lacunar infarction (LI). Fifty-one patients with LI and 64 healthy controls were analysed. Genotypes were determined through polymerase chain reaction with or without restriction endonuclease digestions. Genotype distribution was significantly different between patients with LI and controls for 27 bp repeat in intron 4, 27 bp deletion/deletion genotype frequency being 1.7% and 17.2%, respectively (odds ratio, 0.47; 95% CI, 0.26–0.84; P = 0.011). Haplotypes with the deletion polymorphism were significantly higher in controls when compared with the LI group (75% versus 25%, P = 0.001). In conclusion, 27 bp deletion genotype in intron 4 of eNOS gene seems to be protective for isolated lacunar infarction.

PP-345
The effects of antioxidant quercetin on membrane electrical properties in the presence of some heavy metals

A. Popescu, D. Ionescu, M. Dragusin and C. Ganea
Biophysics Department, University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania. E-mail: ancappsc@gmail.com

Quercetin, a flavonoid synthesized by plants and found in various foods, has remarkable properties concerning its interactions with biological mechanisms of protection against pathological circumstances, due to its strong antioxidant properties. The compound has different affinities to biological and artificial membranes, in which it inserts or to which it attaches. Quercetin has a heavy-metal binding activity, which can be explained by the appearance of the flavonoid-metal complex, due to the affinity of the catechol group – the binding unit of the benzene ring, as well as of the ketonic groups near the OH radicals. This might be the mechanism by which quercetin protects the living cell against the free radicals induced by heavy metals intoxication. Our results show that the insertion of quercetin in artificial bilayers, due to its planar structure, leads to the augmentation of lipid bilayer electrical parameters, such as conductance and capacitance, monitorized by the electrophysiological BLM (Black Lipid Membrane) method. We are measuring the variations of these electrical properties of the artificial lipid membranes in the presence or absence of heavy metal ions and in the presence or absence of quercetin. We are interested in obtaining important clues on the mechanisms by which flavonoids and heavy metals interact with the membranes, clues that are essential for understanding the way the same processes develop *in vivo*.

PP-346
Oxidative stress in testicular tissues of rats exposed to cigarette smoke and protective effects of CAPE

Huseyin Ozyurt[1], Hidir Pekmez[2], Suha Parlaktas[3], Ilter Kus[4], Birsen Ozyurt[5], Semsettin Sahin[1] and Mustafa Sarsilmaz[4]
[1]Departments of Biochemistry, School of Medicine, Gaziosmanpasa University, Tokat, Turkey, [2]School of Health Sciences, Firat University, Elazig, Turkey, [3]Departments of Urolog, School of Medicine, Gaziosmanpasa University, Tokat, Turkey, [4]Department of Anatomy, School of Medicine, Firat University, Elazig, Turkey, [5]Departments of Anatomy, School of Medicine, Gaziosmanpasa University, Tokat, Turkey. E-mail: huseyinozyurt@hotmail.com

To show the oxidative stress after cigarette smoke exposure in rat testis and to evaluate the effects of caffeic acid phenethyl ester (CAPE). Twenty-one rats were divided into three groups of seven. Animals in Group I were used as control. Rats in Group II were exposed to cigarette smoke only and rats in Group III were exposed to cigarette smoke and received daily intraperitoneal injections of CAPE. After 60 days all the rats were killed and the levels of NO and anti-oxidant enzymes such as SOD, CAT and GSH-Px and the level of MDA were measured in the testicular tissues of rats. There was a significant increase in CAT and SOD enzymes activities in Group II when compared to the controls, but the levels of both decreased after CAPE administration in Group III. GSH-Px activity was decreased in Group II but CAPE caused an elevation in GSH-Px activity in Group III. The difference between the levels of GSH-Px in Group I and Group II was significant, but the difference between groups II and III was not significant. Elevation of MDA after smoke exposure was significant and CAPE caused a decrease to a level, which was not statistically different to the control group. A significantly increased level of NO after exposure to smoke was reversed by CAPE administration and the difference between NO levels in groups I and III was statistically insignificant. Exposure to cigarette smoke causes changes in the oxidative enzyme levels in rat testis, but CAPE can reverse these harmful effects.

PP-347
The increased genotoxicity following inhibition of the glutathione S-conjugate transport in K562 cells

S. Ozdas[1], I. Onaran[2] and G. Kanigur[2]
[1]College of Engineering, Biology and Chemistry Department, Koc University, Istanbul, Turkiye, [2]Department of Medical Biology, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkiye. E-mail: sozdas@ku.edu.tr

Elimination of the products of xenobiotic metabolism is an important step in cellular detoxification and involves a specific transport system or export pump. ATP-dependent transport of glutathione S-conjugates has previously been demonstrated in a variety of tissues and cell lines, such as He-La, HL 60.The aim of this study is to investigate the cytotoxic and genotoxic effects of the GSH S-Conjugate transport inhibition on K562 cell line. For this purpose, transport in H2O2-pretreated cells was inhibited by S-Hexyl GSH, which is inhibitor of Glutathione S transferase (GST) activity. The different concentrations of Shexyl GSH and different time of culture were used to treat K562 cells. Oxidative stress effects were analysed by measured of the lipid peroxidation products, MDA and 4 HNE. Genotoxic effects of S-Hexyl GSH was analysed by cellular DNA fragmentation by ELISA and Comet Assay and cell morphology was also detected. Although the activity of GST increased in H2O2 treated K562 cells whereas decreased after S-Hexyl GSH treatment. After incubation of the S-Hexyl GSH, the mean levels of MDA and 4-HNE in cells was significantly higher than those H2O2 treated K562 cells. After GSH S-Conjugate transport inhibition, it was found that cellular DNA fragmentation and tail factor % were significantly increased in K562 cell line. Taken together, our data suggest that the inhibition of GSH transport may increase the cell cytotoxicity in relation to DNA damage and apoptosis induction in K562 cells.

PP-348
Effect of taurine on mesenteric blood flow decrease and organ injury in septic shock

A. Erdem[1], A. M. Sevgili[1], F. Akbiyik[2], A. B. Iskit[3], Z. D. Balkanci[1] and M. O. Guc[3]
[1]Physiology, Hacettepe University Faculty of Medicine, Ankara, Turkey, [2]Biochemistry, Hacettepe University Faculty of Medicine, Ankara, Turkey, [3]Pharmacology, Hacettepe University Faculty of Medicine, Ankara, Turkey. E-mail: aerdem@hacettepe.edu.tr

Endotoxin decreases mesenteric blood flow and inflicts organ injury via free radicals. We investigated if taurine, an endogenous antioxidant and vasodilator, could attenuate the deleterious effects of endotoxin in a murine model of septic shock. Swiss albino mice were allocated into four groups (n = 5–9, each) and treated with taurine (150 mg/kg, ip at 0th, 8th, 16th h) or its solvent sterile saline (NaCl 0.9%, w/v) while Escherichia coli endotoxin (Lipopolysaccharide, O55:B5; 20 mg/kg, ip) or its solvent saline was also given at 8th h. At 24th h animals were anaesthetized, mesenteric blood flow was measured by using perivascular ultrasonic Doppler-flowmeter. Then the animals were exsanguinated, the spleen, liver, and kidneys were isolated and weighed. The organs were also examined for thiobarbituric acid-reacting substances (TBARS), glutathione, and myeloperoxides (MPO) activities. Statistical analysis was performed by using Instat® software and significance was accepted when $P < 0.05$. Endotoxin significantly decreased the mesenteric blood flow (ml/min, control: 5.1 ± 0.4, n = 5; endotoxin: 1.6 ± 1.4, n = 6) and glutathion levels in liver (mmol/mg protein, control: 0.045 ± 0.008, n = 6; endotoxin: 0.0102 ± 0.001, n = 12) and kidney without altering the organ weights while TBARS and MPO activity were increased. However, taurine did not significantly modify the deleterious effects of endotoxin. Therefore, we concluded that endotoxin-induced organ injury via free radicals is resistant to blockade by taurine.

PP-349
Effects of some metal ions on yeast glutathione reductase

N. N. Ulusu and B. Tandogan
Department of Biochemistry, Faculty of Medicine, University of Hacettepe, Sihhiye, Ankara, Turkey.
E-mail: nnulusu@hacettepe.edu.tr

Glutathione reductase (GR, type IV, from Baker s yeast, EC 1.6.4.2) catalyses the reduction of oxidized glutathione to its reduced form (GSH). Glutathione is an essential for antioxidant enzymes and protects the cells from the lethal effects of reactive oxygen species (ROS) and xenobiotics. Glutathione reductase have key role in the scavenging of the radicals. Free radicals are playing a significant role in a variety of diseases; neurodegenerative disorders, atherosclerosis, and cancer. Because of glutathione reductase that protects cells from ROS-induced damage, inhibition of this enzyme has a vital role for the organism. In this study we have tested some of the heavy metal ions on the glutathione reductase enzyme such as lithium, manganese, molybdate,

aluminium, barium and nickel. Only Ni^{2+} (0.1 between 5 mM) showed an inhibition effect on this enzyme. We have also found that glutathione reductase enzyme IC 50 is 0.8 mM. An inhibition effect of Ni^{2+} on glutathione reductase activity, when NADPH is the varied substrate, is also consistent with an uncompetitive inhibition pattern and competitive inhibition is found when oxidized glutathione is the varied substrate. Nickel is a naturally occurring metal and is used in a wide variety of metallurgical processes. Although nickel is an essential cofactor for a number of enzymatic reactions in both prokaryotes and eukaryotes, this metal can inhibit glutathione reductase in a concentration dependent manner.

PP-350
The effects of streptozotocin induced diabetes on some oxidative biomarkers of rat liver

G. Sadi[1], G. Görgülü[2], T. Güray[1]
[1]*Department of Biochemistry, Middle East Technical University, Ankara, Turkiye,* [2]*Research and Development Center, Suleyman Demirel University, Isparta, Turkiye. E-mail: sadi@metu.edu.tr*

Diabetes mellitus which is a glucose metabolism disorder is associated with consequences of oxidative stress due to non-enzymatic protein glycation, glucose autoxidation and polyol pathway, which augments the free radical production. In this study, effects of diabetes on microsomal lipid peroxidation, cytosolic protein oxidation, level of glutathione and lipid protein ratios were evaluated and compared in both streptozotocin induced diabetic ($n = 9$) and control ($n = 7$) Wistar rats. It has been observed that microsomal lipid peroxidation and cytosolic protein oxidation were significantly ($P < 0.05$) higher in diabetic animals than controls, on the other hand, diabetic glutathione concentration were significantly lower then controls. Also, diabetic microsomal membrane lipid to protein ratio was significantly higher compared to control group. Antioxidant profiles of groups were studied by measuring the antioxidant enzyme activities namely superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferase (GST). CAT activities were found to be 28% lower in diabetes, however 29 and 31% higher SOD and GPx activities were observed in diabetes compared to controls, respectively. All these changes, except GST activities, were statistically significant ($P < 0.05$). Furthermore, mRNA expressions and western blot analysis of some of the antioxidant enzymes were also studied. Results showed that not all the antioxidant enzyme mRNA levels were increased in diabetes.

PP-351
Phosphodiesterase (PDE) IV inhibitor rolipram (ROL) diminishes endothelial dysfunction in experimental diabetes

M. Kamper, O. Tsimpoukidi, **A. Chatzigeorgiou**, M. Lyberi, L. Kopeikina and E. Kamper
Department of Experimental Physiology, Medical School, University of Athens, Athens, Greece.
E-mail: ekamper@med.uoa.gr

Accelerated atherosclerotic vascular disease due to prolonged and excessive inflammatory processes in vascular wall is the leading cause of mortality in diabetic patients. The aim of this study was to evaluate whether rolipram (ROL), the specific PDE IV inhibitor, can improve endothelial dysfunction and inflammation in streptozotocin (STZ)-induced diabetes in rats. Sprague Dawley male rats were divided into control group, untreated STZ-induced diabetes group and STZ-induced diabetes group treated with ROL over a period of 1 week according to dose dependent protocol (3.5, 7 and 10.5 mg/kg) The degree of insulitis was evaluated histochemically. Plasma nitrate/nitrite (NOx) and sICAM measurements were used to evaluate the state of endothelial dysfunction. ROL treatment improved diabetes clinical features, lowered plasma glucose levels and diminished insulitis severity. NOx and sICAM levels in STZ rats were significantly higher than those in controls. The strong positive correlation of NOx with sICAM ($rs = 0.414$, $P = 0.044$) and both of them with glucose level ($rs = 0.576$, $P = 0.006$ and $rs = 0.695$, $P < 0.0001$, respectively) were found in STZ rats. A significant correlation between NOx or sICAM level with the degree of insulitis was found in diabetic rats. ROL treatment reduced NOx and sICAM levels in diabetic animals in a dose dependent manner. The results indicate that ROL can protect diabetic patients from atherosclerotic vascular complications reducing hyperglycaemia-induced oxidative stress and vessel wall inflammation.

PP-352
The effect of exercise and melatonin administration on oxidative stress in rats

M. Serter[1], S. Temocin[2], E. Demir[1], **K. Ergin**[3], D. Kozaci[1] and T. Boylu[3]
[1]*Biochemistry, Adnan Menderes University, Medical School, Aydin, Turkey* [2]*Phisiology, Adnan Menderes University, Medical School, Aydin, Turkey* [3]*Histology, Adnan Menderes University, Medical School, Aydin, Turkey. E-mail: cancemdr@yahoo.com*

Introduction: Many studies have reported that physical exercise induces the oxidative stress and melatonin decreases that. To minimize the oxidative stress and remove the free radicals the body uses a very effective antioxidative defence system. Erythrocyte catalase activity, reduced glutathione and serum nitric oxide level were investigated to determine the effects of longer time exercise and melatonin administration on the oxidative stress in rats.
Material and methods: The rats were randomly divided into four groups; group 1: exercise + melatonin, group 2: exercise + serum physiologic, group 3: serum physiologic alone and group 4: melatonin alone. Erythrocyte catalase activity was determined by the method of Aebi. Reduced glutathione and serum nitric oxide levels were determined spectrophotometrically.
Results: In analyzation of the results with Mann-Whitney U test, the catalase activity was significantly higher in group 1 ($P = 0.045$), and in group 3 ($P = 0.028$), in comparisons of group 1 to 2 and group 2 to 3 respectively.
Conclusion: In conclusion, while the exercise induced oxidative stress, melatonin reduced these effects in our study.

PP-353
Correlation between CRP and nitric oxide in patients with total abdominal hysterectomy

S. Kirdar[1], M. Serter[2], H. Yuksel[3], A. Odabasi[3], E. Demir[2], T. Ozan[3], B. Gultekin[1] and S. Demircan[3]
[1]*Department of Microbiology, Adnan Menderes University Hospital, Aydin, Turkey,* [2]*Department of Biochemistry, Adnan Menderes University Hospital, Aydin, Turkey,* [3]*Department of Obstetrics and Gynecology, Adnan Menderes University Hospital, Aydin, Turkey. E-mail: smukadder02@yahoo.com*

Introduction: Total abdominal hysterectomy (TAH) cause inflammation and oxidant stress due to post-operative trauma. C reactive protein (CRP) is accepted as inflammation marker. Nitric oxide (NO) is a free radical, which causes oxidative stress and may also be used as inflammatory marker. The aim of this

study is to evaluate presence of a correlation between the levels of CRP and NO among patients with hysterectomy.

Methods: Ten female patients who underwent total abdominal hysterectomy were studied. The concentration of CRP and NO were assessed in sera collected before operation and 1, 2 and 7 days after operation using nephelometric and Griess methods, respectively. Spearman method is used for statistical analysis.

Results: CRP level before operation was 0.94 ± 1.01. Twenty-four hours and became 35.52 ± 25.21, 46.11 ± 20.58, and 13.23 ± 17.11, 24, 48 h and 7 days after operation, respectively. NO level was 14.18 ± 2.7 before operation, and became 13.53 ± 2.9, 19.84 ± 8.85, and 23.11 ± 8.34, 24 h, 48 h, and 7 days after the operation, respectively. Correlation between NO and CRP levels was positive and significant before operation correlation coefficient (CC = 0.586, P = 0.097). But there was no correlation 24 h after operation (CC =0.109, P = 0.763). Among levels after 48 h the correlation was positive and moderate (CC = 0.321, P = 0.365). At 7th day correlation was negative and weak.

Conclusion: A moderate correlation was found among NO and CRP levels only after 48 h.

PP-354
The effect of phosphodiesterase IV inhibitor rolipram on enzymatic antioxidant defence system in experimental animals

O. Tsimpoukidi, M. Kamper, A. Chatzigeorgiou, M. Lyberi, L. Kopeikina and E. Kamper

Departments of Experimental Physiology, Medical School, University of Athens, Athens, Greece. E-mail: ekamper@med.uoa.gr

Oxidative damage has been suggested to be a contributing factor in the development of vascular complications in diabetes. The aim of this study was to evaluate whether ROL, the specific phosphodiesterase IV inhibitor, can improve the function of enzymatic antioxidant defence system (EADS) in experimental diabetes. Sprague Dawley male rats were divided into control group, untreated streptozotocin (STZ)-induced diabetes group and STZ-induced diabetes group treated with rolipram (ROL) over a period of 1 week according to a dose dependent protocols (3.5, 7 and 10.5 mg/kg). Plasma glutathione peroxidase (GPx) and glutathione reductase (GR) were measured to evaluate the state of EADS. All STZ rats exhibited biochemical signs of diabetes. GPx and GR activities in diabetic group were significantly increased with respect to control group 10 days after STZ administration (P = 0.021and P = 0.048, respectively). There was significant positive correlation between GPx or GR activity and glucose level or the degree of insulitis in diabetic rats. ROL treatment improved clinical and biochemical signs of diabetes in dose dependent manner. A dose dependent decrease in GPx and GR activities was observed in ROL-treated STZ rats compared to the untreated STZ rats, reaching statistical significance at dose of 7 mg/kg. Our results indicate that the administration of ROL to STZ rats over a period of 1 week, in addition to the antidiabetic effects, causes significant improvement of the EADS function.

PP-355
The role of UCP 2 in mitochondrial ROS production

N. Raju[1], F. Leinisch[1], T. Špaček[2], P. Ježek[2] and **W Trommer**[1]

[1]Department of Chemistry, Technical University Kaiserslautern, Germany, [2]Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic. E-mail: trommer@chemie.uni-kl.de

Thermogenin from brown adipose tissue, or uncoupling protein 1 (UCP1) as it is now called is responsible for non-shivering heat generation by dissipating energy stored in the protein gradient over the inner mitochondrial membrane. Some time ago we provided evidence that UCP1 does not transport protons from the intermembrane space into the mitochondrial matrix but rather translocates negatively charged fatty acids which get protonated at the lower pH in the intermembrane space, diffuse back freely into the matrix and thus, mediates net transport of protons. We have now shown that UCP2 from human liver that is thought to play a role in controlling mitochondrial ROS (reactive oxygen species) production functions in a similar way.

We have compared the formation of superoxide anion radical by spin-trapping in mitochondria from normal rats and a strain deficient in UCP 2.

PP-356
Catalase over-expression in the heart of desmin null mice

K. Rapti and Y. Capetanaki

Cell Biology, IIBEAA, Athens, Greece. E-mail: crapti@bioacademy.gr

Desmin is the major muscle specific intermediate filament protein. Mice null for desmin develop dilated cardiomyopathy and heart failure characterized by mitochondrial defects and cardiomyocyte death accompanied by extensive calcification and fibrosis. The cellular and biochemical alterations in the hearts of these mice strongly suggest that oxidative stress is one of the mechanisms contributing to the pathogenesis of the phenotype. Using desmin null mice as a heart failure model, our therapeutic approach focuses on the enhancement of the antioxidant defence system in their heart. Towards this goal we have generated transgenic mice that overexpress in the heart the antioxidant enzyme catalase under the control of the cardiac specific promoter aMHC. In total, six transgenic lines were created. All of them have higher protein levels compared to wild type hearts. The measured catalase enzymatic activity was higher than that of liver, which is the highest catalase expressing tissue. Three of the transgenic lines that cover the broadest spectrum of catalase activity, were crossed to desmin null mice, in order to study the protective effect of catalase overexpression in the development and progress of the desmin deficient cardiomyopathy. Initial histochemistry data that show decrease in fibrotic lesions in the myocardium of the transgenic line with the lowest catalase levels, are very promising and confirm that oxidative stress is part of the pathogenesis mechanism.

PP-357
Stobadin prevents doxorubicin-induced apoptosis by inhibiting caspase 3 in P815 cells

E. U. Bagriacik[1], C. Karasu[2], K. Uslu[3], M. Stefek[4] and V. Bauer[4]

[1]Immunology, Faculty of Medicine, Gazi University, Ankara, Turkiye, [2]Medical Pharmacology, Faculty of Medicine, Gazi University, Ankara, Turkiye, [3]Microbiology, Faculty of Medicine, Gazi University, Ankara, Turkiye, [4]Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovak Republic. E-mail: umit_bagriacik@yahoo.com

Stobadin has been classified as a pyridoindole antioxidant. However, there is no evidence to explain at molecular level how stobadin may exert its antioxidant effects on apoptosis. To investigate mechanism(s) by which stobadin may exert its antioxidant effects, we used an *in vitro* doxorubicin-induced apoptosis model of P815 cells. First, IC_{50} values of doxorubicin for P815 cells were titrated and found to be (2×10^{-7}M). Stobadin at various concentrations was tested against doxorubicin. Apoptosis

was measured by flow cytometry using propidium iodine (PI)/Annexin-V-FITC. Caspase activity was also studied by colorimetric assays. Stobadin inhibited doxorubicin-induced apoptosis even at very low doses (10^{-7}M). We also found that stobadin prevented apoptosis inhibiting activities of caspase-3 and caspase-9 by 59 ± 4% and 37 ± 4%, respectively. Effects of stobadin were comparable to those of a wellknown antioxidant, N-acetyl L-cysteine. Our results suggested that stobadin might be used as an antioxidant to prevent doxorubicin-induced cellular damage such as cardiotoxicity, which is frequently observed following chemotherapy in cancer patients. This study was supported by TUBITAK (SBAG-SLOVAK-2 No: 103S180).

PP-358
Effects of oxidized dietary fat on antioxidant status in broilers fed with vitamin E supplemented diet

S. Tanriverdi Akhisaroglu[1], Z. S. Altun[1], Z. Acikgoz[2], O. Altan[2] and H. Bayraktar[2]
[1]*Institute of Oncology, Dokuz Eylul University, Izmir, Turkey,* [2]*Faculty of Agriculture, Department of Animal Science, Ege University, Izmir, Turkey. E-mail: serpil.tanriverdi@deu.edu.tr*

Broilers have fast growth capacities so that lipid supplementation in mixed diets must be done for their increased energy requirements. The aim of this study was to determine superoxide dismutase (SOD), glutathione peroxidase (GPX) activities and nitric oxide (NO), malondialdehyde (MDA) levels in broilers fed with oxidized fat and vitamin E supplemented diets. Thirty-six one-day-old male broiler chicks were used. The study groups were: control group, oxidized dietary fat group and oxidized dietary fat with vitamin E supplemented diet group. Blood samples were divided in two groups; 1-Plasma extraction for MDA, NO, glucose (Glu), uric acid (UA), triglyceride (TG) and cholesterol (Chol), 2-Whole blood for SOD and GPX assays. Broilers fed with oxidized fat diets had increased concentrations of TG, Chol, Glu and NO and increased activity of GPX as compared with animals fed the fresh fat diets. The oxidized dietary fat with vitamin E supplemented diet group showed decreased levels in all these parameters. TG, Chol and NO concentrations were significantly different. UA and MDA levels showed no difference among the groups. SOD activity was reduced in broilers fed with oxidized fat diets.
In conclusion, this study shows that the sunflower oil oxidized at low temperature did not effect the antioxidant status sufficiently. Since formation of secondary lipid peroxidation products may occur at high temperatures we suggest for further studies using these kind of fats.

PP-359
Involvement of adrenergic receptor balance in gender-dependent response to caloric restriction in brown adipose tissue

A. Valle, F. J. García-Palmer, J. Oliver and **P. Roca**
Department de Biologia Fonamental i Ciències de la Salut, Universitat de les Illes Balears, Palma de Mallorca, Spain. E-mail: pilar.roca@uib.es

Gender-dependent deactivation of brown adipose tissue (BAT) has been shown to be involved in the greater ability of female rats to conserve energy when faced with caloric restriction (CR). Facultative thermogenesis carried out in brown adipose tissue (BAT) implies a great energy cost. Noradrenaline is the main physiological regulator of thermogenesis through the presence in the brown adipocyte of adrenergic receptors (AR). Previous results in our group showed gender differences in adrenergic regulation in housing conditions and in response to overfeeding. Thus, we decided to examine the effects of CR on AR balance and the main proteins involved in thermogenic and lipolytic pathway in male and female rats subjected to 40 % CR for three months. CR decreased uncoupling protein 1 and lipolysis-related enzymes in female and protein kinase A in male rats. β_3-AR was decreased in both genders, whereas α_2-AR decreased only in males. This higher α_2/β_3 ratio is designed to favour inhibition of lipolysis and thermogenesis in female rats. Our results suggest a cross talking between CR signals and gender at receptor level in which noradrenalin, insulin and sex hormones might be involved. These changes in lipolytic-thermogenic axis could be part of the adaptations that allow females to increase energy efficiency under CR, promoting their own survival and that of the species.

PP-360
Modulation of serum paraoxonase activity during a short-term fasting period in male and female rats

E. Thomàs-Moyà, M. Gianotti, I. Lladó and **A. M. Proenza**
Biologia Fonamental i Ciències de la Salut, Universitat de les Illes Balears, Palma de Mallorca, Spain. E-mail: ana.proenza@uib.es

Paraoxonase 1 (PON1) associates with specific high-density lipoproteins (HDL) containing apolipoproteins A-I and J (apoA-I and apoJ), and is largely responsible for their antiatherogenic properties. We have previously reported that serum PON1 activity strongly dropped in rats subjected to 14 weeks of 40% energy restriction and also, that the adaptive response to food deprivation was gender-dependent. Unlike prolonged partial caloric restriction, fasting represents an extreme and acute situation of energy restriction. The aim of this work was to investigate the effect of 12-h food deprivation on serum PON1 activity in male and female rats. Thus, lipid profile, lipid peroxide levels, PON1 activities and PON1, apoA-I and apoJ content were measured in fed and fasted rats. Lipid peroxide levels significantly dropped with fasting, whereas serum paraoxonase and arylesterase activities showed a great increase. ApoJ levels also increased with fasting, although it was only significant in female rats. These data reveal that the rise in PON1 activity with fasting is a result of a higher content of PON1 and apoJ in HDL. In addition, our results confirm that the response to an acute period of food deprivation is also gender-dependent. A 12-h food deprivation caused a strong response in PON1 activity to face up to the increased oxidative stress, whereas a prolonged partial energy restriction period would imply an adaptive response that would have a protective function to increase survival.

PP-361
Caloric restriction decreases age-dependent decline in thermogenic capacity of brown adipose tissue

A. Valle, R. Guevara, F. J. García-Palmer, P. Roca and J. Oliver
Department of Fundamental Biology and Health Science, University of Balearic Island, Palma de Mallorca, Spain. E-mail: jordi.oliver@uib.es

Caloric restriction (CR) has been shown to be the most effective way to extend longevity, ameliorate age-related decline in physiological function and the incidence of age-related disease. Thermogenesis and energy expenditure are attenuated during the senescence process, leading to a loss in the capacity to maintain homeothermy and allowing higher fat accumulation. In rodents, brown adipose tissue (BAT) is the main effector of non-shivering thermogenesis, a major component of energy expenditure to control cold adaptation and body weight. In order to study the undergoing changes in thermogenesis during CR, we examined

the effects of long-term CR on body composition, energy expenditure and BAT features of adult male rats. Twelve-month-old rats were fed ad libitum or 40% CR for 10 months and body mass, food intake and oxygen consumption were monitored. Organ weight, and BAT composition, uncoupling protein 1 (UCP1) and hormone sensitive lipase (HSL) content were determined. Animals showed 35% weight loss, showing a high decrease in fat. No differences were found in oxygen consumption. BAT weight was decreased by CR, showing a great decrease in fat content, whereas total and mitochondrial protein per cell was increased. Specific UCP1 and HSL were higher in restricted rats. These results indicate lower age-related decline in the thermogenic capacity of restricted animals, suggesting that BAT may be involved in the anti-aging effects underlying CR.

PP-362
The effect of haem oxygenase-1 induction by octreotide on radiation enteritis

M. Giriş[1], S. Doğru Abbasoğlu[1], Y. Erbil[2], T. Eren[2], V. Olgaç[3], M. Uysal[1] and G. Aykaç Toker[1]
[1]*Biochemistry, Istanbul Medical Faculty, Istanbul, Turkey,* [2]*General Surgery, Istanbul Medical Faculty, Istanbul, Turkey,* [3]*Oncologic Pathology, Istanbul Medical Faculty, Istanbul, Turkey.*
E-mail: mgirism@gmail.com

Radiation enteritis occurs as a response to abdominal radiation, which can cause mucosal damage in the gastrointestinal mucosal epithelium. The small intestine is one of the most radiosensitive organs in the abdomen. The present study was undertaken to investigate the effect of octreotide (OCT) administration on haem oxygenase-1 (HO-1) expression of the radiation enteritis model. Rats received 50 mg/kg/day OCT for 4 days before irradiation and continued for 3 days after irradiation. Intestinal myeloperoxidase (MPO) activities, malondialdehyde (MDA) levels are indicators of oxidative damage while caspase-3 activities reveal apoptosis degree of the small intestine. At histological examination, the terminal ileum tissue was analysed for morphological changes. Irradiation significantly increased the intestinal MPO and caspase-3 activities, MDA levels and HO-1 expression in comparison to sham control group. OCT treatment was associated with increased HO-1 expression and caspase-3 activity, decreased MPO activity and MDA levels. Histological examination revealed that the intestinal mucosal structure was preserved in the OCT treated group. OCT appears to have protective effects against radiation-induced intestinal damage. This protective effect is, in part, mediated by modification of the inflammatory response and the induction of HO-1 expression.

PP-363
Nitric oxide levels in pseudoexfoliation syndrome

Z. Yildirim[1], N. Kilic[1], N. I. Ucgun[2] and E. Gursel[2]
[1]*Medical Biochemistry, Gazi University Faculty of Medicine, Ankara, Turkey,* [2]*Second Eye Clinic, Ankara Numune Education and Research Hospital, Ankara, Turkey.*
E-mail: zyildirim2004@yahoo.com

Purpose: To study the role of the nitric oxide (NO) pathway in pseudoexfoliation syndrome (PSX) pathogenesis by measuring nitric oxide levels in the cataract patients with and without pseudoexfoliative syndrome.
Material and methods: The study involved 29 cataract patients with pseudoexfoliation syndrome and 27 cataract patients without PSX matched for sex and age. All subjects underwent a complete ophthalmic examination and NO levels were measured in serum. We used a method for simultaneous evaluation of nitrate and nitrite concentrations in a microtiter plate format for evaluate NO levels.
Results: The mean NO levels in the serum were 131.17 ± 34.6 μM in cataract patients with PSX and 136.5 ± 58.57 μM in cataract patients without PSX. There was no statistical difference between two groups ($P > 0.05$).
Conclusions: NO levels were not found different between PSX and control patients. NO is implicated in a variety of ocular pathophysiological states including glaucoma, uveitis, retinal ischaemic disease and diabetes mellitus. However NO levels in serum of PSX patients warrant further evaluation in a larger study.

PP-364
In prefrontal cortex of rat brain in an experimental psychosis model and the protective effects of melatonin

B. Ozyurt[1], H. Ozyurt[2], **S. Sahin**[2], I. Kus[3], O. Akyol[4], M. Sarsilmaz[5] and I. Zararsiz[5]
[1]*Department of Anatomy, Medical Faculty, Gaziosmanpasa University, Tokat, Turkey,* [2]*Department of Biochemistry, Medical Faculty, Gaziosmanpasa University, Tokat, Turkey,* [3]*Department of Anatomy, Medical Faculty, Firat University, Elazig, Turkey,* [4]*Department of Biochemistry, Hacettepe University Medical Faculty, Ankara, Turkey,* [5]*Department of Anatomy, Medical Faculty, Firat University, Elazig, Turkey.*
E-mail: semsettinsahin@hotmail.com

The aims of this study are to demonstrate the contribution effect of oxidative stress to the neuropathophysiology of schizophrenia and that prevention of oxidative stress may improve prognosis. MK-801 induced selective neurotoxicity has been proposed as an animal model for psychosis. Healthy adult and male Wistar Albino rats were obtained Firat University Biomedical research Unit and 30 rats divided randomly into three groups. MK-801 was given intraperitoneally for 5 days in the experimental psychosis group. Melatonin was given to the treatment group 50 mg/kg/day for 6 days by intraperitoneally. In control group, saline was given in the same way. In 7th-day from the beginning of the experiments, rats were killed by decapitation and prefrontal cortex was removed immediately. Malondialdehyde (MDA) and protein carbonyl (PC) analyses were made by spectrophotometric methods. Malondialdehyde, as an indicator of lipid peroxidation, as well as protein carbonyl, as an indicator of protein oxidation, levels was found to be increased significantly in prefrontal cortex of MK-801 group compared to control group. In melatonin treated rats, prefrontal tissue malondialdehyde and protein carbonyl levels were decreased significantly when compared to MK-801 group. These results indicate that MK-801 can increase oxidant parameters in prefrontal cortex, in addition to reduced antioxidant parameters. On the other hand, melatonin may indirectly enhance the activity of antioxidant enzymes.

PP-365
Oxidative stress in smokers and non-smokers

F. B. Isik[1], A. Ceylan[2] and **R. Isik**[3]
[1]*Department of Biochemistry, Faculty of Medicine, Dicle University, Diyarbakir, Turkey,* [2]*Department of Public Health, Faculty of Medicine, Dicle University, Diyarbakir, Turkey,* [3]*Department of Chest Disease, Faculty of Medicine, Dicle University, Diyarbakir, Turkey. E-mail: birgul60@hotmail.com*

Oxidative stress plays an important role in the pathogenesis of some diseases such as lung cancer, chronic obstructive pulmonary disease and atherosclerosis.
Smoking may enhance oxidative stress not only through the production of reactive oxygen radicals in smoke but also through

weakening the antioxidant defence systems. In the present study, we aimed to investigate the effects of smoking on lipid peroxidation and paraoxonase activity in healthy population. The study consisted of (n = 30) smokers and (n = 30)non-smokers. The mean age of the population we studied was 44.74 ± 10.59 years. The levels of serum malonlydialdehyde (MDA) and paraoxonase (PON1) activities were measured by the modified Buege method and the Eckerson method, respectively. The student's t-test was used to analyse the data. The levels of serum MDA were significantly higher p el 0.001 and the activities of serum PON1 were significantly lower p el 0.001 in smokers than non-smokers. Increased levels of serum MDA and decreased PON1 activities may be important in determining the oxidant/antioxidant imbalance in smokers.

PP-366
In prefrontal cortex of rat brain in an experimental schizophrenia model and the protective effects of CAPE

B. Ozyurt[1], H. Ozyurt[2], O. Atis[2], N. Akpolat[3], A. Akbas[2] and M. Sarsilmaz[4]
[1]*Department of Anatomy, Gaziosmanpasa University Medical Faculty, Tokat, Turkey,* [2]*Department of Biochemistry, Gaziosmanpasa University Medical Faculty, Tokat, Turkey,* [3]*Department of Pathology, Firat University Medical Faculty, Elazig, Turkey,* [4]*Department of Anatomy, Firat University Medical Faculty, Elazig, Turkey. E-mail: mdbirsen@hotmail.com*

Healthy adult and male Wistar Albino rats were obtained Firat University Biomedical Research Unit and 30 rats divided into three groups. MK-801 was given intraperitoneally for 5 days in experimental psychosis group. CAPE was given to treatment group for 6 days by intraperitoneally. In control group, saline was given in the same way. In 7 days the beginning of the experiments rats were killed by decapitation. Brain were removed and prefrontal part of the brain was divided for histological and biochemical analyses. Histological preparats were stained with HE and analysed. Malondialdehyde and protein carbonyl analyses were made by spectrophotometric methods. The histological examination demonstrated that MK-801 induced prefrontal apoptosis. A similar series of experiments has shown that CAPE decreased the apoptotic cell account in prefrontal cortex after MK-801 injection. Malondialdehyde, protein carbonyl levels were found to be increased significantly in prefrontal cortex of MK-801 group ($P < 0.0001$) compared to the control group. In CAPE treated rats, prefrontal tissue malondialdehyde and protein carbonyl levels were decreased significantly when compared to MK-801 group ($P < 0.0001$).
These results indicate that MK-801 can increase reactive oxygen species (ROS), and induce apoptotic changes in prefrontal cortex of rats. This experimental study also provides some evidences for the protective effects of CAPE on MK-801-induced changes in prefrontal rat cortex.

PP-367
The effects of ginkgo biloba extract (EGb 76) and selenium against rat cerebral ischemia reperfusion injury (IR/I)

S. Ozbal[1], G. Erbil[1], **H. Kocdor**[2], K. Tugyan[1], C. Pekcetin[1] and C. Ozogul[1]
[1]*Department of Histology and Embryology, School of Medicine, Dokuz Eylül University, Izmir, Turkey,* [2]*Department of Basic Oncology, Institute of Oncology, Dokuz Eylül University, Izmir, Turkey. E-mail: hilal.kocdor@deu.edu.tr*

Brain shows high sensibility to oxidative stress IR/I. It has been suggested that, both ginkgo biloba (GB) and selenium (Se) have tissue protective properties against oxidative stress. Neuroprotective effects of Se and GB combination against brain IR/I are not known. Here, we aimed to investigate the effects of GB extract, Se and combination on hippocampus in the rats subjected to IR/I. 35 male wistar rats were divided into five groups and each group consisted of seven rats. Except sham, the rats were underwent brain IR/I model. Se (0.625 mg/kg/d), GB (50 mg/kg/d) and same dose drug combination were administered via intraperitoneal injections (ip) followed by IR/I for 14 days. After the sacrification, hippocampus was dissected for biochemical analysis. proNGF and mature NGF, TNF-α and IL-1β were analysed as ischaemic and pro-inflammatory markers in fresh hippocampal tissues. Hippocampal proNGF and mNGF levels were significantly lower in IR/I group and high NGF levels were remarkable in GB-administered groups (IR/I + GB, IR/I + GB + Se). Whereas, hippocampal TNF and IL-1 levels were high in IR/I group. But, combined administration was not enhanced proinflammatory cytokine production. Histological results were consistent with the biochemical parameters. Serum TNF and IL-1 levels were also similar to hippocampal cytokine release. On conclusion, Se and GB extract were found to be effective against brain IR/I, but this effects was not enhanced by combination.

PP-368
Protection against liver ischemia-reperfusion injury in rats by silymarin or glutamine

D. Sahin[1], M. Kisakurek[2], A. Demirag[3], A. Sepici Dincel[1], I. C. Sogukpinar[2], M. Gonultas[4], S. Karakayali[2] and N. Altan[1]
[1]*Department of Biochemistry, Faculty of Medicine, Gazi University, Ankara, Turkey,* [2]*Department of Surgery, Ankara Teaching and Research Hospital, Ankara, Turkey,* [3]*Department of Surgery and Transplantation, Yeditepe University Hospital, Istanbul, Turkey,* [4]*Department of Pathology, Ankara Teaching and Research Hospital, Ankara, Turkey. E-mail: asepici@yahoo.com*

Liver injury after cold or warm ischemia, followed by reperfusion, remains one of the major obstacles in liver surgery, especially in transplantation. Various mechanisms have been implicated in liver ischemia-reperfusion (I-R) injury including ROS generation, lipid peroxidation, mitochondrial dysfunction and cytokine production. Silymarin (SLY), a flavonoid extract has been shown to have protective effects, due to its ability to remove free radicals, prevent lipid peroxidation and depletion of glutathione. Supplementation with glutamine (GLN) has been reported to have beneficial effects on all of these protective pathways. Although the possible cytoprotective effect of SLY and GLN has been studied, there is no information about the comparative effects of these drugs for over a period of time I-R by methods that can evaluate antioxidant and cytoprotective capacity. The aim of this study was to evaluate preventive effect of SLY and GLN supplementation against I-R injury.
Methods: Sprague-Dawley rats were assigned to five groups: (a) sham (Group I); (b) control with hepatic ischemia (clamping on the portal pedicle for 45 min and reperfusion) (Group II); (c) SLY + ischemia (Group III); (d) GLN + ischemia (Group IV); SLY and GLN were given 2 h before ischemia. Samples were obtained after 45 min, 2 h and 24 h of reperfusion and MDA, SOD, TNF-alpha levels and histopathologic findings were evaluated.
Results: The liver MDA, SOD, and serum TNF-alpha ($P < 0.05$) levels were significantly lower in Group VI than in Group II at 45 min, 2 h and 24 h of reperfusion. and the similar effects were seen in group III when compared with group II at 45 min and 24 h of reperfusion. While the effect of GLN on tissue MDA levels was significantly different than SLY group in all time frames, the difference between these two groups was less

significant for SOD. The most prominent ischaemic injury was observed in Group III in which SLY was administered.

Discussion: These results suggest that GLN and SLY are effective in attenuating liver ischemia/reperfusion injury and antioxidant effect of both medications could be responsible for the cytoprotection.

PP-369
Reduction of cisplatin-induced nephrotoxicity by L-arginine + N-acetylcysteine

M. Erdinc[1] and L. Erdinc[2]
[1]*Department of Pharmacology, Faculty of Medicine, Dicle University, Diyarbakır, Turkey,* [2]*Department of Biochemistry, Faculty of Medicine, Dicle University, Diyarbakır, Turkey.*
E-mail: merdinc@dicle.edu.tr

Cisplatin (CDDP) is an effective antineoplastic drug used against various human malignancies. However, it induces nephrotoxicity, which is its dose-limiting side effect. It has been suggested that oxygen free radicals play an important role in that severe nephrotoxicity. The aim of this study was to investigate the effect of L-arginine, which is a nitric oxide precursor, and N-acetylcysteine (NAC), which replace intracellular stores of reduced glutathione and has antioxidant effects.

Method: Male Wistar albino rats were divided into five groups: (a) control group (saline injection); (b) CDDP group: a single injection of CDDP (5 mg/kg , i.p.); (c) L-arginine group: injection of L-arginine for 5 days (10 mg/kg i.p.) 4) NAC group: injection of NAC for 5 days (300 mg/kg i.p.) and (e) L-arginine (10 mg/kg i.p.) + NAC (300 mg/kg i.p.) group. After 5 days in CDDP group, acute nephrotoxicity was demonstrated by an increase in serum creatinine and blood urea nitrogen levels. Malondialdehyde(MDA) levels were significantly increased. In group 3, 4 and particularly in group 5 serum creatinine and blood urea nitrogen and MDA levels were significantly decreased.

Conclusion: As a result it was demonstrated that L-arginine and NAC have protective effects against CDDP-induced nephrotoxicity.

PP-370
Effects of the methanol extract of *Lobaria pulmonaria* on antioxidant system and gastric damages in rats

Y. Bayir[1], B. Karakus[1], F. Odabasoglu[1], H. Suleyman[2], A. Cakir[3], A. Aslan[4], O. Yucel[5] and M. Halici[1]
[1]*Department of Biochemistry, Ataturk University-Faculty of Pharmacy, Erzurum, Turkey,* [2]*Department of Pharmacology, Ataturk University-Faculty of Medicine, Erzurum, Turkey,* [3]*Department of Chemistry, Ataturk University-Kazim Karabekir Education Faculty, Erzurum, Turkey,* [4]*Department of Biology, Ataturk University-Kazim Karabekir Education Faculty, Erzurum, Turkey,* [5]*Department of Biochemistry, Ataturk University-Tech. High school Health Serv., Erzurum, Turkey.*
E-mail: mesut@halici.com

In the present study, the gastroprotective effects of methanol extract obtained from a lichen species, *Lobaria pulmonaria* (L.) Hoffm. was investigated in indomethacine (IND)-induced gastric ulcer models in rats. Gastroprotective effect of 50, 100, 200, 500 and 1000 mg/kg body wt. doses of the extract were determined by comparing with control groups. The gastric lesions were significantly reduced by all doses of the extract as compared with the IND (25 mg/kg body weight) group, the highest gastroprotective effect was observed for 1000 mg/kg body wt. dose with 77.2% inhibition. The *in vivo* antioxidant levels in the stomach tissues of all animal groups were also evaluated. The administration of IND decreases superoxide dismutase (SOD), glutathione peroxidase (GPx) activities and reduced glutathione (GSH) levels, and increase the lipid peroxidation (LPO) level and catalase (CAT) activity ($P < 0.05$). The administration of all doses of the extract reversed the trend, inducing a significant increase of SOD, GSH and GPx levels and a reduction in LPO level and CAT activity in tissues. In addition to *in vivo* antioxidant activity, the *in vitro* antioxidant properties of the extract were determined. Similarly, the extract showed significant antioxidant activity. The present results indicate that the methanolic extract of *L. pulmonaria* has a gastroprotective effect against gastric ulcer induced by IND, which can be attributed to its antioxidant potential.

PP-371
Effects of drotrecogin alpha alone and drotrecogin alpha plus meropenem on liver antioxidant system in rats with sepsis

A. Yildirim[1], H. Turhan[1], Y. Sahin[1], N. Bakan[1], S. Yildirim[2] and S. Boy[3]
[1]*Department of Biochemistry, Medical Faculty, Ataturk University, Erzurum, Turkey,* [2]*Department of Physiology, Medical Faculty, Ataturk University, Erzurum, Turkey,* [3]*Department of Anaesthesiology and Reanimation, Medical Faculty, Ataturk University, Erzurum, Turkey. E-mail: kadir@atauni.edu.tr; kadiryildirim@hotmail.com*

Free radicals are believed to be important mediators of cellular injury contributing to the development of sepsis. The aim of this study was to compare the effects of drotrecogin alpha (DA) and DA plus meropenem treatment on rat liver tissue oxidant and antioxidant systems in experimental sepsis induced by cecal ligation and puncture (CLP). Twenty-four animals were equally divided into four groups: group I (sham operated), group II (sepsis), group III [DA (100 μg/kg, i.v.) and meropenem (30 mg/kg, i.v.) administered immediately before CLP] and group IV [DA (100 μg/kg, i.v.) administered immediately before CLP]. All animals were killed after 48 h and the liver tissue malondialdehyde (MDA), glutathione (GSH) and nitric oxide (NO) levels and glutathione peroxidase (GPX), glutathione-S-transferase (GST) and superoxide dismutase (SOD) activities were measured in all groups. DA alone (group IV), and plus meropenem (group III) administrations significantly decreased MDA values, and considerably increased GSH levels and GPX and GST activities compared to group II; the changes were more pronounced in group III than in group IV. These results suggest that the liver tissue MDA levels in rats with sepsis by CLP that were pretreated with DA were significantly decreased, possibly by providing GSH augmentation and GPX and GST activities. However, DA plus meropenem treatment may have a significant effect on the prevention of liver damage by free oxygen radicals.

PP-372
Effects of activated protein C alone or in combination with meropenem on oxidative stress parameters in rat lung with sepsis

A. Yildirim[1], H. Turhan[1], S. Yildirim[2], E. Bakan[1], N. Dogan[3] and F. Akcay[1]
[1]*Department of Biochemistry, Medical Faculty, Ataturk University, Erzurum, Turkey,* [2]*Department of Physiology, Medical Faculty, Ataturk University, Erzurum, Turkey,* [3]*Department of Anaesthesiology and Reanimation, Medical Faculty, Ataturk University, Erzurum, Turkey. E-mail: hamdullahturhan@hotmail.com*

Free oxygen radicals play an important role in the development of multiple organ failure in sepsis. Drotrecogin alpha (DA) is a

recombinant form of human activated protein C, which is the new drug for the treatment of sepsis. We investigated the effects of DA in addition to meropenem treatment in experimental sepsis on the oxidant and antioxidant systems in the rat lung tissue. Twenty-four rats were divided into four groups of six; group I: sham operated, group II: sepsis induced by cecal ligation and puncture (CLP), group III: received a combined therapy of DA (100 μg/kg, i.v.) and meropenem (30 mg/kg, i.v.) immediately before CLP, group IV: received only DA (100 μg/kg, i.v.) before CLP. All rats were killed after 48 hours and lung tissues were removed. The lung tissue malondialdehyde (MDA), glutathione (GSH) and nitric oxide (NO) levels and glutathione peroxidase (GPX) and superoxide dismutase (SOD) activities were measured in all groups. MDA and NO values were significantly higher in rats with sepsis than in sham operated rats. DA alone (group IV), and plus meropenem (group III) administrations significantly decreased MDA values, and considerably increased GSH levels and GPX and SOD activities compared to group II; the changes were more pronounced in group III than in group IV. These results suggest that DA plus meropenem treatment may have a significant effect on the prevention of lung tissue damage by free oxygen radicals in rats with experimental sepsis by CLP.

PP-373
Alcohol-induced oxidative stress and reduction in oxidation by ascorbate/l-cys/l-met in the liver of rat

O. K. Tunçel[1], R. Amanvermez[1], S. Ankaralı[2] and **M. Alvur**[1]
[1]Department of Biochemistry, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey, [2]Department of Physiology, Faculty of Medicine, Mersin University, Mersin, Turkey. E-mail: aramazan@omu.edu.tr

Chronic exposure to high dose of alcohol causes pathophysiological changes in the liver function due to alcohol itself and/or the effects of its metabolism (i.e., generation of acetaldehyde, NADH, free radicals and oxidative stress). The role of each of these effects in alcohol-associated pathologies is still subject to search on the liver in chronic alcoholism. It is hypothesized that cysteine-methionine and vitamin C might neutralize harmful compounds and also potentiate the antioxidant capacity of the cell or tissue. In research, the rats were fed regular diets and maintained in the following groups for 90 days: Control; Alcoholic [2.5 g of 50% ethanol/kg, (i.g.)]; Alcoholic with antioxidant supplement [2.5 g of 50% ethanol/kg + a solution contained to 200 mg vitamin C, 100 mg cysteine, 100 mg methionine administered (i.g.) every other day]. According to the results, oxidized protein and lipid content in the liver were low in the control group, higher in the antioxidant-supplemented group and the highest in the alcoholic group. Interestingly, the level of total thiol in the liver of antioxidant-supplemented group was higher than the other groups. In conclusion, chronic alcohol administration led to a significant increase in the level of lipid and protein oxidation in the liver of rats. Simultaneous intake of ascorbate/l-cys/l-met along with ethanol attenuated the amount of lipid and protein oxidation in the liver on oxidative stress resulted by alcohol consumption.

PP-374
The effect of licopen on liver damage of alcoholic rats

T. Asicioglu[1], **F. Turgay**[1], B. Aslan[1], N. Yucel[1], S. Buyukisik[1], N. Eren[1] and F. Kabukcu[2]
[1]Biochemistry Department, Sisli Etfal Education and Research Hospital, Istanbul, Turkey, [2]Pathology Department, Sisli Etfal Education and Research Hospital, Istanbul, Turkey. E-mail: nezaketeren@yahoo.co.uk

Alcohol creates oxidative stress and has toxic damage on liver. 30 Winstar albino rats were used to study the protective effects of licopen, which is known for its antioxidant properties. For 10 weeks 500 ml of ethanol per day was given to the first group to create chronic alcoholism. Second group, which received licopen in addition to alcohol was given a solution of 20% ethanol, 50% tomato juice and 30% tap water. Control group received 500 ml tap water. Blood and liver samples of rats were taken 24 h after the last application. AST and ALT levels were found out to be high when the control group was compared to the first group ($P < 0.05$). When the second and the control groups were compared, no significant difference was present. Histopathological sections of liver were stained with Haemotoxylin-Eosin and studied under light microscope. Among the group, 10% of macrovesicular steatosis, 30% of microvesicular steatosis and 60% of increase in sinusoidal cells in addition to the swelling in parenchymal cells were observed. Forty percent of the Alcohol + licopen group had normal parenchymal structure whereas 20% had microvesicular steatosis and 40% had hepatocyte swelling. Normal parenchymal structure was observed in the control group. In conclusion, ability of licopen to prevent the oxidative stress caused by alcohol was proved both by the AST, ALT levels and histopathological examination of liver sections.

PP-375
Oxidized LDL, homocysteine and glutathione in postmenopausal women treated with hormone replacement therapy

G. Andican[1], R. Gelisgen[1], L. Koldas[2], B. Cimen[1], M. T. Agac[2] and G. Burcak[1]
[1]Department of Biochemistry, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey, [2]Department of Cardiology, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey. E-mail: andican@e-kolay.net, remisagelisgen@hotmail.com

Hormone Replacement Therapy (HRT) is suggested to lower total homocysteine (tHcy) and oxidized low density lipoprotein (oxLDL) concentrations and alter the Hcy/glutathione(GSH) balance in favour of GSH. The purpose of this study was to determine plasma tHcy, oxLDL and erythrocyte GSH concentrations in a population of postmenopausal women with cardiovascular complaints treated and nontreated with HRT, and to observe the relations between them. The lipid profiles of the patients were also examined. The study was conducted on 46 postmenopausal women receiving neither statins nor antioxidants. The subjects were divided into two groups: HRT (+) group ($n = 20$), treated with HRT for at least two years and HRT (−) group ($n = 26$). In HRT (+) group triglyceride concentration was lower ($P < 0.01$) and high density lipoprotein cholesterol (HDL-C) higher ($P < 0.001$) than in HRT (−) group. No significant differences were observed in total cholesterol or LDL-C concentrations. oxLDL concentration was lower ($P < 0.05$) in HRT (+) group than HRT (−) group. tHcy and GSH concentrations did not display significant differences between the groups. Our results reflect that HRT has favourable effects on lipid profile: reduction in triglycerides and increase in the antiatherogenic HDL-C.

Importantly the concentration of oxLDL, which enhances foam-cell formation and lipid accumulation in the vasculature, was lower in postmenopausal women treated with HRT. However HRT did not appear to affect tHcy or erythrocyte GSH concentrations.

PP-376
Evaluation of lipid peroxidation and protein oxidation in hyper- and hypothyroid patients

A. Kahraman[1], F. Karabağ[1], M. Maralcan[2], S. Demir[2], R. Demirel[3] and T. Köken[1]
[1]Department of Biochemistry, The School of Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey, [2]Department of Internal Medicine, The School of Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey, [3]Department of Public Health, The School of Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey. E-mail: ahmetkah@aku.edu.tr

Objective: Our study was designed to compare the levels of plasma malondialdehyde (MDA) as an index of lipid peroxidation, protein carbonyl content (PCC) as a marker of free radical-mediated protein oxidation and protein sulfhydryl groups (SH) as a non-enzymatic antioxidant in hyperthyroid and hypothyroid patients.
Design and methods: We examined 25 hyperthyroid, 21 hypothyroid patients, and 15 euthyroid healthy controls.
Results: In the hypothyroid patients plasma malondialdehyde (MDA) levels were significantly high as compared to the control group ($P < 0.05$). There was a significant increase in protein carbonyl content in the hyperthyroid and hypothyroid patient as compared to euthyroid healthy controls ($P < 0.05$ and $P < 0.01$, respectively). There was significant decrease in protein sulfhydryl groups in hyperthyroid and hypothyroid patient as compared to control group ($P < 0.001$).
Conclusion: Our study shows that both hyperthyroidism and hypothyroidism are associated with enhanced oxidative stress as reflected by increased MDA levels, protein carbonyl content and decreased protein sulfhydryl groups.

PP-377
Advanced glycation and oxidation products in type 1 diabetic patients and relatives

A. Kural[1], C. Coskun[1], Y. Doventas[1], H. Seval[1], M. Koldas[1], F. Basinoglu[1], **E. Yetkin**[1] and Z. Saglam[2]
[1]Department of Biochemistry, Haseki Education and Research Hospital, Istanbul, Turkey [2]Department of Internal medicine, Haseki Education and Research Hospital, Istanbul, Turkey. E-mail: alevkural@hotmail.com

Objective: The link between hyperglycaemia and the complications of diabetes is unknown. It is still discussed whether oxidative stress precedes or merely reflects diabetic complications. To search for a familial predisposition to oxidative stress, we investigated indexes of glucose, lipid, protein metabolism markers of plasma and advanced glycation endproducts and advanced oxidation protein products in type 1 diabetes and their relatives.
Methods: We examined 45 type 1 diabetic subjects (30 without diabetic complications, 15 with retinopathy or nephropathy or neuropathy), 12 nondiabetic parents and 12 nondiabetic siblings of type 1 diabetic subjects. Levels of blood creatinine, glucose, HbA1c, cholesterol, triglyceride, total protein, albumin, AGE and AOPP were determined. AGE's were estimated spectrofluorimetrically (360 nm/460 nm) whereas AOPP were determined spectrophotometrically (340 nm).
Results: There was a correlation between AGE's and glucose in both type 1 DM and nondiabetic siblings and parents groups. ($r = 0.25$, $P < 0.05$) AOPP correlated with triglycerides in Type 1 DM ($r = 0.60$, $P < 0.05$). Slight elevated but not statistically significant differences in AOPP and AGE levels in diabetic patients with and without complication and their relatives ($P > 0.05$).
Conclusion: We can conclude that oxidative stress probably also plays a more important role in AGE's formation in other types DM than in type 1 DM, where AGE's formation depends more on the compensation of the disease.

PP-378
The protective role of N-acetylcysteine on cyclosporine A-induced nephrotoxicity in rats

H. Oksuz[1], S. Sogut[2], A. Koc[3], E. Uz[4], C. Tamer[1], H. Yilmaz[4], M. Duru[5], G. Kuvandik[5], H. Kaya[6], G. Genctoy[7] and Y. Akaydin[3]
[1]Department of Ophthalmology, Faculty of Medicine, Mustafa Kemal University, Hatay, Turkey, [2]Department of Biochemistry, Faculty of Medicine, Mustafa Kemal University, Hatay, Turkey, [3]Department of Histology and Embryology, Faculty of Veterinary, Mustafa Kemal University, Hatay, Turkey, [4]Department of Medical Biology and Genetics, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey, [5]Department of Emergency Medicine, Faculty of Medicine, Mustafa Kemal University, Hatay, Turkey, [6]Department of Internal Medicine, Faculty of Medicine, Mustafa Kemal University, Hatay, Turkey, [7]Department of Internal Medicine, Faculty of Medicine, Mersin University, Mersin, Turkey. E-mail: sadiksogut2@hotmail.com

Cylosporine-A (CsA) is effective in the treatment of severe intraocular inflammations and the major side effect is renal toxicity. The aim of our study was to investigate the possible role of oxidative stress in the pathogenesis of CsA-induced nephrotoxicity and the effect of N-acetylcysteine (NAC) fort he prevention of the toxicity. Rats were divided into four groups: control, NAC, CsA and CsA + NAC group. MDA, NO levels, SOD and XO activities were measured in kidney tissues of the rats. Renal impairment was assessed by blood urea nitrogen (BUN) and serum creatinine (SCr) levels as well as changes in kidney histology. Administration of CsA resulted in the increase of BUN and Cr as well as MDA, NO levels and XO activities. CsA also caused reduction of SOD activities in the kidney tissues. Morphological changes including tubular epithelial atrophy, vacuolization and cell desquamation were clearly observed in the rats treated with CsA alone. The cellular debris in the proximal tubules was prominent. NAC administration concurrently during CsA injections improved kidney functions, as indicated by lower of BUN and creatinine levels. Moreover, NAC significantly reduced MDA, NO levels and XO activities, and increased SOD activity provided a histologically-proven protection against CsA-induced nephrotoxicity. These results indicate that NAC is a protective agent against CsA nephrotoxicity and suggest there is a possible role of oxidative stress in the pathogenesis.

PP-379
Lipoic acid attenuates oxidative and nitrosative stress, simultaneously sialic acid content in liver tissues of diabetic rats

P. Atukeren[1], Z. Ozturk[1], M. K. Gumustas[1], S. Aydin[1], I. Isler Butun[1], U. Aksu[2], H. Sonmez[1], T. Altug[3] and **E. Kokoglu**[1]
[1]Department of Biochemistry, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey, [2]Department of Biology, Science Faculty, Istanbul University, Istanbul, Turkey, [3]Animal Production and Research Laboratory, Istanbul University, Istanbul, Turkey. E-mail: p_atukeren@yahoo.com

Increased oxidative and nitrosative stress and impaired antioxidant defence mechanisms are important factors in the pathogenesis of diabetes mellitus. On the other hand, alterations in the contents of sialic acid in certain tissues of diabetic patients were reported. This study was designed to determine whether lipoic acid (LA), which has been shown to have substantial antioxidant properties, would prevent oxidative and nitrosative injury and have influence on sialic acid levels in liver tissues and sera of diabetic rats. Wistar Albino rats were divided into three groups; as two diabetics and one control. Diabetes was induced by single dose STZ injection. LA was supplemented in one of the diabetic groups for 4 weeks. Liver tissue and plasma levels of nitrotyrosine; as nitrosative stress marker, TBARS and total sialic acid, and SOD activity in liver tissue and erythrocyte samples were measured. Both in the liver tissues and blood samples, levels of nitrotyrosine and TBARS and levels of total sialic acid and SOD activity were significantly increased in LA administered diabetic group when compared with the controls and decreased when compared with the diabetic group. It was concluded that, due to its antioxidant property, LA has a protective effect not only in oxidative and nitrosative stress but also in influencing the sialic acid content against hepatotoxicity caused by diabetes.

PP-380
Free radical and antioxidant enzyme levels on exposure to volatile organic compounds in workers

Ms. Bayıl[1] and Dr Celik[2]
[1]Department of Biochemistry, Health institution, Gaziantep, Turkey, [2]Department of Biochemistry, Health institution, Gaziantep, Turkey. E-mail: siybil2004@yahoo.com

Objectives: The aim of this study was to determine volatile organic solvent-associated effects on free radical level and antioxidant enzyme activities in workers who work at a paint factory.
Methods: There were twenty workers included in this study, who worked at manufacture of paint factory. Control group has twenty healthy people. Serum MDA concentration of this group was analysed as an indicator of the lipid peroxidation. Erythrocyte superoxide dismutase (SOD), serum TAC (total antioxidant capacity) were measured as an indicator of antioxidant activities.
Results: At paint group MDA level was found to be elevated than control group ($P < 0.01$). Erythrocyte SOD activity was significantly increased in paint group compared. Serum TAC level was lower in paint group compared to control ($P < 0.01$).
Conclusion: Elevated MDA levels in paint group indicate to increase oxidative stress whereas elevated SOD activity in paint compared to control expose that antioxidant system is worked. However, decreased TAC level may be indicated that other antioxidant system is used in paint group.

PP-381
Investigation of Na^+/K^+ ATPase enzyme activity, MDA level in static magnetic field

P. Uslu, C. Topcu, M. Aköz, İ. Öztok, Ö. Köylü and **M. Gürbilek**
Biochemistry, Selcuk University, Turkey. E-mail: gurbil@yahoo.com

Investigation of the occupational exposure effects of NIR (non-ionized radiation) among electric utility workers and the relationship of altering levels of MDA (malondialdehyde) and Na^+/K^+ ATPase function versus a control group which is not working in the same occasion. Our study includes 50 workers group and 50 control group. The workers group was found in the risk of area because of their days per week. The other group where we call control was not working in a specific exposure area. In both of the groups the blood samples were taken with anticoagulant and worked immediately for the three parameters, which we analysed. The mean ± SD of the parameters as follows; MDA worker 5.183 ± 0.370 nmol/ml, MDA control 3.77 ± 0.645 nmol/ml; Na^+/K^+ ATPase worker 0.128 ± 0.022 μmol.prt-1.10 min. Na^+/K^+ ATPase control 0.141 ± 0.023 μmol.prt −1.10 min. We adapt our data on SPSS version 12 statistic program and we chose *t*-test. We found that there is no significant difference between the parameters that we worked for the two groups ($P < 0.05$). So, we can say as a conclusion; occupational exposure to NIR, changes the levels of Na^+/K^+ ATPase and MDA levels of the individuals what we can do for the problem is obvious we should take into account the limits and standards of NIR. This is so important for both work and workers health and as for our next generation we should avoid magnetic sound pollution for our earth.

PP-382
Does melatonin have any effect on liver preservation in ringer lactate and University of Wisconsin solutions?

A. Coskun[1], E. Celik[1], O. Gunal[2], O. Yavuz[1], A. Aslaner[2], O. Belenli[3] and R. Memisogullari[1]
[1]Department of Clinical Biochemistry, Abant Izzet Baysal University, Duzce School of Medicine, Duzce, Turkey [2]Department of General Surgery, Abant Izzet Baysal University, Duzce School of Medicine, Duzce, Turkey, [3]Department of Pathology, Abant Izzet Baysal University, Duzce School of Medicine, Duzce, Turkey. E-mail: coskun_a2002@yahoo.com

Melatonin has antioxidant activity and reacts with singlet oxygen, hydroxyl radical, peroxynitrite and nitric oxide. Ischemia and reperfusion is a major problem in organ preservation before transplantation and cause graft failure. Thus it may be useful to add antioxidants such as melatonin – which passes membranes freely and distributes all body compartments – to preservation solutions. University of Wisconsin (UW) solution has been accepted as a gold standard solution for hypothermic liver preservation. We aimed to investigate the effect of melatonin on cold ischaemic injury of the liver. Forty Winster-Albino rats were divided into control (ringer lactate solution (RL)), RL plus melatonin, UW and UW plus melatonin groups. Liver grafts were preserved in 40 ml of solutions and ALT, AST, LDH activity, ALT/AST ratio (de Ritis) at 1, 24, 48 and 72 h were measured. Tissue injury was also evaluated on histological examination. Melatonin did not prevent elevation of enzymes and ALT/AST

ratio effectively in comparison to control group ($P > 0.05$). However histological examination revealed that melatonin added RL solution prevented congestion, peliosis, Kupffer cell infiltration, portal ven dilatation, and in UW solution prevented Kupffer cell infiltration, central and portal ven dilatation, inflammation and otolyzis. We concluded that melatonin could be added to preservation solution such as University of Wisconsin to protect tissue damage during cold ischemia period.

PP-383
The evaluation of the oxidant injury as a function of time following brain irradiation in a rat model

H. S. Erkal[2], K. Batcioglu[2], M. Serin[1], B. Uyumlu[2] and **N. Yucel**[3]
[1]Department of Radiation Oncology, Faculty of Medicine, Inonu University, Malatya, Turkey, [2]Department of Biochemistry, Faculty of Pharmacy, Inonu University, Malatya, Turkey, [3]Department of Emergency, Faculty of Medicine. Inonu University, Malatya, Turkey. E-mail: kadirbatci@inonu.edu.tr

This study presents the evaluation of the oxidant injury as a function of time following brain irradiation in a rat model. Thirty-six Wistar rats were divided into seven groups. The rats in Group 1 through Group 6 underwent irradiation, whereas the rats in Group 7 underwent sham irradiation. The rats in Group 1 through Group 6 underwent euthanasia at 1 h through 48 h following irradiation, whereas the rats in Group 7 underwent euthanasia immediately following sham irradiation. At the time of euthanasia, the brain tissue was dissected for evaluation of the malondialdehyde level and the superoxide dismutase, catalase and glutathione peroxidase activities. The mean malondialdehyde levels were increased and the mean superoxide dismutase, catalase and glutathione peroxidase activities were decreased at all of the time points for evaluation for the rats that underwent irradiation as compared to the rats that underwent sham irradiation, substantial for Group 1 and gradually stabilizing through Group 6. This study suggests that the oxidant injury might be evaluated at its best through the first several hours following brain irradiation.

PP-384
The protective effect of trapidil on ischemia reperfusion injury

S. Somuncu[1], M. Cakmak[1], **G. Dikmen**[2] and P. Dogan[2]
[1]Department of Paediatric Surgery, University of Kirikkale, Faculty of Medicine, Kirikkale, Ankara, Turkey, [2]Department of Biochemistry, Faculty of Medicine, University of Hacettepe, Sihhiye, Ankara, Turkey. E-mail: gunnur@hacettepe.edu.tr

Trapidil (5-methyl-7-diethylamino-s-triazolopyrimidine), a phosphodiesterase and platelet-derived growth factor (PDGF) inhibitor, is known to have a vasodilatator effect, anti-aggregan and antioxidant activities. The objective of the study was to investigate the effects of Trapidil on the oxidant/antioxidant status of ovarian tissues after ischemia reperfusion (IR) injury caused by adnexal torsion. In this study, 38 pubertal female New Zealand albino rabbit were assigned to four groups. In the IR group (Group 1), 3 h adnexal torsion and 3 h detorsion was performed. In the study group (Group 2), adnexal torsion was performed for 3 h and Trapidil (40 mg/kg) was administered intraperitoneally 1 hour before detorsion. In the sham group (Group 3), the left adnexa was brought through the incisions and replaced. Normal ovarian tissues were evaluated as the control group (Group 4). Left oopherectomy was done in all groups and the tissue levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and Glutathione peroxidase (GSH-Px) were determined. Intraperitoneal administration of Trapidil (40 mg/kg) in Group 2 significantly inhibited the increases of ovarian MDA content and prevented the activities of SOD, CAT and GSH-Px from declines caused by IR compared to Group 1. These results suggest that Trapidil has protective effects against ischemia-reperfusion injury due to unilateral adnexal torsion by increasing the levels of antioxidant enzymes.

PP-385
Protective effect of dehydroepiandrosterone on testicular torsion/detorsion injury

H. Aksoy[1], **T. Yapanoglu**[2], H. Turhan[1] and Y. Aksoy[2]
[1]Department of Biochemistry, Ataturk University, Erzurum, Turkey, [2]Department of Urology, Medical School, Ataturk University, Erzurum, Turkey. E-mail: aksoyhulya@yahoo.com

In the present study, we aimed to evaluate the effects of dehydroepiandrosterone (DHEA), on the antioxidant enzymes activities, lipid peroxidation and histopathology in both testes after unilateral testicular torsion and detorsion. Twenty-four adult male Sprague-Dawley rats were randomly divided into four groups ($n = 6$ for each group). Sham operation, torsion/detorsion (T/D), T/D + vehicle, and T/D + DHEA. Three hours before detorsion, 50 mg/kg DHEA was given intraperitoneally to T/D + DHEA group and 0.2 ml propylene glycol to T/D + vehicle group. Testicular ischemia was achieved by twisting the left testis 720 degrees clockwise for 3 h, and reperfusion was allowed for 24 h after detorsion. In all groups, bilateral orchiectomies were performed to determine the testicular tissue glucose 6 phosphate dehydrogenase (G6PDH), catalase (CAT), superoxide dismutase (SOD) activities and malondialdehyde (MDAlevels, and histopathologic examination). Contralateral testicular CAT, SOD and MDA values in the T/D + DHEA group were similar but G6PDH activity was higher ($P < 0.05$) when compared to the values in the ipsilateral testes. Compared with ipsilateral testes, contralateral testicular G6PDH ($P < 0.01$), CAT ($P < 0.01$) and SOD ($P < 0.05$) activities were significantly higher and MDA ($P < 0.01$) values were lower in the T/D group. Also there were significant differences in the G6PDH, CAT, SOD and MDA values between ipsilateral and contralateral testes in the T/D + vehicle group ($P < 0.01$ for all). Compared with the sham group, G6PDH and CAT activities in the ipsilateral testis obtained from T/D group were significantly lower and MDA was significantly higher ($P < 0.05$ for all). Administration of DHEA caused a decrease in MDA levels and increases in ipsilateral G6PDH, CAT and SOD activities compared to T/D group. Specimens from T/D and T/D + vehicle had a significantly greater histologic injury than sham and T/D + DHEA groups.

PP-386
Malondialdehyde and nitric oxide levels in metabolic syndrome

A. Gemalmaz[1], A. Karul[2], G. Discigil[1], S. Gurel[1], T. Kavak[2], M. Serter[2] and **O. Basak**[1]
[1]Department of Family Medicine, Adnan Menderes University, Faculty of Medicine, Aydin, Turkey, [2]Department of Biochemistry, Adnan Menderes University, Faculty of Medicine, Aydin, Turkey. E-mail: abkarul@yahoo.com

The metabolic syndrome (MS) characterized with insulin resistance consists of several cardiovascular risk factors. Oxidant stress plays an important role in various pathologies especially in MS. The aim of the study is to determine the difference of an indirect

and direct oxidant stress markers, MDA (malondialdehyde) and NO (nitric oxide) values between a group of patients with MS (n = 26) and controls (n = 34) in Umurlu/Aydin. Sixty subjects were included in the study. Of the total 37 were women and 23 were men. The mean age was 46.2 ± 14.5 years. Mean Body Mass Index (BMI) and waist circumference were found 29.1 ± 5.7 kg/m^2, 95.2 ± 14.8 cm respectively. The difference was not significant for MDA (P = 0.902) and NO (P = 0.447) between the groups.

PP-387
Escherichia coli endotoxaemia decreases the plasma L-arginine/asymmetrical dimethylarginine ratio in experimental inflammation

H. Yaman[1], Y. Kurt[1], N. Turkozkan[2], **E. Cakir**[3], H. Erdamar[2], O. Ozcan[3], E. O. Akgul[1], C. Bilgi[3] and M. K. Erbil[1]
[1]GATA Medical School, Department of Biochemistry and Clinical Biochemistry, Ankara, Turkey, [2]Department of Biochemistry, Faculty of Medicine, Gazi University, Ankara, Turkey, [3]GATA Medical School, Department of Emergency Medicine, Ankara, Turkey. E-mail: ygkurt@yahoo.com

Nitric oxide (NO) is a short-lived effector molecule derived from the enzymatic oxidation of L-arginine by nitric oxide synthase (NOS). However, over production of NO causes various inflammatory injuries. Asymmetrical dimethylarginine (ADMA) is the endogenous NOS inhibitor. Taurine is an abundant free amino acid in inflammatory cells that protects cells from inflammatory damages. The aim of the present study was to evaluate the effects of Escherichia coli (E. coli)-derived lipopolysaccharide (LPS) by measuring the ratio between L-arginine and ADMA. Guinea pigs were pretreated with taurine and saline (for the control) followed by intraperitoneal administration of LPS. Levels of plasma L-Arginine and ADMA were quantified by HPLC with fluorescence detector. Results are expressed as the median (range). LPS administration decreased plasma concentrations of L-arginine from 8.89 (4.46–11.54) μg/ml at baseline to 2.00 (0.37–4.76) μg/ml after 6 h (P < 0.001), but did not affect ADMA concentrations. Consequently, the L-arginine/ADMA ratio declined significantly. After taurine administration, the L-arginine/ADMA ratio increased significantly (P < 0.05). Acute inflammation reduces the L-arginine/ADMA ratio, which could contribute to inflammatory injury. Taurine may offer an advantage in because of it increase the reduced L-arginine/ADMA ratio. Thus, taurine protects cells from inflammatory injury resulting from overproduction of NO.

PP-388
The effects of vitamin A, vitamin C and melatonin on 3-nitrotyrosine formation in guinea pig hearts in lipopolysaccharide-induced stress

H. Yaman[1], E. Cakir[2], N. Turkozkan[3], Y. Kurt[1], B. Cimen[4], E. O. Akgul[1], O. Ozcan[2] and M. K. Erbil[1]
[1]GATA Medical School, Department of Biochemistry and Clinical Biochemistry, Ankara, Turkey, [2]GATA Medical School, Department of Emergency Medicine, Ankara, Turkey, [3]Department of Biochemistry, Faculty of Medicine, Gazi University, Ankara, Turkey, [4]Department of Chemistry-Biochemistry, Faculty of Science-Art, Kahramanmaras Sutcu Imam University, K. Maras, Turkey. E-mail: ffhalil@yahoo.com

The aim of the present study was to evaluate the effects of *Escherichia coli* (*E. coli*)-derived lipopolysaccharide (LPS) on guinea pig heart tissues by measuring 3-nitrotyrosine (3-NT) (an indicator of protein nitration) levels. In addition, the possible protective effects of vitamin A and melatonin against LPS-mediated peroxynitrite formation and the therapy effect of vitamin C against LPS-mediated peroxynitrite formation were assessed. Guinea pigs were pretreated with vitamin A, melatonin and saline (for the control) followed by intraperitoneal administration of *E. coli*. Six hours after the administration of *E. coli*-derived LPS, 3-nitrotyrosine levels in guinea pig heart tissues were measured by reverse phase high performance liquid chromatography (HPLC). In the LPS group, 3-NT levels were significantly increased compared with the groups of control, vitamin A + LPS-treated, vitamin C + LPS-treated and, melatonin + LPS-treated (P < 0.001). Vitamin A and melatonin pretreatment and vitamin C treatment prevented 3-NT formation significantly. Vitamin A, vitamin C and, melatonin may offer an advantage in that they could improve hemodynamics as well as reduce the formation of peroxynitrite.

PP-389
Antioxidant changes in coronary artery surgery with and without cardiopulmonary bypass

A. Hacışevki[1], A. Gönenç[1], H. Karagöz[2], M. Torun[1] and B. Şimşek[1]
[1]Department of Biochemistry, Faculty of Pharmacy, Gazi University, Ankara, Turkey, [2]Department of Cardiac Surgery, Güven Hospital, Ankara, Turkey. E-mail: abozkir@gazi.edu.tr

Ischemia and reperfusion is characterized by both a significant oxidative stress and characteristic changes in the antioxidant defence. Serum alpha-tocopherol, beta-carotene and ascorbic acid levels were measured to investigate effect of on (CABG) and off-pump (OPCAB) coronary artery bypass surgery and diabetes mellitus on antioxidants in 30 patients undergoing OPCAB, 12 patients undergoing CABG and 18 healthy controls. In OPCAB group, there was no difference among the periods in terms of vitamin E and C. For beta-carotene, it was observed significant difference between before anaesthesia and anaesthesia induction, and reperfusion times (P < 0.01). In CABG group, vitamin E and beta-carotene levels were showed significant differences between before anaesthesia and ischemia, and reperfusion (P < 0.01), between anaesthesia induction and ischemia, and reperfusion (P < 0.01, P < 0.05). In accordance with vitamin C levels there was found a significant relation between before anaesthesia and ischemia, and reperfusion (P < 0.01) and between ischemia and reperfusion (P < 0.05). These results support the role of oxidative stress in ischemia-reperfusion injury and emphasize the importance of antioxidant mechanisms in cardioprotection. In addition, we observed that CABG operation had more effects on coronary artery patients compared with OPCAB operation.

PP-390
Effects of fluvastatin on H_2O_2-induced oxidative stress in carotid arteries of rabbit

G. Sevin, **G. Gökçe**, G. Ozsarlak Sozer, M. Yasa and Z. Kerry
Department of Pharmacology, Faculty of Pharmacy, Ege University, Izmir, Turkey. E-mail: gokselgokce@hotmail.com

Intracellular reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) have been implicated in the pathogenesis of cardiovascular diseases. Fluvastatin (FLU), a HMG-CoA reductase

inhibitor, has not only lipid-lowering effects but also antioxidant properties. We investigated the effects of FLU on vascular responses in isolated rabbit carotid artery subjected to H_2O_2-induced oxidative stress. Endothelium-dependent relaxations in response to acethylcholine (ACh) were attenuated in rings exposed to H_2O_2 (87.5 ± 4.4%; 39.5 ± 4.9%, $P < 0.05$, $n = 5$, control versus H_2O_2). This effect was prevented by FLU (39.5 ± 4.9%; 81.5 ± 6.1%, $P < 0.05$, $n = 5$, H_2O_2 versus H_2O_2 + FLU). LNA reversed the increased ACh relaxations induced by FLU (81.5 ± 6.1%; 51.1 ± 4.3%, $P < 0.05$, $n = 5$, H_2O_2 + FLU versus H_2O_2 + FLU + LNA). Contractile responses to serotonin (5-HT) increased in H_2O_2-treated rings (113.9 ± 7.3; 131.1 ± 7.3, % of 60 mM potassium chloride-induced contraction, $P < 0.05$, $n = 5$, control versus H_2O_2). FLU decreased the augmented 5-HT contractility (131.1 ± 7.3; 51.3 ± 5.2, $P < 0.05$, $n = 5$, H_2O_2 versus H_2O_2 + FLU). LNA inverted the inhibitory effects of FLU on 5-HT contractions in rings under oxidative stress (51.3 ± 8.5; 110.9 ± 8.3, $P < 0.05$, $n = 5$, H_2O_2 + FLU versus H_2O_2 + FLU + LNA). Consequently, FLU has protective effects on H_2O_2 -induced changes in vascular reactivity. These effects of the drug may possibly be related with NO activity.

PP-391
Oxidative status, nitric oxide metabolites, and asymmetric dimethylarginine levels in obstructive sleep apnea syndrome

Y. Özkan[1], H. Fırat[2], B. Şimşek[1], S. Ardıç[2] and S. Yardım-Akaydın[1]
[1]Department of Biochemistry, Faculty of Pharmacy, Gazi University, Ankara, Türkiye, [2]Department of Chest Diseases, Hospital of Social Insurance Educational of Ankara, Ankara, Turkey. E-mail: yesim@gazi.edu.tr

Obstructive Sleep Apnea Syndrome (OSAS) is characterised by repetitive obstruction of the upper airway often resulting in oxygen desaturation, apnea/hypopnea attacks and arousal from sleep. Because of hypoxia/reoxygenation, OSAS is linked to oxidative stress and endothelial dysfunction. Also there is a rapid accumulation of evidence pointing at links between OSAS and cardiovascular morbidity and mortality. On the basis of these considerations, we measured oxidative status, nitric oxide metabolites (nitrite and nitrate), and asymmetric dimethylarginine (ADMA), which is nitric oxide synthase inhibitor, levels in OSAS patients and compared their results with those of healthy controls. Subjects were divided into groups according to apnea/hypopnea index (AHI) and cardiovascular disease history. Group I: Healthy controls, AHI < 5 ($n = 21$); Group II: Controls having CVD; history, AHI < 5 ($n = 15$); Group III: OSAS patients, AHI > 5 ($n = 45$); Group IV: OSAS patients with CVD history, AHI > 5 ($n = 12$). We observed significantly increased oxidative status, and decreased nitric oxide metabolites in Group III, especially in severe OSAS patients, compared with Group I ($P < 0.05$). Serum ADMA levels did not show any significant difference between groups. There was a significant negative correlation between oxidative status and nitric oxide levels in OSAS patients. Our result indicate that nitric oxide production is impaired, and there is an increased reactive oxygen species production in OSAS patients.

PP-392
The effects of the stobadine and taurine on renal ischemia/reperfusion injury

G. Guz[1], E. Oz[2], N. Ulusu[3], S. Omeroglu[4], N. Lordlar[4], B. Demirogulları[5], M. Stefek[6] and **C. Karasu**[7]
[1]Nephrology, Faculty of Medicine, Gazi University, Ankara, Turkey, [2]Physiology, Faculty of Medicine, Gazi University, Ankara, Turkey, [3]Biochemistry, Faculty of Medicine, Hacettepe University, Ankara, Turkey, [4]Histology, Faculty of Medicine, Gazi University, Ankara, Turkey, [5]Paediatric Surgery, Faculty of Medicine, Gazi University, Ankara, Turkey, [6]Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovak Republic, [7]Medical Pharmacology, Faculty of Medicine, Gazi University, Ankara, Turkey. E-mail: karasu@gazi.edu.tr

Ischemia-reperfusion (I/R) injury is one of the significant causes of renal dysfunction. Cellular oxidative stress is controlled primarily by the action of glutathione (GSH), and the regeneration of GSH is carried out by glutathione reductase (GR). A pyridoindole antioxidant stobadine has been demonstrated to repair oxidized amino acids and to preserve oxidation of SH groups. Taurine, an endogenous antioxidant and a membrane stabilizing amino acid, may protect tissues against I/R injury. This study investigated the effects of taurine and stobadine against renal I/R injury by evaluating tissue GR activity, serum GSH and malondialdehyde (MDA) levels, and kidney P-selectin immunoreactivity. Wistar rats were allocated into six groups: Sham, I/R, stobadine-treated, I/R + stobadine-treated, taurin-treated, and I/R + taurin-treated rats. Stobadine HCL 2.0 mg/kg, and taurine 7.5 mg/kg were given to the rats as a single dose (i.p.). Renal I/R was achieved by occluding the renal arteries bilaterally for 40 min. Following 6 h of reperfusion, blood and tissue samples were harvested. Pretreatment with stobadine or taurine was restored MDA and GSH levels determined after renal I/R. GR activity did not significantly change in I/R, IR + stobadine treated or I/R + taurine-treated group compared to sham. The high P-selectin immunoreactivity observed after I/R was significantly decreased by stobadine or taurine administration.

Acknowledgment: Supported by TUBITAK/SBAG-SLOVAK-2 (103S180) and Gazi University-BAP (01/2004-83).

PP-393
The effects of diagnostic ultrasound on oxidant/antioxidant status of rat foetus liver tissues

N. Nurlu[1], M. Kavutçu[1], A. Biri[2], İ. Karagöz[3] and O. Canbolat[1]
[1]Department of Medical Biochemistry, Gazi University Medicine Faculty, Ankara, Turkey, [2]Department of Obstetrics and Gynecology, Gazi University Medicine Faculty, Ankara, Turkey, [3]Department of Electrical and Electronics Engineering, Gazi University Faculty of Engineering and Architecture, Ankara, Turkey. E-mail: nilhannurlu@yahoo.com

The routine use of ultrasound for antenatal examination is today a virtually universal procedure. Because of the increasing ultrasonic power penetrating the body, diagnostic techniques cannot always be applied without risk. Despite its widespread use, many authors continue to express concern about the potential risks to the foetus. The embryo and foetus are susceptible to damage by physical agents such as heat and mechanical stress. In this study, we have shown the evaluation of the thermal effects of the ultrasound exposure to the rat foetal liver tissues at different observation points.24 pregnant rats were obtained, and randomly divided into three groups: Control group, B-mode (by using 3.75 MHz probe for 20 min) and Doppler (by using 16 PWR colour Doppler) ultrasonography groups. At 20th day of pregnancy

the delivered foetal liver tissues were obtained. The tissues were grouped as 23 samples for each group. To investigate the biological effects of the USG exposure to the liver, malondialdehyde (MDA) levels and the antioxidant enzymes such as Glutathione peroxidase (GSH-Px) and Catalase (CAT) activities of the liver tissues were evaluated .As a result the MDA levels were found significantly higher in doppler group versus control group ($P < 0.005$). GSH-Px activities were found significantly elevated in doppler group compared to controls ($P < 0.025$). Catalase activities were significantly increased in B-mode and doppler groups compared to controls ($P < 0.01$).

PP-394
Total phenolics content and free radical scavenging capacities of *Aesculus hippocastanum* L. bark extracts

N. Özdoğan[1] and N. Çoruh[2]
[1]*Department of Biochemistry, Middle East Technical University, Ankara, Turkey,* [2]*Department of Chemistry, Middle East Technical University, Ankara, Turkey. E-mail: nozdogan@metu.edu.tr*

Aesculus Hippocastanum L. (AH) is a well-known medicinal plant and ornamental tree, which is natural to Europe and Asia minor. In this study a high free radical scavenging capacity was encountered in the AH bark extracts which could be considered as a favourable feature to decrease the oxidative stress, and related malformations. Bark samples were extracted in 1:10 ratio of water, ethanol and ethyl acetate solvents. Each one was examined for their radical scavenging capacity using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. Percent radical scavenging data were used to calculate the 50% inhibitory concentrations (IC_{50}) for each extract. Ethyl acetate extract demonstrated the highest antioxidant capacity with an IC_{50} value of 0.009 mg/ml. Total phenolics content of the extracts were determined according to their gallic acid equivalents. Total amount of the phenolic compounds were in the range of 0.2-0.3 mg of gallic acid equivalents per mg of extracts. Ethyl acetate extract was also analysed with high pressure liquid chromatography (HPLC), equipped with, both diode array and fluorescence detectors, to isolate and characterize the active compounds in bark. HPLC analysis resulted in three separable compounds, one of them displayed florescent character with excitation and emission wavelengths at 285 and 445 nm respectively.

PP-395
Effects of propofol and dexmedetomidin on oxidative stress in rat lung and kidneys

M. Yavuz Taslipinar[1], A. Urfalioglu[2], E. Y. Akcaboy[2], N. Bayraktar[3] and N. Kilic[1]
[1]*Department of Medical Biochemistry, Gazi University Medicine Faculty,* [2]*Department of Anaesthesiology, Numune Hospital, Ankara, Turkey,* [3]*Biochemistry Laboratory, Başkent University Ankara Hospital, Ankara, Turkey. E-mail: minecu2002@yahoo.com*

The aim of the present study was to investigate whether treatment with propofol and dexmedetomidin modifies the levels of oxidative stress and antioxidant enzyme activity in rats subjected to ischemia-reperfusion injury. For this purpose, rats were divided into five groups: surgery insition group (group 1), surgery insition + ischemia-reperfusion group (group 2), dexmedetomidin 25 mcg/kg group (group 3), dexmedetomidin 10 mcg/kg group (group 4) and propofol group (group 5). In all groups except group 1, portal triad was clamped for 30 min followed by 45 min reperfusion. Fifteen minutes before clamping, group 3 was given dexmedetomidin 25 mcg/kg intraperitoneally, group 4 was given dexmedetomidin 25 mcg/kg intraperitoneally and group 5 was given 30 mg/kg intraperitoneally. At the end of the experimental procedure, rat lung and kidneys were taken to determine MDA levels and SOD activities. MDA levels, after ischemia and reperfusion in group 2 were increased significantly compared with the other groups (ple0.05). In group 5 MDA levels were significantly lower than the other groups (ple0.05). In group 3 MDA levels were lower than group 1, group 2 and group 4 but not group 5 (ple0.05). Changes in SOD activity were opposite to those in MDA contents in various groups (ple0.05). We conclude that oxidant injury occurs during ischemia and reperfusion and propofol and dexmedetomidin provides protection primarily by enhancing tissue antioxidant activity and reducing lipid peroxidation.

PP-396
The protective effects of allopurinol on glycerol-induced acute renal failure in rats

L. Erdinc[1] and M. Erdinc[2]
[1]*Department of Biochemistry, Faculty of Medicine, University of Dicle, Diyarbakır, Turkey,* [2]*Department of Pharmacology, Faculty of Medicine, University of Dicle, Diyarbakır, Turkey. E-mail: lerdinc@edu.tr*

Glycerol-induced acute renal failure (ARF) has been used as a model for myoglobinuric ARF in experimental studies. It has been suggested that, among the different pathological mechanisms, oxygen free radicals may also play a role in this model of myoglobinuric ARF. In various studies, it has been shown that Allopurinol (Allp), a xanthine oxidase inhibitor, is a free radical scavenger and has beneficial effects against ischemia reperfusion injury in different organs. In this study we aimed to investigate the effect of Allp on Gly induced ARF. Experiments were performed on wistar-albino rats, which were divided in three groups. In the first group (Control), rats were injected with saline (10 mg/kg, i.p.), in the second group(Gly), animals were injected with Gly 50% v/v in saline (10 mg/kg, i.p) and in the third group (Allp), animals were injected with Allp (50 mg/kg, s.c.), 30 min before Gly injection (10 mg/kg, i.p.). In all groups, 3 h after Gly injections, animals were anaesthetized and kidneys were removed. In Gly group, ARF was demonstrated by an increase in serum creatinine, blood urea nitrogen and malondialdehyde (MDA) levels which were significantly decreased in Allp group. As a result it was demonstrated that Allp has protective effects on Gly-induced acute renal failure in rats.

PP-397
The effect of sildenafil citrate in colonic anastomotic healing

S. Erkan[1], H. Surer[2], E. Ogus[2], A. Hasanoglu[1] and D. Yucel[2]
[1]*Department of General surgery,* [2]*Department of Biochemistry, Ministry of Health, Ankara Training Hospital, Ankara, Turkey. E-mail: haticesurer@worlcard.com.tr*

Aim: Gastrointestinal system anastomosis are the most often performed surgical procedures in general surgery departments

clinics. The studies on healing of colonic anastomosis was attracted by many colorectal surgeons. Sildenafil citrate is contributed with wound healing by increasing the microcirculatory haemodynamic effects. The proven effect of sildenafil citrate in erectile dysfunction and pulmonary hypertension was with vasodilation. In this study the effect of sildenafil citrate on anastomatic wound healing in colon was investigated.

Material and method: Twenty-four rats were divided into four groups, six in each group. Following the colonic anastomosis, anastomotic bursting pressure and the tissue level of hydroxyproline was determined at the postoperative third day in Group 1, postoperative seventh day in Group 3. For groups 2 and 4, peroral Sildenafil Sitrate was administrated 10 mg/kg per day for 1 week. After colonic anastomosis, the anastomotic bursting pressure and the tissue Hydroxyproline levels were determined at postoperative third day in Group 2, postoperative seventh day in Group 4.

Results: When the results of the bursting pressures of anastomotic site evaluated, in treatment groups the pressure levels were statistically significant higher versus control groups ($P < 0.05$).

Conclusion: Although sildenafil citrate has no comparable effects on collagen accumulation at the anastomotic area, increasing colonic bursting pressure in treatment groups may be attributed to sildenafil citrate's potential regulatory effects on haemodynamic microenvironment at the anastomotic site.

PP-398
Preconditioning effects of sildenafil citrate, valdenafil HCL and Gingko glycosides on testicular ischemia reperfusion injury

H. Surer[1], A. Ayyıldız[2], E. Ogus[1], C. Germiyanoglu[2] and D. Yucel[1]

[1]Department of Biochemistry, Ankara training Hospital, Ankara, Turkey, [2]Department of Urology, Ministry of Health, Ankara training Hospital, Ankara, Turkey. E-mail: hogus@hacettepe.edu.tr

Aim: In this study, Sildenafil Sitrate, Valdenafile HCl and Gingko glycosides administrated by oral route for 1-month up to experimental testicular torsion. After testicular torsion, in all groups malondialdehyde, nitrate and nitrite levels were determined to defining of the severity of ischemia-reperfusion injury and the comparative effects of drugs in long-term use on it.

Material and method: Eight-six male Wistar Albino rat was randomly seperated into five equal groups (in control which no drug was administrated and sham groups there were six rats). To group 1 isotonic NaCl 10 ml/day, to group 2 Valdenafile HCl 2 mg/day orally, to group 3 Sildenafil Sitrate 2 mg/day orally was administrated during one month. No drug was administrated to the rats in group 5. At the end of 1 month, left testis of all rats were torsioned 720°. After 1-h testis detortioned for two hours for reperfusion injury. At the end of third hour, both testis of the all rats were removed. Malondialdehyde, nitrate and nitrite levels were determined in testicular tissues.

Results: After testicular ischemia-reperfusion injury, there is no significant difference was found in levels of malondialdehyde, nitrate and nitrite ($P > 0.05$) in both in-groups or inter-groups.

Conclusion: The administration of Sildenafil Citrate, Valdenafile HCl and Gingko glycosides were found ineffective in prevention of testicular ischemia-reperfusion injury due to testicular torsion.

PP-399
The comparison of the nitric oxide levels before and after treatment in osteoporotic patients

M. Akoz[1], M. Portakal[2], C. Topcu[1], M. Gurbilek[1] and O. Koylu[1]

[1]Selçuk Üniversitesi Meram Tıp Fakültesi, Biyokimya Anabilim Dalı, Meram, Konya, [2]Kızılay Dispanseri F. T. R Polikliniği, Meram, Konya. E-mail: mehmetakoz2003@ yahoo.com

This research was purpose to compare the effect of calcitonin treatment on plasma nitric oxide (NO) levels in osteoporotic patients. The patients were divided into two equal groups randomly and treatment protocols were as follows:

(a) Group I: Calcitonin 200 IU + Calcitriol 0.5 mcg + Calcium 1000 mg;

(b) Group II: Calcitriol 0.5 mcg + Calcium 1000 mg.

The patients were followed for a year regularly and blood specimens of the each of the two groups before and after treatment were obtained and plasma NO values were measured. In the Group I, there was difference in plasma NO before and after treatment, while there was no significant difference in Group II patients who have not treated by calcitonin. Finally, our finding suggests that beneficial effects of calcitonin in the treatment of osteoporosis might be mediated by NO.

PP-400
The role of prostaglandin E (1) on acute ischemia reperfusion-induced renal failure in rats

N. Orhan[1] and S. Bolkent[2]

[1]Department of Neuroscience, Istanbul University Institute of Experimental Medicine, Istanbul, Turkey, [2]Department of Biology, Faculty of Science, Istanbul University, Istanbul, Turkey. E-mail: norhan@istanbul.edu.tr, sbolkent@istanbul.edu.tr

The aim of this study was to investigate the role of PGE (1) on renal ischemia reperfusion-induced acute kidney injury in rats with respect to histological, immunohistochemical and biochemical. Sprague Dawley rats were randomly divided into four groups. First group: control animals given physiologic salt solution; second group: control animals given only PGE (1) (20 mug/kg); third group: experimental animals carried out ischemia reperfusion model; and fourth group: experimental animals given PGE (1) and applied ischemia reperfusion. Kidney samples were taken for histological examination and determination of biochemical parameters. Streptavidin-Biotin-Peroxidase technique was applied as an immunohistochemical method for proliferating cell nuclear antigen (PCNA) and caspase 3. Lowry's method for protein, Beutler method for glutathione, Ledwozyw's method for lipid peroxidation and Diphenylamine method for DNA were applied in homogenized kidney tissue samples. A decrease in the histological damage; a decrease in PCNA and caspase 3 immunoreactivities; an increase in glutathione and DNA levels and a decrease in lipid peroxidation level were observed in kidneys of the experimental group with ischemia reperfusion models and given prostaglandin E (1). As a result, PGE (1) has a protective effect on acute renal ischemia reperfusion-induced renal injury in rats.

PP-401
Bismuth and medazepam-mediated interference of malondialdehyde measurement by TBA test: a cautionary note

S. Atasayar, H. Ozgunes and **H. Orhan**
Toxicology Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey. E-mail: atasayar@hacettepe.edu.tr, ozgunes@hacettepe.edu.tr, hilmi@tr.net

The thiobarbituric acid (TBA) reaction with malondialdehyde (MDA) is commonly used to measure free radical-mediated oxidative changes in lipids containing polyunsaturated fatty acids. This test has a broad range of application since it has been introduced in 1949; for proving quality of fatty foodstuff, for measuring degree of lipid peroxidation in experimental animals and in humans, and for screening *in vitro* oxidant/antioxidant capacity of synthetical or natural compounds. This broad application proves the analytical importance of TBA test in terms of reliability. The test has been critized because of its low specificity, however. We present here that in the presence of bismuth or medazepam, the test gives erroneous results in spectrophotometric assay. Among the tested compounds, bismuth inhibits the formation of MDA-TBA complex. In contrast, medazepam caused two-fold higher absorbance compared to the absorbance obtained in the absence of the drug. UV spectrum analysis of the reaction revealed that another unknown product is formed with a maximum absorbance wavelength of 458 nm. This product significantly interferes the absorbance of MDA-TBA complex at 532 nm. We have succeeded to separate this unknown product chromatographically and quantify MDA-TBA complex accurately by a HPLC method. Though it requires more sophisticated equipments, we suggest using HPLC for MDA quantification especially in biological samples, which might contain interfering compounds.

PP-402
Induction of oxidative damage by halogenated biphenyls and biphenyl ethers in rat hepatocytes

H. Gurer-Orhan[1], H. Hilmi[1], N. Nico P.[2] and J. John H.[2]
[1]*Department of Toxicology, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey,* [2]*Division of Molecular Toxicology, Department of Pharmacochemistry, Vrije Universiteit, Amsterdam, The Netherlands. E-mail: hgurer@gmail.com, hilmi@tr.net, npe.vermeulen@few.vu.nl, meerman@lacdr.leidenuniv.nl*

The present study was designed to investigate the generation of reactive oxygen species (ROS) by several mono- and di-halogenated biphenyls and biphenyl ethers and subsequent induction of ROS-mediated lipid peroxidation (LPO) in rat hepatocytes *in vitro*. For this aim, 4-chloro- and 4-bromo biphenyl (4-CB and 4-BB), 4-OH, 4?-BB, 4-bromo diphenylether (4-BDE), 4,4?-dichlorobiphenyl (4,4?-DCB), 4,4?-dibromobiphenyl (4,4?-DBB), 3,4-DCB were incubated with freshly isolated rat hepatocytes. Their oxidative potential was evaluated by both detecting the intracellular ROS formation by oxidant-sensing fluorescent probes (2?, 7?-dichlorofluorescein diacetate and C11-BOD-IPY581/591) using a multiplate reader and determining the levels of eight LPO products (formaldehyde, malondialdehyde, propanal, butanal, pentanal, hexanal, octanal and nonanal) by a gas chromatography-electron capture detection method. Mono-bromo-BBs and 4-BDE were found to induce the formation of ROS more than the mono-chlorinated congeners. 4-BDE was found to be the most effective one among all tested compounds in generating ROS and inducing LPO. Lactate dehydrogenase leakage analyses indicate that all tested compounds are cytotoxic where 4-BDE has the highest effect among all mono-halogenated congeners. Our results suggest that oxidative damage induced by these industrial pollutants is well correlated with their cytotoxic effects.

PP-403
Nitric oxide and oxidant/antioxidant status of patients with systemic sclerosis

E. Devrim[1], Ş. Erten[2], İ. B. Ergüder[1], M. Namuslu[1], M. Turgay[2] and İ. Durak[1]
[1]*Department of Biochemistry, Ankara University School of Medicine, Ankara, Turkey,* [2]*Department of Clinical Immunology and Rheumatology, Ankara University School of Medicine, Ankara, Turkey. E-mail: erdincdevrim@yahoo.com*

It was aimed to investigate oxidant/antioxidant status and possible role of nitric oxide (NO) in the etiopathogenesis of systemic sclerosis (SSc). For this aim, 29 patients diagnosed with SSc and 16 volunteer healthy subjects (as control group) participated in the study. Blood specimens were obtained from the patients and healthy subjects into anticoagulated tubes to get erythrocyte sediments. Malondialdehyde (MDA) and NO levels were measured in these specimens. Malondialdehyde levels were measured by the thiobarbituric acid reactive substances method and NO levels by the method based on Griess reaction. It was observed that NO level increased significantly (160.9 ± 33.4 versus 98.9 ± 35.1 μmol/ml, respectively; $P < 0.001$) in the erythrocyte sediments of the patients as compared to that of the control group. It was also found that MDA level increased significantly (285.1 ± 34.3 versus 261.4 ± 31.4 nmol/ml, respectively; $P = 0.047$) in the erythrocyte sediments of patient group as compared to that of the controls. There was weak positive correlation between NO and MDA levels ($r = 0.30$; $P = 0.15$ in the patient group and $r = 0.27$; $P = 0.49$ in the control group). In conclusion, the results suggest that NO level increases in the erythrocytes of patients with SSc. The increase in NO level might be due to switching of NO synthase from endothelial form (eNOS) to inducible form (iNOS) in SSc. The MDA level might be increased due to the increased NO level or some other unknown factors like decreased antioxidant capacity or increased oxidant stress.

PP-404
The effects of vitamin E and *Hippophae rhamnoides* L. on nicotine-induced oxidative stress in rat heart

S. Taysi[1], K. Gumustekin[2], B. Demircan[3], O. Aktas[2], N. Oztasan[4], F. Akcay[3], **H. Suleyman**[5], S. Akar[2], S. Dane[2] and M. Gul[2]
[1]*Department of Biochemistry, Nenehatun Obstetrics and Gynecology Hospital, Erzurum, Turkey,* [2]*Department of Physiology, Faculty of Medicine, Ataturk University, Erzurum, Turkey,* [3]*Department of Biochemistry, Faculty of Medicine, Ataturk University, Erzurum, Turkey,* [4]*Department of Physiology, Faculty of Medicine, Afyon Kocatepe University, Afyon, Turkey,* [5]*Department of Pharmacology, Faculty of Medicine, Ataturk University, Erzurum, Turkey. E-mail: mustafagul@hotmail.com*

The aim of this study was to investigate the effects of vitamin E and Hippophea rhamnoides L. extract (HRe-1) on nicotine-induced oxidative stress in rat heart. There were eight rats per group and supplementation period was 3 weeks. The groups were: nicotine (0.5 mg/kg/day, intraperitoneal); nicotine plus vitamin E [75 mg/kg/day, intragastric (i.g.)]; nicotine plus HRe-1 (250 mg/kg/day, i.g.); and the control group. The parameters were measured spectrophotometrically in tissue homogenate. Data were

analysed by one-way ANOVA with post-hoc LSD test. Nicotine increased MDA level in heart tissue compared with the control group. This nicotine induced increase in lipid peroxidation was prevented by both vitamin E and HRe-1. Glutathione peroxidase activity in nicotine plus vitamin E supplemented group was higher than others. Glutathione S-transferase activity in nicotine plus HRe-1 supplemented group was higher than the control group. Catalase activity was higher in nicotine group compared with other groups. Superoxide dismutase activity was higher in nicotine plus HRe-1 supplemented group compared with other groups. Total and non-enzymatic superoxide scavenger activities in nicotine plus HRe-1 supplemented group were higher than nicotine plus vitamin E supplemented group. Glutathione reductase activity and nitric oxide level were not affected by any of the treatments. Our results suggest that both vitamin E and HRe-1 can protect the heart against nicotine-induced oxidative stress.

DNA Damage Processing

PP-405
Toxicity induced in cultured vero cells exposed to the zearalenone, apoptosis or mutagenesis?

I. Ayed, Z. Ouanes, H. Bacha and **S. Abid**
Laboratoire de Recherche sur les Substances Biologiquement Compatibles (LRSBC), Faculté de Médecine Dentaire, Monastir, Tunisia. E-mail: salwaabid@yahoo.fr

Mycotoxins are secondary metabolites produced by *Aspergillus*, *Penicillium* and *Fusarium*. They have become a worldwide preoccupation for human and animal health and raise serious economic problems. Zearalenone (ZEN) is a fusarotoxin produced mainly by *Fusarium graminearum* in template and warm countries (Eriksen and Alexander, 1998). ZEN has several adverse effects in humans and animals. ZEN has a strong estrogenic activity associated with hyperestrogenism and several physiological alterations of the reproductive tract. Mutagenic and genotoxic proprieties of ZEN were described recently, the molecular mechanisms of its action are not yet well understood. The aim of this study was to determine the involvement of other possible mechanisms in ZEN induced toxicity. Cytotoxicity, cell cycle perturbation, genotoxicity and mutagenicity were monitored in Vero cells exposed to ZEN. Our results showed that ZEN reduced cell viability correlated to cell cycle perturbation, induces DNA fragmentation resulting in DNA laddering patterns on agarose gel electrophoresis. This observation is consistent with apoptosis, which was confirmed by observation of apoptotic bodies. Moreover, ZEN induces concentration dependant micronuclei and chromosomic aberration. This apparent contradiction between apoptotic effect and mutagenic effect of ZEN can be explained by this toxin to modify the normal cellular regulation inducing the apoptotic or the anti-apoptotic factors witch decide cell destiny.

PP-406
Systemic analysis of genetic factors that predispose to DNA damage in human placenta

O. P. Martsenyuk
Institute of Molecular Biology and Genetics National Academy of Sciences, Kyiv, Ukraine. E-mail: o.p.martsenyuk@imbg.org.ua

We have focused investigation on DNA damage and detoxification efficiency in human placenta. Damage to placental DNA has been assumed to result from the maternal exposure, maternal detoxification efficiency and can serve as a surrogate marker for DNA damage in the foetal tissues. We have revealed inverse correlation between the amount of polycyclic aromatic hydrocarbons (PAH) adducts in DNA and the cytosolic glutathione S-transferase (GST) activity. The study was conducted on 156 placental samples collected in Ukraine. We genotyped GSTP1 (A313G), GSTM1 (present or deleted allele), cytochtome P450 1A1 (A4889G) and methylenetetrahydrofolate reductase (MTHFR) (C677T) enzymes. MTHFR as a key enzyme of one-carbon unit cycle may regulate GSTP1 transcription via methylation of its GC-rich promoter. We have confirmed the association of GSTP1 mutated allele (104 Val) with the decrease of GST activity. Mutated forms of CYP1A1 and MTHFR are associated with increase of GST activity. The stimulation of GSTP1 transcription by by-products of CYP activity and by potential hypomethylation of its promoter is suggested. Analysis of GST activity at different combination of investigated genotypes has revealed the prominent role of GSTM1 deletion and MTHFR mutation in decrease and increase of GST activity, correspondingly. Thus the individual genetic peculiarities of metabolically related systems make their input in detoxification and genotoxicity and define the risk factors for newborns.

PP-407
XRCC1 forms covalent adducts with AP site

Zh. Nazarkina[1], S. Marsin[2], O. Lavrik[1], S. Khodyreva[1] and P. Radicella[2]
[1]*Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russian Federation,* [2]*Departement de Radiobiologie et Radiopathologie, Commissariat a l'Energie Atomique, Fontenay aux Roses, France. E-mail: joanna_naz@ngs.ru*

A basic sites are common products in DNA, arising either spontaneously, via accelerated base release due to chemical modification, or through glycosylase-catalysed removal of a damaged base. If unrepaired, AP lesions present mutagenic and cytotoxic challenges to the cell. The X-ray repair cross-complementing group 1 protein (XRCC1) plays a major role in facilitating the single-strand break and base excision repair (BER) in mammalian cells, via its ability to modulate and interact with multiple enzymatic components of repair reactions. XRCC1 is thought to coordinate the first stages of repair of base damages, interacting with DNA glycosylases and APE1. Here we show that XRCC1 can form covalent complex with DNA harbouring an AP site via Schiff base formation. The specificity of binding of XRCC1 to AP sites was shown by competition assays. DNA duplex containing THF residue (synthetic analogue of AP site) was shown to reduce cross-linking of XRCC1 to AP site more efficiently than regular DNA duplex. hOGG1 facilitates binding of XRCC1 with AP site possibly through protein-protein interactions. Conversely, APE1, the next player in the BER process, reduces the level of XRCC1 cross-linking to AP sites. With lower efficiency, XRCC1 can form covalent products with AP site incised by hOGG1. The domain of XRCC1 required for Schiff base formation with AP sites was determined.

The research was supported by RFBR grant no 04-04-48525, INTAS YS grant no 03-55-1683, ECO-NET grant no 10262VF.

PP-408
Trapping of topoisomerase I on nick-containing DNA in cell free yeast extracts

N. Lebedeva[1], P. Auffret Van Der Kemp[2], O. Lavrik[1], S. Boiteux[2]
[1]*Institute of Chemical Biology and Fundamental Medicine, Prospect Lavrentieva, Novosibirsk, Russia,* [2]*CEA, UMR217 CNRS 'Radiobiologie Moléculaire et Cellulaire', route du Panorama, BP6, Fontenay aux Roses, France. E-mail: nataleb@niboch.nsc.ru*

The aim of the present study was to identify proteins that bind nicked DNA intermediates formed in the course of the base excision repair process in cell free extracts of *Saccharomyces cerevisiae*. One of the most promising methodologies to trap proteins that interact with damaged DNA lies in using the photocrosslinking technique with photoactivable dNTP analogues. Using this approach, we identified a major covalent DNA-protein adduct of a molecular mass of around 100-kDa. Unexpectedly, the formation of the 100-kDa adduct does not require the incorporation of the photoreactive dNMP residue at the 3'-margin of the nick nor exposure to near UV-light. However, the formation of the 100-kDa adduct strictly requires a nick or a short gap in the DNA probe. Furthermore, the 100-kDa adduct was not detected in yeast extract lacking the Topoisomerase I. To prove further the nature of crosslinked protein, yeast TopI was tagged with a Myc-epitope. In this case, the mobility of TopI-DNA adduct was increased by 7-kDa. Therefore, our data speak in favour that TopI can be trapped by nicked DNA. In contrast, undamaged, uracil- and abasic site-containing DNAs are unable to trap TopI under the same assay conditions. Since, nicked DNA structures are frequently formed in the course of BER, their covalent adducts with TopI can potentially interfere with this process *in vivo*.

Acknowledgment: This work was supported partially by the grant from RFBR 05-04-48319.

PP-409
Exonuclease activity of APE1 towards DNA containing DNMP and its modified analogs at the 3′ end

N. Dyrkheeva, A. Lomzov, D. Pyshnyi, S. Khodyreva and O. Lavrik
Institute of Chemical Biology and Fundamental Medicine. E-mail: elpis@gorodok.net

Human apurinic/apyrimidinic endonuclease 1 (APE1) is involved in the DNA base excision repair (BER). In addition to its endonuclease activity APE1 is able to remove mispaired or modified nucleotides from the 3' end. This exonuclease activity of APE1 is considered important in proofreading of DNA synthesis during BER. We study the removal of the modified dNMP bearing different photoreactive groups at the base in comparison with natural matched and mismatched dNMP. To investigate whether the ability of APE1 to excise nucleotides from the 3' end depends on the thermal stability of the DNA duplex, we studied this characteristic of the DNA. We determined a direct correlation between exonuclease activity of APE1 and the thermal stability of DNA duplexes. The efficiency of the removal and the thermal stability of DNA duplexes varied depending on the reaction conditions and the nature of the group at the 5' end of the nick. This activity of APE1 shows a preference for nicked DNA with a hydroxyl group at the 5' end of the nick, flapped and recessed DNA. On the basis of our results, we selected the best photoreactive dNTP analogs for photoaffinity modification in APE1-containing systems. Different DNA structures containing these modified dNMP moieties at 3' end of the primer were used for photoaffinity modification of purified APE1 and proteins of cell extracts.

Acknowledgment: The research was supported by RFBR 04-04-48525, 05-04-48319, MCB program of the Presidium RAS, CRDF Y2-08-03.

PP-410
Molecular stability of Huerin's carcinoma nuclear DNA during oncogenesis

I. Shmarakov, G. Kopylchuk and M. Marchenko
Chernivtsy National University. E-mail: igor_shmarakov@mail.ru

High proliferation and metabolic activity of tumour cells demands maximum effective functioning of their genetic apparatus. One of the factors, providing this efficiency, is molecular stability of nuclear DNA, which is defined as a ratio of its biosynthesis and degradation processes. The purpose is to determine molecular stability of Huerin's carcinoma nuclear DNA during oncogenesis. It is shown, that the periods of active tumour growth, accompanying with logarithmic increase of its sizes and active invasion, are characterized by the absence of Huerin's carcinoma nuclear DNA fragmentation attributes and intensive 3-thymidine incorporation. To infringe molecular stability of tumour nuclear DNA for tumour growth oppression, a nucleotide structure preparation 3-(5-aminouracil)-5,6-benzocoumarin (BCU) developed by us was introduced. Per os introduction of BCU in a doze of 1/2 LD50 during 7 days (the total doze 42 mg/kg) to rats with a tumour causes malignant neoplasm growth inhibition on 74%. In nuclear fraction of tumour tissue high-molecular DNA fragments (sizes from 50 kbp to 10 kbp) were observed, alongside with increased in four times DNase I activity. Thus, the periods of Huerin's carcinoma active growth are characterized by nuclear DNA degradation processes oppression and increasing the intensity of its synthesis. BCU introduction provides strengthening of degradation processes, which is accompanied by tumour regression and its elimination from the organism.

PP-411
Impact of benzo[*a*]pyrene diol epoxide-DNA adducts on DNA methylation

E. Gromova[1], O. Subach[1], V. Baskunov[1], M. Dariy[1], D. Maltseva[1], A. Jeltsch[2], A. Kolbanovskiy[3], M. Kolbanovskiy[3] and N. Geacintov[3]
[1]*Chemistry Department and Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russian Federation,* [2]*School of Engineering and Science, International University Bremen, Bremen, Germany,* [3]*Department of Chemistry, New York University, New York, USA. E-mail: gromova@genebee.msu.ru*

DNA methylation is an epigenetic alteration of the genome that plays an important role in the regulation of gene expression in eukaryotes. The benzo[a]pyrene (BP) is ubiquitous environmental pollutant. It is metabolized *in vivo* to highly genotoxic dihydrodiol epoxides (BPDE), which bind to DNA causing mutations, thus contributing to the initiation of tumorigenesis. A question how DNA methyltransferases (MTases) work when carcinogen lesions are introduced in DNA is open. Our purpose is to reveal the relationship between DNA methylation and carcinogenic DNA modifications. The influence of the carcinogen on DNA methylation was studied taken C5 prokaryotic MTases HhaI and SssI

and catalytic domain of mammalian MTase Dnmt3a as examples. DNA duplexes were obtained which contain (+) or (−)-trans-anti-BP-dG residues replacing individually dG in the MTase recognition sites. The BP lesions affect primarily the methylation reaction by decreasing/blocking the efficiency of the catalytic step rather than affecting the binding of the MTases to the carcinogen-DNA adducts. The methylation efficiency is strongly dependent on adduct conformation, its position within the DNA substrate and the nature of the MTase. In (+) or (−)-trans-anti-BP-dG adduct BP fits into the minor groove of double helix. The impact of BPDE-DNA adducts on DNA methylation manifests as disturbance of DNA-MTase contacts in the minor groove of double helix.

Acknowledgment: Supported by FIRCA (TW05689) and RFBR (04-04-49488, 05-04-49690).

PP-412
DNA-PK regulates cathepsin D expression via nuclear factor-κB activity

Y. S. Hah and D. R. Kim
Department of Biochemistry and Institute of Health Sciences, Gyeongsang National University, JinJu, South Korea. E-mail: drkim@gsnu.ac.kr

DNA-dependent protein kinase (DNA-PK) plays a major role in the repair of DNA double-strand breaks produced by genotoxic agents such as ionizing radiation (IR). DNA-PK-defective cells display defect in DNA double-strand break repair (DSBR) and general radiosensitivity. In the present study, we found that Cathepsin D precursor was severely down-regulated in DNA-PK deficient human malignant glioma M059J cells in comparison of total proteins of M059K cells expressing wild type of DNA-PK. Cathepsin D (CatD), a lysosomal aspartic proteinase plays an essential role in the multiple steps of tumour progression and apoptosis induction. However, the relationship between DNA-PK and Cat D during apoptosis is poorly understood. We observed that M059J cells showed the increased levels of NF-κB and ROS but decreased sensitivity to DNA damaging reagents such as doxorubicin and etopocide. Recently, some reports have shown that DNA-PK can phosphorylate 36 serine residue and 273 threonine residue of IkBα and IkB phosphorylation by DNA-PK triggers increase of its interaction with NF-κB, resulting in reduction of the DNA binding activity of NF-κB. We found a putative NF-κB binding site (core binding sequence GGGACTTT) in the promoter region of Cat D gene. These findings suggest that cathepsin D expression is regulated by DNA-PK via NK-κB although further studies are still required.

PP-413
DNA damage induces change of cellular localization of responding proteins

Y. Choi[1], J. Park[2] and D. R. Kim[1]
[1]Department of Biochemistry and Institute of Health Sciences, College of Medicine, Gyeongsang National University, JinJu, South Korea, [2]Department of Physiology and Institute of Health Sciences, College of Medicine, Gyeongsang National University, JinJu, South Korea. E-mail: drkim@gsnu.ac.kr

DNA damage is one of the most serious threats to cell because it can result in loss or rearrangement of genetic information, that lead to cell death or carcinogenesis. Cells typically respond to DNA damage via two major pathways: DNA damage repair and programmed cell death. Upon DNA damage, cells sense damage and decide on whether to repair and survive or to die and keep secure inheritance. Cellular mechanism about this decision is not fully understood. Many DNA damage-related genes have been identified, but their cellular function in DNA damage repair still remain unclear. We sub-cloned about 40 DNA damage-related genes into the EGFP-fusion vector using the gateway cloning system and analysed change of their cellular location upon DNA damage induction by various reagents (e.g. IR, doxorubicin) in various cell types such as ATM-deficient cell, DNA-PK-deficient cell, BRCA1-deficient cell, p53-deficient cell or others. IkBa and AICDA were localized exclusively to the cytoplasm in U2OS cell without DNA damage, but nuclear foci and nuclear retention was observed after IR. RAD51 revealed its nuclear foci formation without DNA damage. However, the structure of these foci was dramatically changed to a filamentous fibre after treatment of IR. TRF, LKB1, Lig4, and FANCC revealed their different cellular pattern and foci formation upon damage induction. Besides, many other DNA damage-related genes switched their cellular location when DNA damage was induced.

PP-414
GALT mutations in a healthy Croatian population

I. Celap, L. Rumora, D. Juretic, T. Zanic Grubisic and K. Barisic
Department of Medical Biochemistry and Haematology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia. E-mail: kbarisic@pharma.hr

An inborn metabolic disorder, galactosemia, is caused by deficiency of galactose-1-phosphate uridyl transferase (GALT) enzyme. This disorder exhibits considerable allelic heterogeneity in different populations and ethnic groups. Recently, numerous mutations of GALT gene have been identified. Two of the most common mutations among Caucasians are Q188R and K285N. Both of these mutations are characterized by complete loss of the GALT enzyme activity in homoallelic individuals. Duarte galactosemia is a form of GALT deficiency, which is induced by N314D mutation. Along with the N314D mutation Duarte variant of galactosemia depends on other genetic changes in alleles, such as intronic sequence variation G1391A in intron V. Heterozygotes for Q188R, K285N and Duarte galactosemia are asymptomatic at birth, but bear higher risk for certain diseases later in life. The aim of our study was to analyse a healthy Croatian population for the frequencies of Q188R, K285N, N314D and intronic sequence variation G1391A. DNA samples from 166 healthy individuals were analysed for all four mutations by polymerase chain reaction and digestion with restriction enzymes (PCR-RFLP). Allele frequencies for Q188R, N314D, intronic sequence variation G1391A and K285N were found to be 0%, 6.6%, 7.2% and 0%, respectively. The obtained results suggest that Q188R and K285N are not the most frequent mutations among the Croatian healthy population.

PP-415
Effects of nick formation on DNA substrates via UV-irradiation on the kinetic parameters of mammalian DNA topoisomerase I

E. Kocadag[1], D. Sarıkaya[2], A. Telefoncu[1] and Z. Topçu[3]
[1]Department of Biochemistry, Ege University Faculty of Sciences, İzmir, Turkey, [2]Department of Bioengineering, Ege University Faculty of Engineering, İzmir, Turkey, [3]Department of Pharmaceutical Biotechnology, Ege University Faculty of Pharmacy, İzmir, Turkey. E-mail: kocadage@yahoo.com

DNA topoisomerases are essential enzymes that regulate the conformational changes in DNA topology by catalysing the

concerted breakage and rejoining of DNA strands. These enzymes are found in prokaryotes, eukaryotes, viruses and organelles, such as mitochondria and chloroplasts with documented essential roles in many genetic processes including DNA replication, transcription, recombination and transposition. In this study, we investigated the DNA binding characteristics of mammalian DNA type I topoisomerase by using intact and UV-irradiated plasmid substrate, pUC19, with ultimate consequences of unusual DNA conformations. The different degrees of nicks, formed on the substrate DNA, drastically changed not only the enzyme's binding affinity but also its tendency to form ternary complex with the compounds reported to interfere with the topoisomerase I activity. Considering the secondary structures of DNA, such as cruciforms and t-loops, formed in a number of *in vivo* conditions, our findings are significant in terms of the affinity of mammalian DNA type I topoisomerase with its substrate DNA within the cell.

PP-416
Analysis of spermatozoa chromatin maturity comparing swim-up and gradient centrifugation methods in assisted human reproduction

A. C. Azevedo, P. Wolff, A. S. Setti, L. R. P. Goncalves, **W. A. P. Carvalho**, E. B. Cordts, A. M. B. Souza, N. Antunes Jr and C. P. Barbosa
Centro de Reproducao Humana, Faculdade de Medicina ABC, Santo André, Brazil. E-mail: alinedecassia@bol.com.br

In the spermiogenesis, sperm chromatin packaging occurs gradually, following replacement of hisotnas by protamines and cross-linking of protamine disulfide bonds, forming a nucleus with the DNA highly condensed. This organization of the DNA not only allows the transference of genetic information to the oocyte but also assures that the correct DNA will be delivered for the correct embryo development.
Aim: To evaluate chromatin maturity in human spermatozoa before and after semen preparation for Assisted Reproductive Technique (ART).
Method: The samples were obtained from patients submitted ART procedures ($n = 40$ swin-up) and ($n = 40$ isolate) and stained with aniline blue. The analysis was made under optic microscope, counting 100 cells and classifying them: stained (histone positive = spermatozoa immature) and not stained (protaminas positive = mature spermatozoa).
Result: A negative correlation was found between the percentage of stained spermatozoa recovered before and after both techniques ($P = 0.0049$).
Conclusion: The swim-up technique should be the choice for semen preparation in ART and both techniques do not recovery spermatozoa according to their chromatin maturity. The immature spermatozoa increased in assisted reproduction procedures can be related to the incorrect fertilization mechanism, embryo implantation failure and possibly recurrent abortions.

PP-417
Relationship between plasma aluminium concentration, lymphocyte DNA damage and oxidative status in persons who have higher levels of aluminium

A. Kocyigit, N. Gunaydin, S. Selek and **O. Erel**
Department of Biochemistry, Harran University, Medical Faculty, Sanliurfa, Turkey. E-mail: hakimcell@yahoo.com

Although it is known that some metals induce DNA damage and oxidative stress, information regarding aluminium is scarce. To explore the effect of higher aluminium levels on basal levels of lymphocyte DNA damage and oxidative status, lymphocyte DNA damage, plasma protein oxidation (PO), malondialdehyde (MDA) and total antioxidative capacity (TAC) and aluminium concentrations were measured in persons who have higher levels of aluminium, and compared the data with those of healthy subjects. Aluminium concentrations were measured by atomic absorption spectrometry (AAS) and lymphocyte DNA damage was determined by the comet assay. The mean values of lymphocyte DNA damage, MDA and PO concentrations were significantly higher in aluminium group than in the control group ($P = 0.001$, $P < 0.05$ and $P < 0.05$, respectively). However, plasma TAC levels of the aluminium group were significantly lower than those of the controls ($P = 0.001$). There were significantly positive correlation between DNA damage and MDA and PO ($P = 0.074$, $r = 0.245$; $P = 0.001$, $r = 0.450$; respectively), and a negative correlation between TAC and DNA damage ($P = 0.001$, $r = (0.506)$ in the aluminium group. The possible mechanisms by which aluminium induces oxidative stress and DNA damage are discussed.

PP-418
Mononuclear leucocyte DNA damage and oxidative status: the association with hand-rolled and manufactured cigarette smoking

S. Selek, H. Celik and S. Guzel
Department of Biochemistry, Harran University, Medical Faculty, Sanliurfa, Turkey. E-mail: kocyigit30@hotmail.com

Based on the use of a filter, cigarette smoke (CS) can be divided into gas and tar phase. Both phases contain different concentrations of free radical and carcinogens. To explore the effects of both filter and non filter cigarette smoking on DNA damage and oxidative status, we measured the level of mononuclear leukocyte DNA damage using single cell electrophoresis (Comet Assay) and, plasma malondialdehyde (MDA), protein oxidation (PO) and total antioxidative capacity (TAC) and nitric oxide (NO) levels in both filter and non filter (hand-rolled) cigarette smokers. Plasma cotinine levels were also measured to estimate the degree of smoking. DNA damage, plasma MDA, PO, NO and cotinine levels were found to be significantly higher both hand-rolled and manufactured cigarette smokers than in controls and, the levels of DNA damage and cotinine were significantly higher in the hand-rolled smokers than in the filter smokers. However plasma TAC was found significantly lower in both non-filter and manufactured cigarette smokers when compared with controls. While there was a positive significantly correlation between MDA and DNA damage, there was a negative correlations between TAC and DNA damage in all smokers. These data suggest that hand-rolled CS can have much more genotoxic effects than in filtered CS. If persons have smoking habits, they should smoke filtered cigarettes, and take vitamins and foods that include antioxidants to prevent genotoxic effects of smoking.

PP-419
DNA binding properties of Xeroderma pigmentosum complementation group E protein and its various complexes

G. Kulaksiz[1], J. T. Reardon[2] and A. Sancar[2]
[1]Department of Biochemistry, Faculty of Medicine, Hacettepe University, Sihhiye, Ankara, Turkey, [2]Department of Biochemistry and Biophysics, School of Medicine, University of North Carolina, Chapel Hill, NC, USA. E-mail: gnihalk@hacettepe.edu.tr

Xeroderma pigmentosum (XP) is a syndrome characterized by increased sun sensitivity and sunlight induced skin cancer, in

some cases with neurological abnormalities. Mutations in XPA-G genes cause XP with defective nucleotide excision repair pathway. However, the exact role of XPE gene product in the nucleotide excision repair mechanism and on tumour prevention is controversial. XPE patients show mild photosensitivity although they have increased risk of skin cancers. Mutations in the small subunit (XPE, DDB2) of UV-damaged DNA binding protein (UV-DDB) cause XPE. XPE protein is implicated in damage recognition step of nucleotide excision repair. In the cell, DDB2 protein exists in 4 forms: as a DDB2 monomer, UV-DDB heterodimer with DDB1, a complex with Cullin 4A which has been shown functioning as an E3 ligase, and as a complex containing a regulatory COP9 signalosome complex. In this study, we purified DDB2 (XPE) and its various complexes and investigated the damage binding properties to better define the role of XPE in the damage recognition step on human nucleotide excision repair. We found that DDB2 has intrinsic binding activity to UV-damage. COP9 and Cullin 4A super complexes containing DDB1-DDB2 (UV-DDB) heterodimer, show similar binding properties with UV-DDB heterodimer in an *in vitro* system.
*G.K. and J.T.R. equally contributed to this work.

PP-420
Restriction enzyme EcoRII interacts with three recognition sites

G. Tamulaitis, G. Sasnauskas and V. Siksnys
Institute of Biotechnology, Vilnius, Lithuania. E-mail: gitamul@ibt.lt

According to the current paradigm Type IIE restriction endonucleases are homodimeric proteins that simultaneously bind to two recognition sites but cleave DNA at only one site per turnover: the other site acts as an allosteric locus, activating the enzyme to cleave DNA at the first. Structural and biochemical analysis of the archetypal Type IIE restriction enzyme EcoRII suggests that it has three possible DNA binding interfaces enabling simultaneous binding of three recognitions sites. To test if putative synapsis of three binding sites has any functional significance, we have studied EcoRII cleavage of plasmids containing a single, two and three recognition sites under both single turnover and steady state conditions. EcoRII kinetic studies reveal that EcoRII requires simultaneous binding of three rather than two recognition sites in cis to achieve concerted DNA cleavage at a single site. Contrary to wt EcoRII, the 1-, 2- and 3-site plasmids are cleaved by the isolated C-terminal EcoRII domain at equal rates similarly to PspGI, an isoschisomer of EcoRII, supporting previous findings that EcoRII-C per se is an orthodox Type II restriction enzyme and does not require interactions with multiple recognition sites for its optimal activity. EcoRII cleavage of plasmid DNA indicates that Type IIE restriction enzymes EcoRII and NaeI follow different reaction mechanisms. We propose that other Type IIE restriction enzymes may employ the mechanism suggested for EcoRII.

PP-421
Novel domain architecture of SDAI restriction endonuclease

G. Tamulaitiene, S. Grazulis and V. Siksnys
Institute of Biotechnology, Vilnius, Lithuania. E-mail: eigie@ibt.lt

Rare cutting restriction enzymes are important tools in genome analysis. We have determined the crystal structure of SdaI restriction endonuclease (recognition sequence 5'-CCTGCA/GG-3') providing the first structure of 8-bp cutter that produces 4-base 3' overhangs. Unlike orthodox Type IIP enzymes, which are single domain proteins, the SdaI monomer is composed of two domains. The N-terminal domain contains a classical winged helix-turn-helix (wHTH) DNA binding motif, while the C-terminal domain shows a typical restriction endonuclease fold. The active site of SdaI is located within the C-terminal domain and represents a new variant of the canonical PD…(D/E) XK motif. SdaI residues involved in DNA sequence recognition are clustered on the recognition helix of wHTH motif at the N-terminal domain. SdaI is cleaved by trypsin into an N-terminal domain, which lacks catalytic activity but binds specifically to the cognate DNA and C-terminal domain that shows neither DNA binding nor cleavage activity. The modular architecture of SdaI resembles that of Type IIS enzyme FokI and suggests a novel mechanism for palindromic nucleotide sequence recognition/cleavage by orthodox Type IIP enzymes.

PP-422
Promoter methylation and the gIVS12-6T/C polymorphism of the hMSH2 gene in head and neck cancer

S. Demokan[1], Y. Suoglu[2], M. Ulusan[2] and N. Dalay[1]
[1]*Oncology Institute, Department of Basic Oncology, Istanbul University, Istanbul, Turkey,* [2]*Istanbul Medicine Faculty, Department of Otorhinolaryngology, Istanbul University, Istanbul, Turkey. E-mail: demokansemra@yahoo.com*

Head and neck cancer is the 6th most common cancer in the world. Previous studies have shown that epigenetic changes and genetic susceptibility play an important role in the risk of developing this disease. The hMSH2 gene plays a central role in the mismatch repair. In this study, an intron splice acceptor site polymorphism (gIVS12-6T/C) in exon 13 and methylation in the promoter region of the hMSH2 gene were investigated in the tumour tissue and peripheral blood samples from 112 patients with head and neck cancer. Methylation in the promoter region was analysed by PCR and digestion with methylation-specific restriction enzymes. Polymorphic regions were amplified by allele specific PCR, the products were separated by electrophoresis and the gels were visualized by using a video gel documentation system. The hMSH2 gene was methylated in 30.3% of the patients. hMSH2 promoter methylation was more frequent in women. Polymorphic distributions in the patients were compared with allele frequencies in healthy individuals. There was no significant difference between the patients and the control group with regard to allele and genotype distributions. The variant allele was not associated with cancer risk or the clinicopathological parameters. We conclude that methylation of the hMSH2 gene may play a role in head and neck carcinogenesis while the polymorphic variant in the splice acceptor site of exon 13 in hMSH2 is not associated with the disease.

PP-423
Investigation of relationships between oxidative DNA damage, lipid peroxidation and antioxidants in lung cancer patients

E. Çalişkan Can[1], H. Firat[2], S. Ardiç[2], B. Şimşek[1] and **S. Yardim-Akaydin**[1]
[1]*Department of Biochemistry, Faculty of Pharmacy, Gazi University, Etiler, Ankara,* [2]*Department of Chest Diseases, Hospital of Social Insurance Educational of Ankara, Dişkapi, Ankara, Turkey. E-mail: emelcan@gazi.edu.tr*

The role of free radicals on the pathology of lung cancer has increasingly being of importance because lungs directly get exposed to either tobacco smoke or toxic gases or oxygen.

Reactive oxygen and nitrogen species might cause DNA damage, formation of lipid peroxidation and destruction of membrane structure by attacking membrane lipids. Nevertheless lungs have developed specific antioxidant defence systems for free radical reactions. We measured plasma MDA levels as a product of lipid peroxidation and urinary 8OHdG levels as a product of DNA damage in patients with lung cancer. In addition, antioxidant vitamin levels and their relations with MDA and 8OHdG have been investigated in lung cancer patients. When the results obtained from patients compared with those of healthy controls, they had significantly higher MDA and 8OHdG levels (2.19 ± 1.19, 1.57 ± 0.86 for patients and 1.54 ± 0.51, 1.09 ± 0.52 for healthy controls, $P = 0.003$ and $P = 0.017$) and had lower vitamin A (2.23 ± 0.89 for patients and 3.29 ± 0.84 for healthy controls, $P = 0.000$), vitamin E (28.82 ± 8.03 for patients and 32.83 ± 7.43 for healthy controls, $P = 0.036$), vitamin C (14.98 ± 4.65 for patients and 47.37 ± 23.97 for healthy controls, $P = 0.000$) and β-carotene levels (0.16 ± 0.11 for patients and 0.36 ± 0.20 for healthy controls, $P = 0.000$). When the correlations between parameters were determined, we found a positive correlation between 8OHdG and MDA levels ($r = 0.463$, $P < 0.010$) and negative correlations between 8OHdG and vitamin A ($r = –0.419$, $P < 0.021$) and β-carotene levels ($r = –0.341$, $P = 0.065$). However, we did not find any correlation between MDA and antioxidants. The results of our study supports other studies on the importance of free radical damage in lung cancer.

DNA Repair in Health, Disease and Aging

PP-424
An improved quantitative detection of telomerase activity in cervical and endometrial carcinoma

U. Eskiocak[1], U. Gündüz[1], H. Zeren[2] and Ma. Vardar[3]
[1]*Department of Biological Sciences, Middle East Technical University, Ankara, Turkey,* [2]*Department of Pathology, Medicine School of Çukurova University, Adana, Turkey,* [3]*Department of Obstetrics and Gynecology, Medicine School of Çukurova University, Adana, Turkey. E-mail: ugureskiocak@gmail.com*

Telomerase is a ribonucleoprotein complex that adds hexameric TTAGGG repeats to the ends of chromosomes in order to prevent their shortening. Telomerase activity has been evaluated for its diagnostic and prognostic value since it is observed in most malignancies but not in most normal somatic tissues. In this study telomerase activity was examined in cancer specimens of cervix, endometrium and their non-cancerous normal counterparts by an improved telomeric repeat amplification protocol (TRAP) – silver staining assay. Appearance of characteristic TRAP leader with six base pair increments indicates a positive result and was observed in all cancerous and most of the non-cancerous lesions. Telomerase activities of carcinoma tissues and normal counterparts were compared by densitometrical analysis. Significantly higher telomerase activity was observed in cervical carcinoma samples compared to normal adjacent tissue. No significant difference was observed between endometrium carcinomas and normal endometria. High telomerase activity in normal endometrium of patients restricts the use of assay for detection of carcinogenesis in this tissue. However, detection of carcinogenesis may be feasible in cervix with accurate quantification of telomerase activity by TRAP – silver stain assay.

PP-425
High expression of the rad52 gene and the capability of tumour cells for differentiation

S. A. Alexandrova and I. N. Shvemberger
Laboratory of Chromosome Stability and Cell Engineering, Institute of Cytology, St. Petersburg, Russia. E-mail: svetal@mail.cytspb.rssi.ru

It has been known that malignant cells under certain conditions can acquire the capability for differentiation and loss of malignancy. Study of molecular mechanisms of these processes can provide a possibility to affect progression of tumour. The goal of the present study was to determine nucleotide sequence of a highly variable DNA fragment amplified by means of RAPD-PCR in hepatoma MH-22a cells after induction of differentiation. The hepatoma MH-22a and its ten clonal lines were transplanted into the eye anterior chamber (EAC) of singene mice for induction of differentiation. Elements of cytotypic and histotypic differentiation were revealed in a half of the studied EAC transplants. All the transplants were studied by RAPD-PCR. In general, changes of amplification of DNA fragments were observed in 80% of the transplants. One of the most variable fragments measuring 850 bp in length was cloned and sequenced. This fragment showed an increase of amplification in tumours of several clones characterized by morphology with elements of differentiation. The sequence analysis has revealed the studied DNA fragment to be highly homologous to the fragment of the mouse gene Rad52 related to the reparation system of two-chain DNA bases. This allows suggesting that the rad52 protein can play an important role in differentiation of tumour cells.

PP-426
Mammalian telomeres: genomic damage sentinels?

N. M. Gomes[1], M. Wang[2], M. Hsieh[2], J. W. Shay[2], W. E. Wright[2]
[1]*Biophysics and Biomedical Engineering Institute, School of Sciences, University of Lisbon, Portugal,* [2]*Cell Biology, UT Southwestern Medical Center at Dallas. E-mail: nuno.gomes@UTSouthwestern.edu*

Apart from being replication counters, human telomeres may measure stress in normal diploid cells due to telomeric DNA low repair efficiency compared to genomic DNA. Growth arrest occurs when damage accumulation leads to the critical shortening of a few telomeres. Nevertheless, cells with good protection against oxidative stress have a long replicative lifespan and low telomere shortening rates. We are investigating the role of telomeres and replicative aging in the mammalian evolutionary tree, and its relationship with other genomic maintenance and repair abilities. We address the question if there is a relationship between a species telomere shortening rate and replicative senescence and the respective genomic DNA antioxidant resistance, damage susceptibility and repair ability. We grew fibroblasts of different species representative of different Orders of mammals and determined their telomere lengths and levels of telomerase activity. We are measuring the levels of cellular indicators of oxidative stress and the response to acute or chronic stress like gamma-irradiation, mild hyperoxia or chemical agents

(Ex: H2O2). The alkaline single-cell gel electrophoresis (Comet) assay is being used to examine DNA damage and subsequent single strand breaks repair efficiency at different time points. The Comet assay is being combined with the fluorescent *in situ* hybridization assay (Comet-FISH) to determine how telomeres are repaired compared to the species overall genomic DNA.

PP-427
Cooperativity in human dUTPase homotrimer

B. Varga[1], J. Kovari[1], O. Barabas[1], E. Takacs[1], A. Malnasi-Csizmadia[2], E. Hunyadi-Gulyas[3], K. Medzihradszky[3], F. Tolgyesi[4], J. Fidy[4] and B. Vertessy[1]
[1]Instituite of Enzymology, BRC, Hungarian Academy of Sciences, Budapest, Hungary, [2]Department of Biochemistry, Eotvos Lorand University, Budapest, Hungary, [3]Mass Spectrometry Facility, BRC, Hungarian Academy of Sciences, Szeged, Hungary, [4]Institute of Biophysics and Radiobiology, Semmelweis University, Budapest, Hungary. E-mail: bvarga@enzim.hu

dUTPase is responsible for preventive DNA repair via exclusion of uracil. The nuclear isoform of the human enzyme was recently implicated as a survival factor in multiresistant cancer cell lines (Chano et al., Oncol Rep., 6: 1257–1263, 2004; Pugacheva et al., Oncogene, 21: 4595–4600, 2002). We generated an efficient overexpression system for thorough characterization of this isoform and initiated cellular studies to identify interacting protein partners of the enzyme in He-La cells. Limited trypsinolysis and differential scanning calorimetry showed that human dUTPase is less ordered and more flexible than the prokaryotic enzyme. Other structural (nucleotide binding, isothermal calorimetry, circular dichroism, and other spectroscopic methods) and kinetic (steady-state and transient using a stopped-flow instrument) analyses, including the determination of the high-resolution 3D crystal structure, indicate significant differences in active site architecture as compared to E. coli dUTPase. Importantly, the three active sites of the human homotrimer seem to show non-identical cooperative behaviour in solution. In agreement with these results, the three active sites are differently ordered in the crystal structure. In physiological studies, beta-actin was identified by multiple approaches as a potential binding partner of dUTPase. Currently, we are investigating the hypothesis that the allosteric behaviour of the enzyme might be involved in its physiological role.

PP-428
O6-methylguanine-DNA methyltransferase and glutathione S-transferase activity in patients with ovarian tumours

T. Akcay, Y. Dincer, Z. Alademir, K. Aydinli, M. Arvas, F. Demirkirkiran and D. Kosebay
Cerrahpaşa Medical Faculty, İstanbul University, İstanbul, Turkey. E-mail: tulakcay@yahoo.com

Glutathione S-transferase (GST) and O6-methylguanine-DNA methyltransferase (MGMT) activity were investigated in the sera of healthy women and patients with malignant and benign ovarian tumours. O6-MGMT activity was measured by the transfer of radiolabelled methyl groups from a prepared O6-MG-DNA substrate to the enzyme fraction of sera. Our work demonstrated that untreated patients with malignant ovarian tumours revealed significantly greater MGMT and GST activities in their sera than did both healthy individuals and patients with benign ovarian tumours, while no significant difference was found between the healthy group and the patients with benign ovarian tumours with respect to their sera MGMT and GST activities. Following chemotherapy, MGMT activity was lower than the postoperative values which preceded the chemotherapy, however, this finding was not statistically significant; GST activity following chemotherapy was significantly lower than the postoperative values preceding chemotherapy. The relationship between sera MGMT and GST activities, tumour histology and pathology was not determined in this study. In conclusion, our work suggests the fact that detection of sera MGMT and GST activities is important in diagnostic and therapeutic approaches during the course of ovarian cancer.

PP-429
Genotyping of alpha-1-antitrypsin in the population of Azerbaijan

A. Ismayilova
Genetics Department, Baku State University, Baku, Azerbaijan. E-mail: aygun_ismayilova@hotmail.com

The results of populational researches of the phenotyping of alpha-1-antitrypsin among population of Siyazan and Gazakh areas of the republic. Using isoelectrofocusing for serum blood proteins to identify in polyacrylamide-ampholine plates with pH 4–6 in practically sound people and the children affected by thalassemia major diagnosis (Coolie anaemia) and their parents and among the sibs, the genotyping of normal and mutant PiM alleles were carried out. Three sound phenotypes of PiM alleles were identified as well as their phenotypic and gene frequencies (M1-38%, M2-38% and M3-24%) had been observed in both homozygous and compound states (M1M2-32%, M1M3-20% and M2M3-12%).

PP-430
ALKB reduces *in vivo* GC→AT mutations level in chloroacetaldehyde treated DNA

A. M. Maciejewska, E. Grzesiuk, A. Karcz, J. B. Lewicka and **J. T. Kuśmierek**
Department of Molecular Biology, Institute of Biochemistry and Biophysics, Polish Academy of Sciences. E-mail: agniesza@ibb.waw.pl

The *E. coli* AlkB protein is a repair enzyme, which removes alkyl lesions from bases via an oxidative mechanism restoring native DNA. Most recently, it was found that AlkB also repairs ethenoadducts (ε-adducts). We generated ε-adducts in plasmid DNA by chloroacetaldehyde treatment; subsequently, the plasmid was transformed into bacterial cells of different genetic background and mutants were selected. The plasmid carried Miller's mutation 102 (GC→AT), which allows studies of mutations caused by εG and εC. In non adapted and adapted to alkylating agents cells we observed a dose dependent increase in mutations in the *alkB* strain, which was correlated with a decrease in transformation efficiency. The double mutant *alkB, mug* (lacking in glycosylase removing εC) showed an enhanced mutagenic effect. The mutagenic effect in *alkA* (lacking in glycosylase removing εG) and *alkA, mug* strains was comparable to that observed in *alkB* and *alkB, mug*, respectively. Interestingly, in *alkB* mutant we also observed a higher mutation level caused by the initially formed $3,N^4$-α-hydroxyethanocytosine (HEC) than by the product of its dehydration, εC. Our results confirm that AlkB repairs εC and suggest that it is also able to repair HEC, indicating that its substrate specificity may be broader than previously reported.

PP-431
Regulation of the human telomerase reverse transcriptase activity by various protein kinases

J. U. Koh and **K. H. Kong**
Department of Chemistry, College of Natural Sciences, Chung-Ang University, Seoul, Republic of Korea. E-mail: khkong@cau.ac.kr

Telomerase, a specialized RNA-directed DNA polymerase that extends telomeres of eukaryotic chromosomes, is repressed in human somatic tissues and becomes active during tumour progression in most human cancer. To date, little is known about how telomerase is activated and controlled in cancer, although activation is thought to be involved in cancer cell immortalization. Here, human telomerase reverse transcriptase (hTERT) was expressed in *E. coli* and characterized to investigate its mechanism by phosphorylation. The full-length hTERT shares structural features with TERTs of other species, including a calculated molecular size of 127 kDa and conserved sequence motifs. Also, the hTERT are phosphoproteins and its phosphorylation is a prerequisite for the activation of telomerase. Thus, phosphorylation of hTERT by protein kinase represents an elemental and essential step in maintenance of telomerase activity. In addition, the activity of hTERT according to pretreatment of various protein kinases is appeared differently.

PP-432
RECQL4: one gene behind three syndromes having an increased risk for cancers

H. A. Siitonen[1], H. Kääriäinen[2] and M. Kestilä[1]
[1]*Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland,* [2]*Department of Medical Genetics, University of Turku, Turku, Finland. E-mail: Annika.Siitonen@ktl.fi*

Three members of RECQ gene family are known to cause syndromes with variable features of premature ageing. Mutations in BLM and WRN genes lead to Bloom and Werner syndromes, respectively. These genes have helical activity and probably have role in DNA replication and recombination. Mutations in the third gene *RECQL4* lead to the RAPADILINO, Rothmund-Thomson (RTS) or Baller-Gerold (BGS) syndromes. RECQL4 does not possess helical activity, but possibly has a role in maintenance of genome stability. We have studied the genotype-phenotype correlation between the *RECQL4* mutations and syndromes. The patients have overlapping clinical features such as growth retardation and radial ray defects. Yet poikiloderma, the hallmark of RTS is never seen in RAPADILINO patients. It seems that mutations, which result in an unfunctional protein cause the Rothmund-Thomson syndrome and high risk for osteosarcoma. Our main focus has been in the RAPADILINO syndrome that is overpresented in the Finnish population. The most common Finnish mutation leads to the in-frame deletion of exon seven and predisposes patients to lymphomas. In our recent studies we have observed the mislocalization of mutant protein when mutant and wildtype constructs were transfected in He-La and COS-1 cells. Other studies at the protein level are in progress. In addition, we are currently analysing genomewide expression arrays in order to further understand the RECQL4 defect in RAPADILINO patients.

PP-433
The frequency of platelet GPIA gene C807T polymorphism in patients with rheumatoid arthiritis

O. Goruroglu Ozturk[1], G. Polat[1], O. Tubay Bagdatoglu[1], G. Sahin[2], **N. Muslu**[1] and U. Atik[1]
[1]*Department of Biochemistry, Faculty of Medicine, Mersin University, Mersin, Turkey,* [2]*Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Mersin University, Mersin, Turkey. E-mail: ozlem_goruroglu@yahoo.com*

The cardiovascular comorbidity and increased mortality from cardiovascular events have been well documented in patients with rheumatoid arthiritis. However it is known that the conservative polymorphism C807T/G873A in the glycoprotein Ia gene was associated with the number of receptors on the platelet cell surface and with the risk of myocard infarction and thrombosis. The purpose of the present research was to find out if the known polymorphisms of the glycoprotein Ia genes are associated with the risk of thrombosis and myocardial infarction in patients with rheumatoid arthiritis. Genomic DNA was obtained from 62 patients with romatoid arthritis, and 106 controls. All cases of patient and control groups' were genotyped for the glycoprotein Ia gene C807T/G873A polymorphism with real-time PCR method (Roche Diagnostics GmbH Mannheim, Germany). Complete linkage between the 807 and 873 sites was found in all samples. The 807CC (873 GG), 807CT (873GA), 807TT (873AA) genotypes were found to be 47.2%, 45.3% and 7.5% in controls (n = 106); 35.5%, 51.6% and 12.9% in patients, respectively. The 807TT genotype frequency of patients with romatoid arthritis was found to be higher than healthy controls (odds ratio 1.81). Glycoprotein Ia gene mutations which is responsible for myocard infarction and thrombosis might be significant in patients with rheumatoid arthiritis.

PP-434
Inhibitory activities of benzoaxol derivatives on mammalian type I DNA topoisomerase

Z. Soyer[1], E. Kocadağ[2], **V. Pabuccuoğlu**[1], A. Telefoncu[2] and Z. Topçu[3]
[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ege University, İzmir, Turkey,* [2]*Biochemistry Department, Faculty of Sciences, Ege University, İzmir, Turkey,* [3]*Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Ege University, İzmir, Turkey. E-mail: zeynep.soyer@ege.edu.tr*

Topoisomerases are DNA-modifying enzymes with essential roles in DNA replication, transcription, recombination and transposition. There has been considerable pharmacological interest in these enzymes because DNA topoisomerases are important therapeutic targets. Numerous studies were conducted to explore new potent inhibitors as a starting point to reach new drug candidates, which led us to evaluate a number of synthetic compounds for their potential to inhibit topoisomerases. We analysed the effects of four 2 (3H)-benzoxazolone derivatives, namely 2-(2-oxo-3H-benzoxazol-3-yl)-N-(o-tolyl)acetamide (1), 3-(2-oxo-3H-benzoxazol-3-yl)-N-(m-chlorophenyl) propionamide (2), 3-(2-oxo-3H-benzoxazol-3-yl)-N-(o-nitrophenyl) propionamide (3) and 3-(2-oxo-3H-benzoxazol-3-yl)-N-(p-nitrophenyl) propionamide (4), via *in vitro* supercoil relaxation assays of mammalian DNA topoisomerase I using plasmid substrate, pBR322. Cytotoxic alkaloid, Camptothecin, a known inhibitor of eukaryotic topoisomerase I, was used as reference compound throughout the assays. Our results showed that in addition to the electronic and hydrophobic features, the conformational preferences may play an important role in determining the inhibitor potency of

w-(2-oxo-3H-benzoxazol-3-yl)-N-phenylacetamide and propionamide derivatives, since the conformational stress of corresponding acetamide derivatives including compound 1 are well documented in NMR experiments by the appearance of rotameric mixtures.

PP-435
Decline of cell cycle and apoptosis controls in the elderly epidermis after a solar light exposure

T. Zuliani[1], N. Lachman-Weber[2], C. Stenger[1], S. Schnebert[2], M. Dumas[2] and Mh. Ratinaud[1]
[1]*Laboratory of Physiologie Mitochondriale EA 3842, Faculté de Médecine , Limoges, France,* [2]*L. V. M. H. Recherche, Saint-Jean de Braye, France. E-mail: marie-helene.ratinaud@unilim.fr*

Following radiation exposures damaged keratinocytes can undergo apoptosis, which removes them from the altered tissue contributing to its homeostasis. Nevertheless, stress accumulation and repair capacity alteration lead to skin photoaging and carcinogenesis. Thus, we have investigated, *in vivo*, cells cycling capacity and apoptosis signalisation during human epidermal ageing. The parameters were assessed on biopsy cryosections provided from the sun-exposed skin of young and aged female volunteers before and 24 h after a single solar simulated radiation exposure (SSRE). Interestingly, the proportion of cycling cell ability (Ki-67 expression) declined with age. After SSRE, the cell cycle arrest was particularly marked in the young skin, since it slightly decreased in the aged one. Apoptosis (DNA fragmentation) was only observed after SSRE, in both groups with a greater sensitivity of the young volunteers; and the proportion of cells with an active caspase-3 and Bax alpha (located in mitochondria) was larger in the basal compartment of elderly. In addition, the great enhancement of p53 expression after SSRE, was similar between young and old subjects; but for these later ones the protein was significantly less phosphorylated at Ser-15. All these data show that the apoptotic response of epidermis to an acute SSRE, is a differentiation-dependent process that declines with the age, due to alterations in the cell cycle control and apoptosis signalisation.

PP-436
Consequences of a fusion protein generation in *E. coli* homologue recombination system

P. Oytun[1], A. Sue[2] and D. Pakize[3]
[1]*Biochemistry Department, Hacettepe University Medical School, Ankara, Turkey,* [2]*Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA,* [3]*Biochemistry Department, Hacettepe University Medical School, Ankara, Turkey. E-mail: oytun@hacettepe.edu.tr*

Double strand breaks are dangerous DNA lesions in all living organism. One of the most important DNA repair mechanism is homologous recombination in which the homology is required between broken and intact duplexes to form a joint molecule. This system contains three stages: presynaptic, synaptic and postsynaptic stages. RecBCD is a principal enzyme in *E. coli* homologue recombination to being in presynaptic stage. RecBCD protein consists for RecB, RecC and RecD subunits, and have unwinding, nuclease, ATPase and Chi genetic activities. In this work, we planned to test the hypothesis that RecD polypeptide regulates the RecA loading activity of RecBCD. We ligated two polypeptides, RecB and RecD, which are the slow and fast helicases of the enzyme, by an insertion of three amino acid and searched whether the RecA loading activity will be lost. Genetic and biochemical analysis showed that the constructed protein is recombination proficient and has RecA loading activity. These findings may suggest that the longer fusion insertion may be required to change the folding of heterotrimer and the enzyme activity.

Diabetes, Obesity & Metabolic Syndrome

PP-437
The effects of *Alimatis rhizoma* on differentiation and triacylglycerol level of 3t3-l1 adipocytes

H. I. Lee, S. M. Lee, K. J. Chung, Y. T. Ro, Y. H. Noh and **J. H. Koo**
Department of Biochemistry, School of Medicine, Konkuk University, Chungju, South Korea. E-mail: jhkoo@kku.ac.kr

The generation of additional adipocytes differentiated from preadipocytes is the one of the major important factors. For finding whether adipocytes differentiation is affected by natural plant extract without any toxicity, we tested the effect of *Alismatis rhizoma* (*A. rhizoma*) extracts, which are classically used for obesity control as Asian herbal medicine on 3T3-L1 preadipocyte differentiation using by microscopical observation and by determining triacylglycerol (TAG) levels in cells. The microscope observation results showed that the water extract of *A. rhizoma* did not affect the adipocyte differentiation even at 100 μg/ml concentration. However, the alcohol extract of *A. rhizoma* showed an inhibitory effect of the adipocyte differentiation at the concentration between 10 and 100 μg/ml. When the fully differentiated 3T3-L1 adipocytes were treated with up to 10 μg/ml of *A. rhizoma* alcohol extract for 24 h, the extract persistently decreased the TAG levels in the cells without showing any cytotoxicity. However, an increasing amount of the alcohol extract to 100 μg/ml showed some detrimental effects on adipocyte proliferation. Interestingly, the *A. rhizoma* alcohol extract significantly decreased the protein levels of C/EBPββ and PPARγ, two important transcription factors involved in adipocyte differentiation, suggesting that the alcohol extract of *A. rhizoma* can be used as a possible drug candidate for obesity treatment.

PP-438
REMOVED

PP-439
Obesity, insulin resistance and gallstone disease

M. González-Hita, B. Bastidas-Ramírez, F. Curiel-López, B. Ruiz-Madrigal, E. Martínez-López and A. Panduro
Department of Molecular Biology and Genomics, University of Guadalajara, Guadalajara-Jalisco, México. E-mail: mhita@hotmail.com

Background: Insulin resistance (IR) is the common factor linking metabolic syndrome, obesity, diabetes and gallstone disease (GD), which are increasing rapidly in Mexico.

Objective: To determine food intake, dietary habits, prevalence of obesity and IR in Mexican patients with GD.
Patients and methods: A cross sectional study was conducted in 99 women with cholelitiasis. A questionnaire including medical history, dietary habits, food consumption and physical activity was carried out prior to cholecystectomy. Obesity and body composition were assessed by bio-impedance. Biochemical determinations were performed.
Results: A high prevalence (63%) of central obesity, was observed. Significant ($P < 0.05$) differences between obese and lean patients were observed in blood glucose (98 mg/dl versus 88 mg/dl), insulin (18 μU/ml versus 10 μU/ml), triglycerides (148 mg/dl versus 107 mg/dl) and HOMA (4.64 versus 2.22), respectively. No statistical differences in total-cholesterol, LDL-cholesterol and HDL-cholesterol were exhibited. Obesity was associated to deficient fibre and excessive saturated fat intake. Also, a regular meal schedule and an adequate exercise practice were lacking in obese patients.
Discussion and Conclusions: Association between IR and central obesity was observed in GD patients. In this study excessive energy intake, poor exercise practice, and no regular meal schedule were the main features of obese patients, which in turn could increase the risk of presenting GD.

PP-440
H6PDH dependent reduction of intraluminal pyridine nucleotides supports 11beta-hsd1 activity in ER

I. Czegle[1], S. Piccirella[2], S. Senesi[2], M. Csala[1], J. Mandl[1], G. Banhegyi[2], R. Fulceri[2] and A. Benedetti[2]
[1]Department of Medical Chemistry, Semmelweis University, Budapest, Hungary, [2]Department of Pathophysiology, University of Siena, Siena, Italy. E-mail: czibolyka@yahoo.com

11beta-Hydroxysteroid dehydrogenase type 1 (11betaHSD1) is a NADP(H)-dependent oxidoreductase of the ER lumen, which may have an important role in the pathogenesis of metabolic syndrome. Here, the functional coupling of 11betaHSD1 and hexose-6-phosphate dehydrogenase (H6PDH) and their cofactor supply was investigated in rat liver microsomal vesicles. Both enzyme activities were latent, in agreement with the intraluminal location of their active sites. A pyridine nucleotide pool was detected fluorimetrically in the lumen of microsomal vesicles, and the microsomal membrane was not permeable toward pyridine nucleotides. Intraluminal pyridine nucleotides were mainly in reduced state. The intraluminal NADPH pool was not accessible for the extravesicular NADPH oxidase activity of microsomes. 11betaHSD1-dependent reduction of cortisone and metyrapone oxidized the intraluminal NADPH pool; on the other hand, the reducing effect of glucose-6-phosphate and cortisol was slightly detectable. However, both compounds could counteract the effect of cortisone and metyrapone. The results demonstrate the existence of a separate intraluminal pyridine nucleotide pool in the hepatic endoplasmic reticulum and a close cooperation between 11betaHSD1 and H6PDH based on their co-localization and the mutual generation of cofactors for each other.

PP-441
Relationship of the Pro12Ala PPAR-γ2 polymorphism with obesity in the adult Turkish population

E. Komurcu-Bayrak[1], V. Sansoy[2], A. Onat[3] and N. Erginel-Unaltuna[1]
[1]Department of Genetics, Institute for Experimental Medical Research, Istanbul University, Istanbul, Turkey, [2]Institute of Cardiology, Istanbul University, Istanbul, Turkey, [3]Turkish Society of Cardiology, Istanbul, Turkey. E-mail: evrimbayrak@yahoo.com

Peroxisome proliferator-activated receptor-γ2 (PPAR-γ2) is a nuclear hormone receptor that stimulates adipocyte differentiation and improves insulin sensitivity. The aim of this study was to investigate the relationship between this polymorphism and obesity in Turkish population. 320 unrelated adults (44.4% male) with a mean age of 53.9 were randomly chosen among subjects selected from the Turkish Adult Risk Factor Study Population (TEKHARF). Clinical and biochemical characteristics included: body mass index (BMI), plasma glucose concentration, total cholesterol, high and low density lipoprotein-cholesterol, triglycerides. PCR-RFLP was used for the genotype determination. The genotype distribution of the Pro12Ala PPAR-γ2 polymorphism in the adult population was 83.4% ($n = 267$), 15.9% ($n = 51$) and 0.6% ($n = 2$) for the Pro12Pro, Pro12Ala and Ala12Ala genotypes, respectively. Obese (BMI≥30) subjects had higher Ala12 allele frequency than in non-obese individuals (21.4% versus 12.8%; OR 1.86, 95% CI 1.03–3.38, $P = 0.04$). In addition, we found a significant association between Ala12 allele and dyslipidemia (coexistence of low HDL-C and hypertriglyceridemia) (OR 2.49, 95% CI 1.37–4.52, $P = 0.002$). In this study we found a relationship between the Pro12Ala polymorphism of the PPAR-γ2 gene and obesity in the adult Turkish population. These results suggest that individuals who are carriers of the Ala12 allele may be at increased risk for the metabolic syndrome.

PP-442
Overexpression of liver CPT1 in rat hepatocytes counteracts insulin effects on lipid metabolism

M. Akkaoui, C. Esnous, J. Girard and **C. Prip-Buus**
Cochin Institute, INSERM U567, CNRS UMR 8104, Department of Endocrinology, Metabolism and Cancer, Paris, France. E-mail: akkaoui@cochin.inserm.fr

Accumulation of excess fatty acids (FA) in most tissues contributes to insulin resistance. To determine whether the liver mitochondrial carnitine palmitoyltransferase 1 (L-CPT1) that is inhibited by malonyl-CoA represents a potential target to increase liver fatty acid oxidation (FAO), we transfected rat hepatocytes in primary culture with adenovirus encoding either wild-type L-CPT1 (CPT1wt) or a mutant form (CPT1mt) that is insensitive to malonyl-CoA. In basal conditions (glucose 5 mM), CPT1wt and CPT1mt overexpression led to: (a) an increased mitochondrial CPT1 protein level and activity, CPT1mt activity being totally insensitive to malonyl-CoA inhibition by contrast to CPT1wt activity; (b) a 2- and 3-fold enhanced FAO, respectively, and (c) a decreased FA esterification into triglycerides (TG). These effects were even more pronounced in the presence of 20 mM glucose/10 nM insulin, which increased the expression of lipogenic enzymes and TG content. Indeed, whereas FAO was inhibited by more than 90% in control cells, CPT1wt and CPT1mt overexpression respectively increased FAO flux by 6- and 13-fold, and inversely counteracts the glucose/insulin-induced TG accumulation. These results clearly demonstrate that L-CPT1 is a prime target to maintain a high liver FAO even in the presence of high glucose/insulin. Further *in vivo* studies will

determine whether this increased FAO can decrease hepatic steatosis and insulin resistance in animal models of obesity and type 2 diabetes.

PP-443
Insights into the mechanisms of action of the anti-diabetic agent sodium tungstate

L. Nocito[1], D. Zafra[1], C. J. Hernandez-Rodriguez[2], L. Yenush[2], J. E. Dominguez[1], J. R. Murguia[2] and J. J. Guinovart[1]
[1]Department of Biochemistry and Molecular Biology and IRB-Barcelona Science Park, Universitat de Barcelona, Barcelona, Spain, [2]IBMCP-CSIC, Universidad Politécnica de Valencia, Valencia, Spain. E-mail: lnocito@pcb.ub.es

The anti-diabetic properties of sodium tungstate have been characterized in several animal models of type 1 and 2 diabetes. Moreover, Phase I of clinical trials has been completed. In contrast, little is known about the molecular mechanisms behind the anti-diabetic actions of tungstate. Our group has shown that it induces the phosphorylation of ERK-1/2 in diverse cellular models. In order to progress in the search for the molecular targets of this compound, we have identified phenotypes in *S. cerevisiae* that are specifically associated with tungstate treatment. Our data show that this compound modifies the transport of potassium in this model and current experiments are underway to characterize its action on potassium transporters. Having established that tungstate alters the transport of potassium in yeast, we studied the effects of this compound on the ionic balance in mammalian cells. Our data show that tungstate-induced activation of ERK1/2 was sensitive to a range of potassium channel inhibitors. Moreover, the data obtained after patch-clamp and transfection experiments indicate that tungstate altered the transport of this ion through the plasma membrane. Our current research focuses on identifying the transporter/s targeted by tungstate and examining the transduction of its effects on intracellular signalling cascades.

PP-444
Hyperinsulinemia in patients with polycystic ovary syndrome

F. B. A. Benli Aksungar[1], I. O. Oz[2], A. E. T. Eren Topkaya[3] and U. O. Oz[2]
[1]Department of Biochemistry, School of Medicine, Maltepe University, Istanbul, Turkey, [2]Department of Gynaecology and Obstetrics, School of Medicine, Maltepe University, Istanbul, Turkey, [3]Department of Microbiology, School of Medicine, Maltepe University, Istanbul, Turkey. E-mail: fehimebenli@gmail.com

Most patients with polycystic ovary syndrome (PCOs) are known to have hyperinsulinemia. In this study we have evaluated serum glucose, insulin and C-peptide levels by means of oral glucose tolerans test – OGTT (100 g for 4 h) in 30 young women (aged between 21–25 years, mean 23, body mass index (BMI) < 25 kg/m^2) who were diagnosed to have PCOs by ultrasonography. The data from 30 age and BMI matched healthy women was used as a control data. Serum luteinizing hormone (LH), follicle stimulating hormone (FSH), free testosterone, sex hormone- binding globulin (SHBG), estradiol, dehydroepiandro sterone-sulphate (DHEA-S), and tumour markers of ovary and mammary glands were also measured in both groups. There were no statistically significant differences in basic clinical and hormonal parameters between two groups but patients with PCOs had abnormal OGTT results together with higher insulin ($P = 0.023$) and C-peptide ($P = 0.018$) levels compared to the control group. These findings suggest that hyperinsulinemia (insulin resistance) found in patients with PCOs can be seen independently from the BMI and hyperandrogenism.

PP-445
High levels of glucose and kcnm genes

E. Leon-Gomez, V. Rivero-Valdenebro, J. Villar and J. Navarro-Antolin
Laboratorio de Investigacion Cardio-Vascular (LICAVA) y Unidad Clínico Experimental de Riesgo Vascular (UCERV), Hospital Virgen del Rocío, Seville, Spain. E-mail: franciscoj.navarro.exts@juntadeandalucia.es

Transient increases in plasma glucose concentrations play an important role in the development of microvascular dysfunction in diabetes. Previous studies in small arteries and arterioles of diabetic subjects have demonstrated that before the appearance of morphological changes a vasomotor dysfunction of microvessels develops. Maxi-K^+ channels, distal effectors of endothelial nitric oxide, are mainly expressed in arterial myocytes. We evaluated the regulation of its regulatory beta1 subunit by high levels of glucose in arterial smooth muscle cells. High levels of extracellular glucose produced decrease of maxi-K^+ beta1 subunit mRNA levels in rat aortic myocytes and in human leukocytes (Jurkat cells) as evaluated by quantitative real-time PCR with Sybr green and TaqMan Probes. This was paralleled by a reduction of beta1 subunit protein level as determined by immunocytochemistry (confocal microscopy and flow cytometry with monoclonal antibodies). In the phenilephrine (Phe)-induced vasoconstriction, the vasorelaxing force of iberiotoxin-sensitive maxi-K^+ channels was diminished in rat and human arterial rings exposed to high levels of glucose. These results indicate that decrease of the maxi-K^+ channel beta1 subunit expression in arterial myocytes is a key factor in the vasomotor alterations induced by high levels of glucose. The data suggest that the maxi-K^+ beta1 subunit should be considered as a possible new therapeutic target to correct hyperglycaemia-derived hypertension.

PP-446
Aspirin, D-penicillamine and vitamin E preserve endothelial cell migration against high glucose

C. Smith, J. Zhang, M. Slevin, Y. Duraisamy, J. Gaffney and N. Ahmed
School of Biology, Chemistry and Health Science, Manchester Metropolitan University, Manchester, UK. E-mail: c.a.smith@mmu.ac.uk

Angiogenesis or the formation of new blood vessels is an important process during wound healing during which endothelial cells undergo proliferation and migration. Angiogenesis declines in patients with diabetes and this may account for the slow wound healing seen in these subjects. Hyperglycaemia has been reported to reduce migration of cultured endothelial cells following wounding *in vitro*. The protective effects of the antiglycation compounds aspirin, D-penicillamine and vitamin E against high (toxic) concentrations of glucose on a monolayer of cultured bovine aortic endothelial cells were investigated. The monolayer was wounded in the presence of different concentrations of glucose with and without these compounds and the recovery of the cells at the wound site measured. The migration of the cells following wounding was inhibited by increasing concentrations of glucose alone. However, migrating activity was maintained in the presence of all three compounds, with vitamin E, affording the

greatest protection against glucose toxicity. This suggests that compounds like vitamin E, which combine antiglycation and antioxidant properties, may have therapeutic potential in protecting diabetics against high glucose toxicity.

PP-447
Antiglycation compounds protect endothelial cell proliferation against glucose-mediated damage

N. Ahmed, J. Zhang, M. Slevin, Y. Duraisamy, J. Gaffney and C. Smith
School of Biology, Chemistry and Health Science, Manchester Metropolitan University, Manchester, UK. E-mail: n.ahmed@mmu.ac.uk

Endothelial cells are prime targets for the toxic effects of hyperglycaemia and advanced glycation endproducts (AGEs), which underlie the pathogenesis of diabetic vascular complications. The protective effects of the antiglycation compounds, aspirin, D-penicillamine and vitamin E against high glucose concentrations and AGE-mediated toxicity on the proliferation of cultured bovine aortic endothelial cells was investigated *in vitro*. The addition of increasing concentrations of glucose to cultured endothelial cells inhibited their proliferation in a dose dependent manner. All three compounds protected against these antiproliferative effects with vitamin E being the most effective. This also give the most protection against the antiproliferative effects of bovine serum albumin derived AGEs (BSA-AGE). D-penicillamine was less protective than vitamin E, whereas aspirin did not offer any significant protection against AGE-induced cellular toxicity. This study suggests that compounds such as vitamin E that posses a combination of antiglycation and antioxidant properties may have therapeutic potential in protecting diabetic patients against high glucose and AGE-mediated cellular toxicities.

PP-448
Analysis in silico of the promoters of the genomic signature of nonalcoholic steatohepatitis (NASH)

F. Aillet[1], F. Zhao[2], A. Rubio[3], M. L. Martinez-Chantar[4], J. M. Mato[4] and S. Rodriguez[1]
[1]Proteomics Unit, CIC BioGUNE, Derio, Spain, [2]Cold Spring Harbor Laboratory, New York, USA, [3]TECNUN, Universidad de Navarra, San Sebastian, Spain, [4]Metabolimics Unit, CIC BioGUNE, Derio, Spain. E-mail: faillet@cicbiogune.es

NASH is a disease of the liver of unknown aetiology characterized by fatty acid accumulation, hepatocyte damage and inflammation. NASH is a critical stage that spans from hepatic steatosis to cirrhosis and liver failure. MAT1A encodes enzymes responsible of S-adenosylmethionine (SAMe) synthesis in liver. MAT1A knockout mice spontaneously develop NASH and hepatocellular carcinoma at about 8 and 15 months of age. A genomic signature of NASH has been obtained from a genome-wide expression profiling of liver patients from NASH and from a mouse model of steatohepatitis. Expression of 81 genes involved in mitochondria, metabolic activities and oxidative stress are deregulated in the pathogenesis of steatohepatitis. Here, we analysed the promoters of these 81 genes up and down regulated by using a transcriptional regulatory element database (http://rulai.cshl.edu/cgi-bin/TRED/). High binding scores for 18 transcription factors have been calculated. Results were presented as overall promoter response or by cellular functions. We found the highest binding scores for Sp1 in the total promoter response. However other transcription factors such as NF-? B, and c-myc showed high binding scores in genes involved in metabolic activities, suggesting that those transcription factors may also be involved in the expression of such genes. The binding of Sp1 to these promoters validates the relevance of this analysis and suggests that Sp1 might be involved in the genesis and development of NASH.

PP-449
Ontogenic development of glucokinase and glucokinase regulatory protein in rat brain

I. Roncero, P. Vázquez, **E. Alvarez**, C. Sanz, P. Barrio and E. Blázquez
Department of Biochemistry and Molecular Biology, Faculty of Medicine, Complutense University Madrid, Spain. E-mail: eao513@med.ucm.es

Our previous description of functional glucokinase (GK) isoforms and its interactions with glucokinase regulatory protein (GKRP) in the brain of human and adult rat, suggested that both might participate in the process of glucose-sensing in the brain. GK is considered as the real glucose sensor localized in the glucose-excited and glucose-inhibited neurons of the hypothalamus because, it is not inhibited by glucose-6-phosphate, has a low affinity for glucose, and its kinetic co-operativity with glucose allows the rate of glucose phosphorylation to be directly proportional to blood glucose concentrations. GK activity may also be regulated by the presence of GKRP. We studied the expression of these molecules during the ontogenic development. Using RT-PCR analysis we found mRNAs coding for the GK pancreatic isoform and GKRP; GK and GKRP proteins were also identified, using western blot in brain extracts of foetal and suckling rats. Low and high Km hexokinases present in the brain were characterized. Thus, we found in the hypothalamus glucose phosphorylating activities with a high apparent Km for glucose and no product inhibition by glucose-6-phosphate. The apparent Km of GK in foetal hypothalamic extracts was 9.1 mM and 7.5 mM in suckling 10-day old rats. These findings indicate that both proteins are functionally active during foetal age, at a period of life before they are needed to act as glucose sensors implied in the control of feeding behaviour.

PP-450
Nonenzymatic glycation of kidney type IV collagen of diabetic rats and the effect of lipoic acid on collagen glycation

S. Bilmen Sarikcioglu[1], O. N. Ozes[2], U. Cakatay[3], T. A. Aksu[4] and S. Gumuslu[4]
[1]Central Laboratory, Akdeniz University Hospital, Antalya, Turkey, [2]Intermune Inc., CA, USA, [3]Department of Biochemistry, Istanbul University, Istanbul, Turkey, [4]Department of Biochemistry, Akdeniz University, Faculty of Medicine, Antalya, Turkey. E-mail: bilmens@akdeniz.edu.tr

Nonenzymatic protein glycation is one of the complications of diabetes and it is shown to be associated with the development of other long-term complications of diabetes. It has been known for a long time that type IV collagen undergoes nonenzymatic glycation in kidney tissues of diabetic patients. It has been reported that alpha-lipoic acid (LA), which has several antioxidant properties, has potential preventing and improving effects against oxidative damage both in type I and type II diabetes. There are also several studies reporting that LA prevents protein glycation *in vitro*. The aim of the present study is to investigate whether LA, the protective effects of which are shown against glycation *in vitro* will exhibit the similar protective effects *in vivo*. For this

purpose, 37 rats were divided into four groups (control, diabetes, lipoic acid + diabetes, lipoic acid). Diabetic rats exhibited impaired renal function where LA administration to the diabetic rats prevented this impairment. Besides, the results of immunoprecipitation, western blot and immunohistochemistry assays have shown that type IV collagen protein of kidneys of diabetic rats underwent glycation and LA administration has prevented this glycation. LA has shown the preventive effect against glycation *in vivo* as well as *in vitro*.

PP-451
Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) activity in diabetic patients

A. Causevic, M. Malenica and T. Dujic
Department of biochemistry and clinical analysis, Faculty of Pharmacy, University of Sarajevo, Bosnia and Herzegovina. E-mail: acausevic5@hotmail.com

Diabetes mellitus represents heterogeneous group of disorders of carbohydrate metabolism characterized by hyperglycaemia. Type I diabetes is being recognized as a disease resulting from cell mediated autoimmune destruction of the pancreatic beta cells, while type II diabetes represents heterogeneous disorder associated with insulin resistance. In majority of patients with type II diabetes, insulin resistance precedes development of diabetes. Recent studies have documented strong relationship between hepatic steatosis and insulin resistance. In this work, activity of enzymes, markers of liver injury (ASAT and ALAT) was tested in 40 patients (both males and females characterized by the absence of hepatitis virus infection or heavy alcohol consumption) classified with diagnosis of diabetes type I and (40 patients, classified in the same manner) with diagnosis of diabetes type II .Aim was to find out possible associations between these markers and type of diabetes. Activity of these enzymes close to upper level of the normal range was obtained in patients characterized with diagnosis of diabetes (both types) when compared to controls. Differences in all the parameters examined were statistically significant. However, our study has pointed out that there is no correlation between concentration of glucose and the activity of examined enzymes in all categories of the patients tested.

PP-452
Histological analyze of pancreas in diet induced obese female rats

B. Z. Altunkaynak, E. Ozbek and M. E. Altunkaynak
Department of Histology and Embryology, Medical School, Ataturk University, Erzurum, Turkey. E-mail: berrinzuhal@yahoo.com

High fat diets are thought to be a risk factor for Type 2 diabetes and associated complications. We investigated effects of high fat diet on the development of diabetes-associated pathologies in female Sprague dawley rats. Animals were divided in two groups as control and treatment. Rats in treatment group were fed with a diet containing 30 kcal (%) fat. Light microscopy was used to examine the structure of the pancreas. According to BMI (Body Mass Index) values, animals fed with high fat diet were obese. Increased fat consumption was associated with severe structural alterations of acinar, islets and blood vessels in the pancreas. Presence of the lymphatic infiltrates in oedematous islets and destruction of the islets were detected. There were lipid droplets in serous acini and there was an increase in interlobular connective tissue content. Also it was found that adipocytes accumulation localized in interlobular area. Between the serous acini, necrotic foci and lymphoid cells were seen. The results show that high fat intake may be induced diabetes and pancreas damage in rats, probably linked to delayed insulin secretion. The model was also associated with the development of a range of pathologies characteristic to human diabetes. Thus, high-fat fed rats provide a model that is likely to be useful in understanding the cellular and molecular mechanisms involved in the pathogenesis of diabetes.

PP-453
Effect of chronic fatty diet induced obesity on structure of the salivary glands

B. Z. Altunkaynak, E. Ozbek and M. E. Altunkaynak
Department of Histology and Embryology, Medical School, Ataturk University, Erzurum, Turkey. E-mail: draltunkaynak@gmail.com

The effect of chronic fatty diet consumption on the structure of the salivary glands was studied. In obese patients there was a significantly reduced size of major salivary glands. Also in literature, it was reported that the saliva excretion was significantly increased and hyperplasia followed by hypertrophy of salivary glands in overweight persons. To investigate effects of obesity on salivary glands, it has been performed a diet induced obesity model in female rats (n = 10 animals) with high fat diet and 10 female rats were utilized as control. In histological procedure, sections were obtained from all parotid and sublingual glands. Then these sections were examined by a light microscope with camera attachment. The parotid and sublingual glands in these animals showed an impressive fatty degeneration. Additionally there was atrophy of the acinar cells. The parotid and sublingual glands of obese animals showed a swelling of the acinar cells and an increase in the interlobular connective tissue. Also in these sections, lymphoid cell infiltrations and necrosis were detected. The results of this study indicate that fatty diet after chronic consumption may be cause severe histological alterations on major salivary glands.

PP-454
Effect of combination of alt-711 and sildenafil on oxidative stress in STZ-diabetic rats

Mf. Usta[1], **G. Sagdic**[2], N. Gurbuz[2], A. Sanli[1], R. Gurkan[1] and A. Baykal[2]
[1]Department of Urology, Section of Andrology, Akdeniz University School of Medicine, Antalya, Turkey, [2]Department of Biochemistry, Akdeniz University School of Medicine, Antalya, Turkey. E-mail: gamzesagdic@akdeniz.edu.tr

Prolonged hyperglycaemia, and oxidative stress in diabetes result in the production and accumulation of AGEs. We aimed to investigate whether a combination of sildenafil and AGE cross--link breaker (ALT-711) could have a synergistic effect on oxidative stress and AGE formation in STZ-diabetic rats. For this purpose, Wistar rats (18–20 weeks old) were used and divided into four groups as, control (n = 10), STZ-diabetic rats (n = 10), STZ-diabetic rats treated with sildenafil (5 mg/kg/day p.o.) (n = 10), STZ-diabetic rats treated with sildenafil (5 mg/kg/day p.o.) + ALT-711 (10 mg/kg/day p.o.) (n = 10). ALT-711 and sildenafil treatment started 1 month after diabetes mellitus induction. 5-Hydroxymethyl-2-furfural (5-HMF) and MDA (malondialdehyde) contents were measured in penil tissues using by spectrophotometric (443 nm) and fluorometric (525/547 nm) methods respectively. We found that MDA and HMF levels was significantly higher in the diabetic group than in the control group. Sildenafil treatment could not prevented the increase of

oxidative stress alone. These alterations were reverted back to near normal levels after the treatment with Sildenafil and ALT-711. Treatment with combination of sildenafil and ALT-711 was more effective on lipid peroxidation and AGE formation.

PP-455
Prevalence intron 1 duplication among patients susceptible to primary hyperoxaluria type 1 in Iran

A. Ghorbani[1], A-A. Ziaee[1] and G. Pourmand[2]
[1]*Institute of Biochemistry and Biophysics ,University of Tehran, Tehran, Iran,* [2]*Urology Research Center, Tehran University of Medical Sciences, Tehran, Iran. E-mail: ghorbani@ibb.ut.ac.ir*

Primary hyperoxaluria type 1 (PH1) is an autosomal recessive metabolic disease. Diagnosis maybe suspected when clinical signs and increased urinary oxalate and glycolate are excreted. The occurrences of this type of the disease is confirmed by measurement of the decrease in Alanin glyoxylate amino transferase (AGT; EC 2.6.1.44) activity in liver samples. In the absence of AGT, glyoxylate is converted to oxalate, which forms insoluble calcium salts. There are two normal haplotypes: the major and minor alleles that commonly found among normal populations. These alleles differ at several polymorphism sites; P11L, I340M and intron 1 duplication. Sixty patients having caox stone several times during their life, with an average age of 46 years were considered and their DNA samples were extracted, then the designed PCR protocol was carried out. Prevalence intron 1 duplication was 1.7% (homozygote) and 30% (heterozygote). Mutation analysis of AGXT gene is accurate and not risky tools for the detection of PH1. Carrying out this test for the minor allele is usually the first step for screening mutation in PH1. This because enzyme activity is not readily available to paediatric nephrologists in many parts of the world and measurement of oxalate is not practical as well.

PP-456
Serum sialic acid, hs-CRP and oxidative stress parameters in obese children

F. M. Yılmaz[1], G. Yılmaz[1], Ş. Erdeve Savaş[2], Y. Dallar[2], B. Ç. Topkaya[1] and **D. Yücel**[2]
[1]*Biochemistry, Ministry of Health Ankara Hospital, Ankara, Turkey,* [2]*Paediatry Clinics Ministry of Health, Ankara Hospital, Ankara, Turkey. E-mail: fatmamericyilmaz@hotmail.com*

Obesity is a common health problem and is increasing among children in the last years. Obesity is accompanied by a high incidence of atherosclerosis, arterial hypertension and type II diabetes mellitus. In this study, we determined sialic acid levels and investigated the correlations with malondialdehyde (MDA), susceptibility to oxidation (SO), total thiol concentrations, glucose and lipid profile in 39 obese (BMI 26.6 ± 3.9) and 33 sex -and age- matched healthy children (BMI 15.9 ± 1.7). MDA concentrations, SO and hs-CRP were significantly higher in obese children than controls ($P = 0.000$, $P = 0.000$ and $P = 0.000$, respectively). Sialic acid and total thiol concentrations were higher in controls but this was not statistically significant ($P > 0.05$, $P > 0.05$, respectively). Total cholesterol, LDL cholesterol and glucose concentrations were significantly higher in obesity group ($P = 0.023$, $P = 0.000$ and $P = 0.000$, respectively) and there was a positive correlation between BMI and MDA, SO, hs-CRP and glucose concentrations and a negative correlation between BMI and HDL cholesterol levels. In conclusion, sialic acid levels were not different between the groups but showed a correlation with hs-CRP. A higher risk was found in obese children in the name of oxidative stress parameters, hs-CRP and lipid profile and this risk showed a positive correlation with BMI. These results are important for children because they will encounter with this increased risk for a longer time than adults.

PP-457
Cystatin C concentration for determination of early renal failure in type 2 diabetes mellitus

G. Can Yilmaztürk[1], D. Aslan[2] and S. Fenkçi[3]
[1]*Medical Biochemistry Laboratory, Uşak State Hospital, Uşak, Turkey,* [2]*Department of Biochemistry, Pamukkale University School of Medicine, Denizli, Turkey,* [3]*Department of Internal Medicine, Pamukkale University School of Medicine, Denizli, Turkey. E-mail: gamzema@yahoo.com*

Background: The earliest clinical evidence of diabetic nephropathy is microalbuminuria. Microalbuminuria and renal function should be checked regularly in patients with diabetes. Cystatin C has been studied intensively in recent years as indices of renal function. We aimed to evaluate the clinical efficiency of cystatin C in type 2 diabetes. Materials and methods: Serum concentrations of cystatin C, beta-2 microglobulin and creatinine were measured in 142 type 2 diabetic patients and 32 healthy men. The results were classified according to 24-h urine albumin and compared with creatinine clearance.
Results: There was a significant difference between means of control group and albumin excretion rate groups' cystatin C, beta-2 microglobulin and creatinine concentrations ($P = 0.000$). Among the creatinine clearance and cystatin C, beta-2 microglobulin and creatinine, a negative and good correlation was detected ($r = –0.671$, $P = 0.000$; $r = –0.589$, $P = 0.000$ versus $r = –0.587$, $P = 0.000$ respectively). ROC curves were drawn at two levels of creatinine clearance for determination of diagnostic utility of cystatin C, beta-2 microglobulin and creatinine in type 2 diabetes. No statistically significant difference was found between areas under curves ($P > 0.05$). Conclusion: Our study shows that serum cystatin C was not superior to serum creatinine for detecting early renal failure in type 2 diabetic patients.

PP-458
Oestrogen receptor alpha polymorphism is associated with cardiovascular diseases in type 2 diabetic men

M. Yuksel[1], S. Sancak[2], A. Velioğlu-Öğünç[1] and D. Yavuz[2]
[1]*Department of Medical Laboratory, Vocational School of Health Related Professions, Marmara University, Haydarpasa, Istanbul, Turkey,* [2]*Section of Endocrinology and Metabolism, School of Medicine, Marmara University, Altunizade, Istanbul, Turkey. E-mail: meralyuksel@marmara.edu.tr*

Estrogens have vasodilatatory, anti-inflammatory and anti-proliferative effects on the cardiovascular system as well as favourable effects on the lipid profile. Additionally oestrogen also may influence insulin secretion in type 2 diabates mellitus. The effects of oestrogens on the vascular system are mediated by oestrogen receptors alpha and beta. Oestrogen receptor alpha (ERalpha) polymorphisms are associated with receptor expression and non-atherosclerotic diseases. In this study we aimed the significance of XbaI and PvuII restriction enzyme polymorphisms of ERalpha gene with lipid profile on male patients with type 2 diabetes mellitus ($n = 47$), diabetic patients with cardiovascular diseases (CVD) ($n = 45$) and healthy controls ($n = 60$). After PCR with specific primers for ERalpha gene, products were digested and electrophoresed in agarose gel and visualized. The homozygous

XX and PP genotype was more prevalent in diabetic patients with CVD. Additionally xx genotype in healthy controls has a higher frequency than diabetic patients. Cholesterol, LDL-cholesterol and Apo B levels are increased in diabetic patients with CVD and associated with XX polymorphism. But an association with PvuII polymorphism was not observed. Triglyceride, HDL-cholesterol, VLDL, Apo A levels have not relation with ERalpha polymorphisms. Our results suggest that ERalpha polymorphisms have a significant effect on cholesterol, LDL-cholesterol and Apo B levels in male diabetic patients with cardiovascular diseases.

PP-459
Association of PAI-1 with impaired glucose tolerance and metabolic syndrome together with cardiovascular risk factors

A. Cavusoglu, A. Dogan, D. Aslanca, F. Yilmaz, S. Bilgili and B. Karaca
Biochemistry, Ministry of Health Izmir Education and Research Hospital, Izmir, Turkiye. E-mail: aylinveaylin@hotmail.com

Aim: Plasminogen activator inhibitor is the primary inhibitor of plasminogen activation *in vivo* and elevated levels of plasminogen activator inhibitor is associated with increased risk for thrombosis, including myocardial infarction and higher incidence of acute cardiovascular complications. Metabolic syndrome is associated with an increased risk for cardiovascular diseases and type 2 diabetes mellitus. Concentrations of plasminogen activator inhibitor-1 are also elevated at the beginning of impaired glucose tolerance and continuing through the development of diabetes mellitus and metabolic syndrome. The aim of this study is to show the association of plasminogen activator inhibitor-1 levels and other cardiovascular risk factors within the impaired glucose tolerance, metabolic syndrome and healthy control groups.
Conclusion: We showed that plasminogen activator inhibitor-1 levels of metabolic syndrome and impaired glucose tolerance groups were significantly higher than the control group, but there were no significant difference among the plasminogen activator inhibitor-1 levels of metabolic syndrome and impaired glucose tolerance groups, so we thought that in progress of impaired glucose tolerance prothrombotic and atherosclerotic process has began and plasminogen activator inhibitor-1 may be an indicator of this.

PP-460
Changes in enzyme levels and serum ion concentrations in diabetic rats exposed to low-frequency magnetic field

I. Ocal[1] and G. Atilla[2]
[1]Department of Biophysics, Cukurova University, Medical Faculty, Adana, Turkey, [2]Department of Biochemistry, Cukurova University, Medical Faculty, Adana, Turkey. E-mail: iocal@cu.edu.tr

Recent investigations revealing changes in enzyme levels and blood parameters in diabetic rats exposed to magnetic field that its depending on parameters and exposed duration. The aim of the present study is to examine the changes of blood parameters, insulin hormone, and levels of alpha amylase enzyme in diabetic rats that they are different exposed durations to alternative magnetic field. The rats were first divided into two groups. The first group was made up diabetic sham group (D1, D7 and D14), the second group was comprised of rats described as diabetic + magnetic field (DMF1, DMF7 and DMF14). The alternative magnetic fields of 5 mT intensity and 50 Hz frequency oriented in the north-south direction was exposed to the diabetic + magnetic field groups for 30 min at 1, 7, 14 days. After the end of the 1, 7 and 14 days, blood was collected from the diabetic rats under ether anaesthesia for biochemical parameters, enzymes and ion concentrations of diabetic rats. While serum glucose and lipid levels are changed that diabetic rats was exposed one day alternative magnetic field, Changes of insulin hormone and enzyme levels are changed end of seventh day. In addition, increased serum K^+ and decreased serum Ca^{2+} concentrations are observed in diabetic rats that they were exposed to 14 days alternative magnetic field. Data are significantly ($P < 0.05$). The magnetic field in diabetic rats that depending on exposed-duration to magnetic field are different affected levels of alpha-amylase enzyme and insulin hormone and ion concentrations.

PP-461
Differential expression of ERP46 under alternating glucose concentration in TC-6 beta cell line analysed by proteomics

A. Alberti, P. Karamessinis, S. Garbis and A. Charonis
Foundation for Biomedical Research of the Academy of Athens. E-mail: aalberti@bioacademy.gr

Proteomic analysis was used to detect differences in protein expression level of murine TC-6 pancreatic beta cells cultured for 5 days under conditions of low glucose (L-G), high glucose (H-G) and alternating glucose (ALT-G) concentrations. ALT-G concentration is relevant to the cellular environment in diabetes but has attracted little attention so far. Using 2-D gel electrophoresis and image analysis software, quantitative differences in 45 spots were encountered. Mass spectrometry analysis by MALDI-TOF-TOF-MS was performed for protein identification. Proteins differentially expressed in ALT-G conditions compared to both the other two conditions include ERp46, ERp44, ERp29 (all endoplasmic reticulum related), ATP synthase alpha chain, prohibition, NADH-ubiquinone oxireductase (all mitochondria related), peroxiredoxin-4, phosphoglycerate mutase 1 and poly (rC)-binding protein. ERp46 was further studied since it is highly expressed specifically in plasma cells producing proteins exhibiting disulfide bonds (as is the case with insulin). The differences in expression of ERp46 were confirmed by Western blotting analysis. There was a 40% increase in expression of ERp46 under ALT-G compared to L-G or H-G conditions. In conclusion, ERp46 is up-regulated in TC-6 pancreatic beta cell line under conditions of ALT-G concentrations. This change may be an important component of the pathogenetic mechanisms contributing to altered islet function in diabetes.

PP-462
The effects of nitric oxide on glycative and oxidative stress in STZ induced diabetic rats

T. Küme[1], H. Resmi[1], A. Solak[1], O. Sayın[1], O. Yılmaz[2], Y. Tunçok[3], S. Sarıoğlu[4] and G. Güner[1]
[1]Department of Biochemistry, Dokuz Eylul University School of Medicine, Izmir, Turkey, [2]Experimental Animals Laboratory, Dokuz Eylul University School of Medicine, Izmir, Turkey, [3]Department of Pharmacology, Dokuz Eylul University School of Medicine, Izmir, Turkey, [4]Department of Pathology, Dokuz Eylul University School of Medicine, Izmir, Turkey. E-mail: tuncay.kume@deu.edu.tr

The aim of this study is to elucidate the antiglycative and antioxidative effects of nitric oxide in diabetic rats. As a preliminary experiment, four rats were included to the study. Rat1

(nondiabetic, control), rat2 (diabetic), rat3 (diabetic, NO donor; NOC18-treated) and rat4 (diabetic, NOC18 and NO scavenger, cPTIO-treated) were made diabetic of which the duration ranged between 75–79 days. Urine microalbumin and creatinine were monitored to observe possibly renal function loses. Renal tissue was also evaluated to elucidate renal alterations. For this purpose percentage of glomeruli with increased basal membrane thickness, diffuse glomerulosclerosis and Kimmelstiel Wilson (KW) nodules were evaluated among 100 glomeruli in PAS stained sections. Serum AGE-specific fluorescence (Ex370/Em440) and m-tyrosine levels were measured to observe glycative and oxidative changes, respectively. Although the AGE-specific fluorescence were not different, m-tyrosine levels support the oxidative stress. NO has reversed this effect. Diffuse glomerulosclerosis and KW rates were lower in NO-treated diabetic rat than diabetic and diabetic + NOC18/cPTIO-treated rats. These data support our hypothesis that NO prevents oxidative reactions, therefore, renal damage. It should be noted that these data are only preliminary and have been obtained restricted number of animals and our study will continue in larger groups sufficient for statistical evaluation.

Acknowledgment: This study is supported by TUBITAK; 104S365.

PP-463
Oxidative stress and metabolic syndrome in obese patients

V. Muzakova[1], J. Skalicky[2] and R. Kandar[1]
[1]*Department of Biological and Biochemical Sciences, University of Pardubice, Pardubice, Czech Republic,* [2]*Department of Clinical Biochemistry, Regional Hospital of Pardubice, Pardubice, Czech Republic. E-mail: vladimira.muzakova@upce.cz*

The aim of this study was to evaluate the effect of oxidative stress in obese people with metabolic syndrome (MS). Free radical plasma levels and total antioxidant capacity were determined in obese people fulfilling at least three criteria of MS (MS, 15 patients), obese ones without MS (OB, 18 patients) and in 48 healthy controls (C). Free radical concentration was determined by direct method based on chlorophyllin acceptance of electrons, total antioxidant capacity using the kit TAS (Randox, UK). Plasma levels of glucose, triglycerides, HDL-ch, LDL-ch, fibrinogen, uric acid and BMI, waistline, blood pressure were followed. The highest level of free radicals was found in patients with metabolic syndrome. MS: 8.68 ± 3.70 mmol/l versus OB: 6.34 ± 1.80 mmol/l ($P \leq 0.05$) versus C: 4.71 ± 0.77 mmol/l ($P \leq 0.01$). Total antioxidant capacity was low in all groups. MS: 0.91 ± 0.12 mmol/l versus OB: 0.73 ± 0.10 mmol/l ($P \leq 0.05$) versus C: 0.81 ± 0.09 mmol/l ($P \leq 0.05$). In MS group significantly ($P \leq 0.05$) higher levels of glucose, triglycerides, uric acid and significantly ($P \leq 0.001$) lower level of HDL-ch were found. Our results confirm high significance of obesity as a risk factor leading to metabolic syndrome and cardiovascular disease, respectively. High levels of free radicals together with low total antioxidant capacity detected in case of patients with metabolic syndrome indicate elevation of the oxidative stress, which potentiates risk of atherogenesis.

Acknowledgment: Supported by MSMT grant 0021627502.

PP-464
Relationship between IL-6 and TNFα in female Turkish subjects with impaired glucose tolerance

D. Konukoglu[1], H. Hatemi[2], H. Bayer[3], N. Bağrıaçık[3] and **S. Fırtına**[1]
[1]*Department of Biochemistry, Istanbul University, Cerrahpasa Medical Faculty, Istanbul, Turkey,* [2]*Department of Internal Medicine, Istanbul University, Cerrahpasa Medical Faculty, Istanbul, Turkey,* [3]*Turkish Diabetes Association, Istanbul, Turkey. E-mail: sinemfirtina@yahoo.com*

Serum interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNFα) concentrations were measured in subjects during two-hour glucose loading in order to investigate the effects of glucose on serum IL-6 and TNFα concentrations. Twenty-six female subjects (mean age: 60 ± 10 years) had normal glucose tolerance (NGT) and nineteen female subjects (mean age: 63 ± 9 years) had impaired glucose tolerance (IGT) according to WHO criteria. Serum IL-6 and TNFα concentrations were measured by chemiluminescent immunometric assay. Subjects with IGT have higher fasting serum TNFα levels than subjects with NGT ($P \leq 0.01$). Serum IL-6 and TNFα concentrations were elevated during glucose loading (for each comparison, $P \leq 0.01$) .The increase in serum TNFα concentrations in IGT was greater than in NGT ($P \leq 0.01$). Serum IL-6 and TNFα concentrations significantly correlated with insulin and glucose in IGT group (for each comparison, $P \leq 0.01$). The correlation between serum glucose and cytokines concentrations were significant in IGT (for each comparison, $P \leq 0.01$). There was also a positive correlation between serum IL-6 and TNFα in NGT and IGT (for each comparison, $P \leq 0.01$). In conclusion, hyperglycaemia is associated with increased circulating cytokine concentrations and fasting TNFα concentrations seem to be more associated with IGT than IL-6.

PP-465
Changes in OGTT performed on patients with HCV infection after interferon therapy

M. Koldaş[1], H. Aral[2], A. İzat[3], E. Altunoğlu[4], C. Müderrisoğlu[4], G. Acar Erdoğan[4], G. Güvenen[2], M. Fincancı[3] and F. Sametoğlu[5]
[1]*Central Clinical Chemistry Laboratory, Ministry of Health Haseki Training and Research Hospital, İstanbul, Turkey,* [2]*Central Clinical Chemistry Laboratory, Ministry of Health İstanbul Training and Research Hospital, İstanbul, Turkey,* [3]*Infectious Disease Clinics, Ministry of Health İstanbul Training and Research Hospital, İstanbul, Turkey,* [4]*Internal Medicine Clinics, Ministry of Health İstanbul Training and Research Hospital, İstanbul, Turkey,* [5]*Ministry of Health İstanbul Training and Research Hospital, Internal Medicine Clinics, Istanbul, Turkey. E-mail: drhalearal@yahoo.com*

We tried to investigate the impact of HCV infection and the therapy with pegylated interferon (IFN)-alpha 2a or 2b together with Ribavirin on glucose metabolism. Eleven non-diabetic patients (nine female, two male) were performed oral glucose tolerance test (OGTT) before and after the therapy over 6 months. They had BMI between 24 and 27. They had no family history of diabetes mellitus (DM). Histological data together with response to antiviral therapy was assessed and no fibrosis was observed in liver. A 75-g oral glucose load was administered and blood specimens were obtained after 1-h and 2-h for serum glucose and insulin concentrations. Glucose levels were measured by standard enzymatic methods. Serum insulin levels were measured by chemiluminescent immunoassay method, using Immulite-2000

(DPC Diagnostic Products Corporation, Los Angeles, CA, USA). Before the therapy, according to ADA criteria of DM (2004), two patients (2/11) were overt DM and three patients (3/11) were IGT (impaired glucose tolerance). After the therapy, one of the diabetics had normal OGTT; the other one was IGT. Three patients defined as IGT had normal OGTT after the therapy. Insulin levels confirmed this observation. Insulin resistance at an early stage in the course of HCV infection was seen in our study group, and this glucose intolerance was regretted with the interferon therapy, but larger study groups are needed to demonstrate this finding better.

PP-466
The investigation of ACE and PON gene polymorphisms relations with MS and CHD in a few part of Turkish population

C. Sesal[1] and I. Peker[2]
[1]Biology, Marmara University, Istanbul, Turkey, [2]Chemistry Engineering, Engineering, Istanbul, Turkey. E-mail: csesal@yahoo.com

Coronary heart disease (CHD) is one of the most important reasons of morbidity and mortality today. Metabolic syndrome (MS) disease is thought to be a potential of CHD. The association of MS, CHD, paraxonase 1 (PON1) and Angiotensin converting enzyme (ACE) genes polymorphisms are investigated and positive association is reported in several case – control studies. In this study, body mass index, triglyceride, cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) like biochemical parameters and ACE and PON1 gene polymorphisms association between CHD (19 people), MS (34 people) and normal healthy subjects (26 people) as control group, are investigated. According to the unrelated group *t*-test results; body mass index, HDL and triglyceride values were found to be associated between MS and control subjects but there is no association between LDL and triglyceride values. According to the Mann Whitney U test results, body mass index, cholesterol, HDL and LDL values are found to be associated between CHD and control group subjects, but there is no association with triglyceride values and ACE polymorphisms. According to the chi-squared test results, PON1 55 and 192 genes polymorphisms are found to have no association between CHD, MS and control groups.

PP-467
The effect of valsartan on serum cystatin C, urine albumin and GFR in diabetic nephropathy

M. Koldaş[1], **H. Aral**[2], A. İzat[3], E. Altunoğlu[4], C. Müderrisoğlu[4], G. Acar Erdoğan[4], G. Güvenen[1], F. Sametoglu[4] and M. Fincancı[2]
[1]Ministry of Health Haseki Training and Research Hospital, Central Clinical Chemistry Laboratory, Istanbul, Turkey, [2]Ministry of Health Istanbul Training and Research Hospital, Central Clinical Chemistry Laboratory, Istanbul, Turkey, [3]Ministry of Health Istanbul Training and Research Hospital, Infectious Disease Clinics, Istanbul, Turkey, [4]Ministry of Health Istanbul Training and Research Hospital, Internal Medicine Clinics, Istanbul, Turkey. E-mail: drhalearal@yahoo.com

We tried to investigate the impact of HCV infection and the therapy with pegylated interferon (IFN)-alpha 2a or 2b together with Ribavirin on glucose metabolism. Ten non-diabetic patients (eight female, two male) were performed oral glucose tolerance test (OGTT) before and after the therapy over 6 months. They had no family history of diabetes mellitus (DM). A 75-g oral glucose load was administered and blood specimens were obtained after 1-h and 2-h for serum glucose and insulin concentrations. Glucose levels were measured by standard enzymatic methods. Serum insulin levels were measured by chemiluminescent immunoassay method, using Immulite-2000 (DPC Diagnostic Products Corporation, Los Angeles, CA, USA). Before the therapy, according to ADA (American Diabetes Association) criteria of DM (2004), two patients (2/10) were overt DM and three patients (3/10) were IGT (impaired glucose tolerance). After the therapy, one of the diabetics had normal OGTT; the other one was IGT. Three of four patients defined as IGT had normal OGTT after the therapy. Insulin levels confirmed this observation. Insulin resistance at an early stage in the course of HCV infection was seen in our study group, and this glucose intolerance was regretted with the interferon therapy, but larger study groups are needed to demonstrate this finding better.

PP-468
Effects of neonatal maternal separation on blood biochemistry parameters in UCH and Wistar rats

O. A. Martins, W. Mello Junior, S. Pereira, R. Kremer, G. R. Teixeira, H. K. Rofatto, A. E. B. Camargo, E. V. Oliveira, C. C. D. Almeida-Francia, S. M. M. Matheus, P. F. F. Pinheiro and F. E. Martinez
Anatomy Department, Instituto de Biociencias, Universidade Estadual Paulista, UNESP, Botucatu, Sao Paulo, Brazil. E-mail: oamartins@ibb.unesp.br

The alcoholism is an old medical problem of great magnitude with important social and economic repercussions. In other hand, obesity, diabetes and a stress life are becoming a public health problem of epidemic proportions, as well. Stress activates the sympathoadrenal system and the hypothalamic-pituitary-adrenocortical (HPA) axis. Complex relationships among HPA axis function, obesity and metabolic parameters have been shown. Alcohol use is also associated with stress, likely related to the individual differences in the HPA axis function. Many developmental outcomes in mammals depend upon the type of maternal care provided by the dams, that regulates the development of individual differences in behavioural and endocrine responses to stress. Maternal care produces complex biological and behavioural interactions that affect the adulthood. In this study, rats of UCh (alcoholic rats) and Wistar strains were submitted to maternal separation (MS) during the stress-hyporesponsive period (4-14 postnatal day), and biochemistry parameters were analysed as adults. The body weight and the plasmatic levels of cholesterol and glucose were elevated in the animals that underwent maternal separation during the neonatal period, in both strains. The pattern of alcohol intake was modified after MS and the plasmatic protein level decreased in UCh strain. Thus, the maternal separation causes metabolic alterations in the adult rat, probably by modifying the HPA axis answer to stress.

PP-469
Investigation of serum nitric oxide, total antioxidant activity and oxidized LDL levels of obese subjects and healthy controls

H. F. Yerlikaya, I. Mehmetoglu, S. Kurban and G. Yilmaz
Biochemistry Department, Selcuk University, Meram Medical School, Konya, Turkey. E-mail: fhumeyray@hotmail.com

This study was performed on 33 obese people and 30 control cases. There was no complaints and symptoms of the obese people other than obesity. Body mass index (BMI) was used as an obesity criteria. Obese cases were composed of eight males and

25 females between the age of 29–69 years whereas control cases were composed of 16 males and 14 females between the age of 28–69 years. Those with BMI more than 35 kg/m^2 were considered as obese. Of the obese cases 20 were morbid obese and 13 were obese. Control cases were selected among healthy people with no complaints and symptoms. Their BMI value was less than 25 kg/m^2. Serum Nitric Oxide (NO), Oxidized LDL (Ox-LDL) and Total Antioxidant Activity (AOA) levels of both groups were measured. NO, Ox-LDL and AOA levels of 0.2276, 0.699 and 0.1467 respectively. The same parameters of the control group were 0.1930, 0.610 and 0.1759 respectively. The data obtained were evaluated by SPSS 10.0 statistics program. Statistically no significant difference was found between serum NO, Ox-LDL and AOA of the groups. Also there was no correlation between NO, Ox-LDL, AOA and BMI. Our study shows that oxidative stress and NO levels don't change obese and non-obese subjects. There is need for more detailed studies to improve our understanding of the role obesity in oxidative stress and NO levels.

PP-470
Lipogenic enzymes in adipose tissue are subjected to the caloric restriction and protein intake

I. Y. Yurtman[1], M. Gultepe[2], O. Ozcan[2], L. Coskuntuna[4], S. Onenc[3] and **K. Avsar**[2]
[1]Department of Zootechnics, Onsekiz Mart University, Canakkale, Turkey, [2]GATA Haydarpasa Training Hospital, Department of Biochemistry, Istanbul, Turkey, [3]Department of Zootechnics, Ege University, Izmir, Turkey, [4]Department of Zootechnics, Trakya University, Tekirdag, Turkey. E-mail: ozcanmd@yahoo.com

The impact of caloric restriction on the metabolism is recently important research topic for understanding the effects of dietary changes on the metabolic process. We aimed to investigate the relationship between caloric restriction and enzymatic control of lipogenesis in an animal model. Male lamps were divided into three groups according to their dietary styles (control group: ad libitum, caloric restriction group: 15% reduced ad libitum and caloric restricted protein enriched group: 15% reduced ad libitum + 20% protein enriched diet). Glucose 6-phosphate dehydrogenase (G6PDH), glycerol-3-phosphate dehydrogenase (G3PDH), malate dehydrogenase (MDH) and fatty acid synthase (FAS) levels of omental and subcutane adipose tissues were determined after 42 and 84 days. Enzyme activities except for MDH were reduced in all groups during time-course. The G6PDH activities of energy-restricted groups were significantly lower than the control group. The decrease in G6PDH, FAS and G3PDH activities were earlier in energy restricted and protein-enriched group than the other groups. In conclusion, Caloric restriction may effect on the G6PDH enzyme, which is the supplier of the 50% of NADPH using in the lipogenesis whereas protein enrichment in caloric restriction seems to be effective on the early decreases of lipogenesis.

PP-471
Coagulative, fibrinolytic and inflammatory changes in insulin resistance syndrome

F. Yağmur Tekeli[1], G. Haklar[1], S. Kara[2] and A. Topuzoğlu[3]
[1]Biochemistry, Marmara University, İstanbul, Turkey, [2]Kirazhtepe Health center, İstanbul, Turkey, [3]Public Health, Marmara University, İstanbul, Turkey. E-mail: feyzaayagmur@yahoo.com

Insulin resistance syndrome (IRS) is a metabolic and low-grade inflammatory state in which atheromatous risk factors come together. We aimed to investigate the interaction between inflammation and oxidation with haemostatic markers. In the study 33 patients with IRS who did not received any treatment and 10 healthy control cases were enrolled. Fasting glucose, cholesterol, triglyceride, HDL, HbA1c, insulin, hsCRP, monocyte respiratory burst were analysed in all individuals. Also prothrombin time, activated partial thromboplastin time, factor VIIa, fibrinogen, thrombin activatable fibrinolytic inhibitor (TAFI), tissue factor pathway inhibitor (TFPI), plasminogen activator inhibitor (PAI-1) and vWF were analysed as haemostatic markers. The comparisons between groups were made by Student's *t*-test and ANOVA, correlation analysis was made by Spearman's Rank test. Significant rises in PAI-1 and TAFI levels explain hypofibrinolysis. In addition to this FVII and TFPI prove the role of endothelial factors in the pathogenesis and explain the increases in coagulation. Correlative increase in hsCRP levels with HbA1c and body mass index indicate that IRS has inflammatory characteristic. On the other hand monocyte respiratory burst levels were significantly lower in IRS group indicating the exhaustion of these cells due to the disease pathology. As a result we can see endothelial damage, oxidant effects, inflammation and coagulation tendency with hypofibrinolysis in IRS patients.

PP-472
11β-HSD type 1 is responsible for low plasma hdl-cholesterol and abdominal obesity in metabolic syndrome patients

F. Atalar[1], B. Vural[1], C. Ciftci[2], A. Demirkan[1], B. Susleyici-Duman[3], P. Cagatay[4], D. Gunay[5], E. Sagbas[5], B. Akpinar[6], U. Ozbek[1] and **A. S Buyukdevrim**[7]
[1]DETAM Genetics Department, Istanbul University, Istanbul, Turkey, [2]Department of Cardiology, Kadir Has University, Medical Faculty, Istanbul, Turkey, [3]Department of Medical Biology and Genetics, Kadir Has University, Medical Faculty, Istanbul, Turkey, [4]Department of Biostatistics, Istanbul University, Cerrahpasa Faculty of Medicine, Istanbul, Turkey, [5]Department of Biochemistry, Florence Nightingale Hospital, Istanbul, Turkey, [6]Department of Cardiac Surgery, Florence Nightingale Hospital, Istanbul, Turkey, [7]Turkish Diabetes Consortium, Istanbul, Turkey. E-mail: tmatalar@yahoo.com

11β-hydroxysteroid dehydrogenase (11β-HSD) isozymes regulate the access of glucocorticoids to their receptors and catalyse the interconversion of active glucocorticoids to their inactive metabolites.11β-HSD type 1 isoform is expressed in liver, brain, lung and adipose tissues. 11β-HSD expression plays an essential role in abdominal obesity which is one of the major causes of metabolic syndrome. We have investigated the role of 11β-HSD type 1 isoform in paired biopsies from epicardial adipose and aorta tissues of 10 metabolic syndrome patients and four control patients undergoing coronary artery by pass operation. 11β-HSD type 1 isoform expression was assessed in whole tissue using the real time PCR. In aorta, 11β-HSD Type 1 isoform showed positive correlation with waist hip ratio (0.579; $P \leq 0.05$)and negative correlation with hypertension (–0.543; $P \leq 0.05$).In epicardial adipose tissue,11β-HSD Type 1 isoform showed negative correlation with HDL (–0.614; $P \leq 0.05$).The analysis indicate that 11β-HSD expression were related to waist-hip ratio and low plasma HDL-cholesterol of our patients, but did not show any correlation with coronary arthery disease. Besides, the negative correlation of 11β-HSD with hypertension does indicate its regulatory role in blood pressure. The outcome of this study shows that 11β-HSD might be responsible for metabolic syndrome without hypertension.

PP-473
The role of FABP4 gene expression in metabolic syndrome patients with previous myocardial infarction

B. Vural[1], F. Atalar[1], A. Demirkan[1], C. Ciftci[2], B. Susleyici Duman[3], P. Cagatay[4], D. Gunay[5], E. Sagbas[5], B. Akpinar[6], U. Ozbek[1] and **A. S. Buyukdevrim**[7]
[1]DETAM Genetics Department, Istanbul University, Istanbul, Turkey, [2]Department of Cardiology, Kadir Has University, Medical Faculty, Turkey, [3]Department of Medical Biology and Genetics, Kadir Has University, Medical Faculty, Istanbul, Turkey, [4]Department of Biostatistics, Istanbul University, Cerrahpasa Medical Faculty, Istanbul, Turkey, [5]Department of Biochemistry, Florence Nightingale Hospital, Istanbul, Turkey, [6]Department of Cardiac Surgery, Florence Nightingale Hospital, Istanbul, Turkey, [7]Turkish Diabetes Consortium, Istanbul, Turkey. E-mail: burcak_2002@yahoo.com

Adipocyte fatty acid binding protein (FABP4) is member of intracellular FABP family and expressed in a differentiation-dependent fashion in adipocytes. In mice, targeted mutations in FABP4 provide significant protection from hyperinsulinemia and insulin resistance in the context of both dietary and genetic obesity. In this study, we compared FABP4 gene expression in 10 metabolic syndrome and control tissue samples ($n = 4$) without metabolic syndrome, in paired epicardial adipose tissue and aorta samples. Gene expressions were determined by real time RT-PCR. Positive correlations were found between body mass index (BMI) and FABP4 gene expression (0.6941, $P \leq 0.05$), 0.6732, $P \leq 0.05$) and between previous myocardial infarction (PMI) and FABP4 gene expression (0.5854, $P \leq 0.05$), (0.6693, $P \leq 0.05$) in both paired epicardial adipose tissue and aorta, respectively. The positive correlation between BMI and FABP4 gene expression, in both paired epicardial adipose tissue and aorta led us to think that FABP4 is responsible for obesity in metabolic syndrome. Whereas, we did not find any correlation between FABP4 gene expression and glycaemia, lipid profile and hypertension. There was no correlation between FABP4 gene expression and coronary artery disease (CAD) or acute myocardial infarction(AMI). The decrease in FABP4 expression might be due to the statin used by all patients. A positive correlation between PMI and FABP4 expression might indicate an association with necrosis in the patients.

PP-474
Body composition and leptin levels were unchanged 48h after a long (Ironman) triathlon competition

I. Ara[1], G. Vicente-Rodriguez[1], **J. Perez-Gomez**[2], J. Chavarren[2], C. Dorado[2], JAL Calbet[2]
[1]Department of Fisiatria y Enfermeria, University of Zaragoza, Spain, [2]Department of Physical Education. University of Las Palmas de Gran Canaria, Spain. E-mail: iara@unizar.es

Seven male triathletes (30.0 ± 5.2 years; 178.9 ± 2.8 cm; 74.9 ± 3.0 kg) who completed an Olympic Triathlon (OT) and eight male triathletes (33.5 ± 8.4 years; 174.5 ± 2.5 cm; 71.9 ± 2.4 kg) who completed the Lanzarote Ironman Triathlon (IT) participated in this study. Before and 48 h after the triathlons a body composition exam (DXA) and a blood sample for leptin, testosterone and dihydrotestosterone assessment were obtained from all subjects. Body composition did not change 48 h after the triathlons. Testosterone concentration in serum was unchanged after the short triathlon, but decreased by 13% after the long triathlon ($P < 0.05$). A trend to lower values was also found in the dihydrotestosterone levels after the long triathlon ($P = 0.11$). Circulating leptin levels remained unchanged after both triathlons. No relationship was found between changes in testosterone and any of the body composition variables assessed before the competitions. However, after 48 h after the Lanzarote Ironman the haematocrit was reduced by 9%, likely due to haemodilution. After accounting for the change in haematocrit no significant effect on serum testosterone or leptin concentration was detected. We concluded that despite the tremendous effort elicited by the Lanzarote Ironman, 48 h later no significant changes in body composition nor leptin levels were detectable in our study. The small effect reduction of testosterone concentration could be accounted for by haemodilution.

PP-475
Quantitative expression analysis of glucocorticoid receptor (GcR) gene in metabolic syndrome

A. Demirkan[1], F. Atalar[1], B. Vural[1], B. Susleyici Duman[2], C. Ciftci[3], G. Gunay[4], P. Cagatay[5], E. Sagbas[4], B. Akpınar[6], U. Ozbek[1] and A. S. Buyukdevrim[7]
[1]DETAM Genetics Department, Istanbul University, Istanbul, Turkey, [2]Department of Medical Biology and Genetics, Kadir Has University Medical Faculty, Istanbul, Turkey, [3]Department of Cardiology, Kadir Has University, Medical Faculty, Turkey, [4]Department of Biochemistry, Florence Nightingale Hospital, Istanbul, Turkey, [5]Department of Biostatistics, Istanbul University Cerrahpasa Medical Faculty, Istanbul, Turkey, [6]Department of Cardiac Surgery, Florence Nightingale Hospital, Istanbul, Turkey, [7]Turkish Diabetes Consortium, Istanbul, Turkey. E-mail: aydemirkan@yahoo.com

Glucocorticoids (GCs) regulate multiple processes in adipose tissue influencing fat cell size and adipose tissue metabolism and they act through a receptor (GcR), which mediates most of their hormone-induced actions. Considering the studies on locally enhanced activity of GCs in adipose tissue, we focused on the GcR mRNA expression in epicardial adipose tissue and aorta of MS patients. Paired aorta and epicardial adipose tissue samples of ten MS patients and four non-MS controls were taken during elective open-heart surgery. Total RNA was isolated and cDNA synthesized by using random hexamers. The real-time RT-PCR assays were performed by LightCycler instrument using lightcycler-DNA master SYBR Green I (Roche, Molecular Biochemicals). Cyclophilin gene was used as reference gene. We used the formula 'target gene quantity/reference gene quantity' to calculate the GcR expression. Aortic expression of GcR was found correlated with body-mass index ($r = 0.6513$, $P \leq 0.005$). Epicardial adipose tissue expression of GcR mRNA was found to be in correlation with carotis artery disease ($r = 0.6121$, $P \leq 0.05$) with coronary artery disease ($r = 0.6478$, $P \leq 0.05$) and also with family history of coronary artery disease ($r = 0.5854$, $P \leq 0.05$), which made us think that epicardial GcR may trigger premature atherosclerosis in both carotis and coronary artery.

PP-476

Paraoxonase, oxidative stress, oxidized low density lipoprotein are associated with macrovascular complications in patients with type 2 diabetes mellitus

S. Kaya[1], C. Sezgin[1], S. Aydın[1], S. Himmetoglu[1], H. Uzun[1], **V. Yumuk**[2] and H. Hatemi[2]
[1]*Department of Biochemistry, Cerrahpasa medical Faculty of Istanbul University, Istanbul, Turkey,* [2]*Department of Internal Medicine, Cerrahpasa medical Faculty of Istanbul University, Istanbul, Turkey. E-mail: drcsezgi@yahoo.com*

In this study plasma glucose, malondialdehyde (MDA), oxidized LDL (oxLDL), monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), and vascular cellular adhesion molecule-1 (VCAM-1), apolipoprotein A1 (ApoA1) levels and paraoxonase-1 (PON1) activity were measured in sixty type 2 diabetic patients (mean age 52.96 ± 12.64 years) 30 of which had macrovascular complications, and 30 healthy controls (mean age 50.63 ± 7.01 years). Diabetic patients had significantly higher levels of MDA, oxLDL, MCP-1, ICAM-1, VCAM-1 than the healthy controls ($P < 0.01$). Diabetics with macrovascular complications had higher levels of MDA, oxLDL, MCP-1, ICAM-1, VCAM-1 than those without, and the difference was significant for all molecules ($P < 0.05$) except for ICAM. PON1 activity and ApoA1 levels of the control subjects were significantly higher than that of the diabetic patients ($P < 0.01$), while PON1 activity and ApoA1 levels in the diabetic patients with macrovascular complications were significantly lower than that in patients without ($P < 0.05$). Hyperglycaemia might play a significant role in generating increased oxidative stress, and decreased PON1 activity, resulting in elevated oxLDL, MCP-1 and VCAM levels might be one of the causal pathogenic factors initiating accelerated atherosclerosis in patients with type 2 diabetes mellitus.

PP-477

Acylation stimulating protein and complement c3 mRNA expression in metabolic syndrome

B. Susleyici Duman[1], C. Ciftci[2], F. Atalar[3], A. Demirkan[3], B. Vural[3], P. Cagatay[4], D. Gunay[5], E. Sagbas[6], B. Akpinar[6], U. Ozbek[3] and **A. S Buyukdevrim**[7]
[1]*Department of Medical Biology and Genetics, Kadir Has University Medical Faculty, Istanbul, Turkey,* [2]*Department of Cardiology, Kadir Has University Medical Faculty, Istanbul, Turkey,* [3]*Department of Genetics, Istanbul University Institute for Experimental Medical Research, Istanbul, Turkey,* [4]*Department of Biostatistics, Istanbul University Cerrahpasa Faculty of Medicine, Istanbul, Turkey,* [5]*Department of Biochemistry, Florence Nightingale Hospital, Istanbul, Turkey,* [6]*Department of Cardiac Surgery, Florence Nightingale Hospital, Istanbul, Turkey,* [7]*Turkish Diabetes Consortium, Istanbul, Turkey. E-mail: bsusleyici@khas.edu.tr*

We have evaluated the mRNA expression of ASP and C3 in human epicardial adipose tissue (EAT) and aorta. Paired aorta and EAT samples were obtained from ten patients and four controls during elective open-heart surgery. All the patients were suffering from metabolic syndrome and have undergone coronary by-pass surgery, whereas all the angiographically normal control patients have undergone valve replacement surgery. None of the patients had acute myocardial infarction (AMI) while three had previous myocardial infarction (PMI). Gene expressions were determined by real time PCR. In the aorta, ASP mRNA decrease with increasing waist ($r = –0.835$, $P \leq 0.01$), BMI ($r = –0.595$, $P \leq 0.05$), family history of coronary artery disease (FHCAD) ($r = –0.647$, $P \leq 0.05$), and CAD ($r = –0.667$, $P \leq 0.01$), whereas increase with elevated HDL-cholesterol ($r = 0.662$, $P \leq 0.01$). In the EAT, ASP mRNA expression has been found to show positive correlation with blood glucose (BG) ($r = 0.784$, $P \leq 0.01$), systolic blood pressure (SBP) ($r = 0.586$, $P \leq 0.05$), FHCAD ($r = 0.641$, $P \leq 0.05$), and CAD ($r = 0.648$, $P \leq 0.05$). C3 expression was found to increase with FHCAD ($r = 0.537$, $P \leq 0.05$) in the aorta, whereas found to decrease ($r = –0.586$, $P \leq 0.05$) with PMI in EAT. These results suggest that ASP produced by EAT may be one of the determinants of CAD and FHCAD. It seems like EAT leads to cardiovascular events by elevating BG and SBP. C3 may be antiatherogenic in MI survivors in EAT.

PP-478

Effect of fenofibrate on oxidant-antioxidant status and endothelial dysfunction in streptozosin induced diabetic rats

M. Olukman[1], **E. D. Sezer**[2], S. Goksel[1], E. S. Sozmen[2] and G. M. Cinar[1]
[1]*Pharmacology Department, Ege University Medical School, Izmir, Turkey,* [2]*Biochemistry Department, Ege University Medical School, Izmir, Turkey. E-mail: ebru.sezer@ege.edu.tr*

Fenofibrate (FF) is a hypolipidaemic agent which is a peroxisome proliferator activated receptor agonist. The aim of this study is to observe the effect of fenofibrate on the endothelial disfunction of strepozosin induced diabetic (DM) rats' aortas and to investigate its reflection on the oxidant-antioxidant status. The rats were divided into four groups (Control, DM, DM + FF, Control + FF). The erythrocyte assays showed that diabetes decreased catalase (4234 versus 5767 U/gHb) and superoxide dismutase activities (1592 versus 2433 U/gHb), while FF treatment increased the activity of these antioxidant enzymes (5552 and 2583 U/gHb). The malondialdehyde levels in DM + FF group (548 nmol/gprot), were significantly lower than the DM group (676 nmol/gHb) both in erythrocytes and aorta homogenates (0.83 versus 1.65 nmol/gprot). The myeloperoxidase (MPO) activities in DM group were higher than the control group and FF significantly decreased MPO activity in the aorta tissues. As for the endothelial functions, the DM group's contraction responses to phenylephrine were higher than the control groups while FF treated group showed a weaker response. The relaxation responses to acetylcholine (Ach) after phenylephrine application were also observed and DM group showed weaker response to Ach than the control group did and FF treatment increased this relaxation response. These results lead to the idea that FF exhibits antioxidant effects on diabetic rats and shows favourable influence on endothelial dysfunction.

PP-479

The impact of bone marrow-derived JNK activity in diet induced systemic insulin resistance

S. Vallerie, G. Tuncman and **G. S. Hotamisligil**
Genetics and Complex Diseases, Harvard School of Public Health, Boston, USA. E-mail: svallerie@yahoo.com

Studies in our lab and elsewhere demonstrated that obesity and type II diabetes are characterized by chronic inflammation. We recently reported a critical role for JNK activity in this process and demonstrated that whole body JNK1 deficiency results in marked protection against diet induced obesity and insulin resistance. To investigate the role of JNK1 activity in bone marrow (BM) derived cells, particularly macrophages, we transplanted

JNK1-deficient (JNK1−/−) or wild type (WT) BM into WT mice and placed the resulting animals on high fat diet. Although there was no statistically significant difference in body weight between the groups, the JNK1−/− mice transplanted with JNK1−/− BM showed improved glucose sensitivity compared to JNK1−/− mice that received WT BM, demonstrating a beneficial impact of macrophage JNK1-deficiency on whole body insulin sensitivity. To address whether this function is only evident in the JNK1−/− setting, we performed preliminary experiments where WT recipients are transplanted with JNK1−/− or WT BM. In this setting we did not observe significant changes in systemic insulin sensitivity between the groups. This latter group is at earlier stages of analysis and therefore a clear conclusion is not yet possible to distinguish the full impact of isolated macrophage versus whole body JNK1-deficiency. Nonetheless, these experiments demonstrate an important role of JNK1 in bone marrow derived cells in mediating diet induced insulin resistance in mice.

PP-480
Additive effects of benzene and alloxan-induced diabetes on carcinogenic potential of nitrosodimethylamine in liver

S. Arslan, O. Adali and E. Arinç
Biochemistry Graduate Programme and Department of Biological Sciences, Middle East Technical University, Ankara, Turkey. E-mail: sevki@metu.edu.tr

N-Nitrosodimethylamine (NDMA) is a procarcinogen that is bioactivated by CYP2E1 dependent NDMA N-demethylase to labile alpha-carbon hydroxylated metabolite further resulting in active methylating agents, which in turn, can methylate DNA and proteins. In this study, the effects of diabetes, benzene and combined effects of both on NDMA N-demethylase activity and on CYP2E1 protein level were investigated in rabbit liver. NDMA N-demethylase activity was increased significantly in diabetic (1.95-fold) and in benzene (2.85-fold) treated rabbits. The activity was further increased by combined treatment of diabetes (alloxan) and benzene (4.45-fold). Besides, immunoblots showed an elevated content of CYP2E1 after treatment with benzene or alloxan, which was further increased when two treatments were combined. The content of CYP2E1 and catalytic activity in the benzene and diabetic group appeared to be additive of the separate treatments. These results indicate that both diabetes mellitus and benzene exposure stimulate metabolic activation of N-nitrosodimethylamine by inducing NDMA N-demethylase, which results in increased amounts of active methylated agents' formation. Besides, exposure of diabetic subjects to benzene shows additive effect on stimulation of NDMA N-demethylase, hence formation of more methylating agents when compared to separate treatments. This may in turn further potentiates the risk of mutagenesis and malignant transformation in liver of these subjects.

PP-481
Plasma leptin response to weight loss in obese patients with coronary artery diseases

A. Kaya[1], G. Karatekin[2], H. Balci[3], A. Arat-Özkan[4] and **Z. Yigit**[4]
[1]Biochemistry Department, Istanbul University Cardiology Institute, Istanbul, Turkey, [2]Şişli Etfal Teaching and Research Hospital, Department of Neonatology, Istanbul, Turkey, [3]Cerrahpaşa Medicak School Center Lab, Istanbul University, Istanbul, Turkey, [4]Cardiology Institute, Cardiology Department, Istanbul University, Turkey. E-mail: stone_64_kaya@yahoo.com

Aim: Leptin, a hormone secreted from adipose tissue, was originally discovered to regulate body weight. This study is designed to evaluate the changes in plasma leptin during weight loss of obese patients with confirmed coronary artery diseases.

Material and methods: The study group consisted of 100 obese patients (48 male, 52 female) with coronary artery diseases, whose body mass index (BMI) mean was ≥35.1 ± 4.3 kg/m^2. During a 6-month period, these patients received orlistate, a 1200 caloric diet, and exercised mildly. The serum leptin, insulin, and some metabolic parameter levels before and after 6 months of treatment were determined.

Results: The patients were 63.35 ± 8.99 years old and reduced their weights by a mean of 8%. Before and after the treatment, the levels of leptin dropped significantly (61.76 ± 34.34 ng/ml versus 41.6 ± 19.66 ng/ml, 45%; $P = 0.0016$). Additionally, serum levels of total cholesterol ($P = 0.0015$), acid uric ($P = 0{,}029$), fasting glucose ($P = 0.0014$), 2 hours glucose ($P = 0.036$), fasting insulin ($P = 0{,}0017$), 2 h insulin ($P = 0{,}0014$), and HbA1C ($P = 0.0077$) decreased in coronary artery disease patients with weight loss.

Conclusion: We conclude that in obese patients with coronary artery diseases, leptin levels are decreased in proportion to weight loss. Our data indicates that serum levels of insulin, glucose, HbA1C, and total cholesterol also decreased in this population. Furthermore, our findings suggest decreased risk of insulin resistance for and obesity-associated cardiovascular disease.

PP-482
Adenovirus mediated TRAIL gene delivery as a model for the treatment of patients with diabetes

E. Dirice[1], A. D. Sanlioglu[1], A. Omer[2], M. K. Balci[1], E. Terzioglu[1] and S. Sanlioglu[1]
[1]Departments of Human Gene Therapy and Medical Biology-Genetics, Akdeniz University Faculty of Medicine, Antalya, Turkey, [2]Section on Islet Transplantation and Cell Biology Joslin Diabetes Center, Harvard Medical School, Boston, USA. E-mail: dercument@akdeniz.edu.tr

Type 1 diabetes is an auto-immune disease which is characterized by T cell-mediated destruction of insulin-producing pancreatic beta cells. While islet transplantation appears to be a promising approach, the graft failure following transplantation is a challenging issue to overcome. Tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) plays essential roles during the course of diabetes as suggested by recent reports. In order to prevent auto-reactive T cells from attacking the islets a novel therapeutic approach is designed. In addition, surgical islet dissecting and transplantation protocols are established through close collaboration with Joslin Diabetes Center of Harvard Medical School. Propidium Iodide (PI) and Fluorescein DiAcetate (FDA) staining is performed on isolated pancreatic islets to determine the cell viability. Islets are also stained with DiThiZone (DTZ) for cell purity. Then pancreatic islets are transduced with an adenovirus vector expressing enhanced green florescent protein (EGFP). Despite the high doses of adenovirus vectors used, pancreatic islets appeared to be relatively resistant to adenovirus infection compared to epithelial cells. This study concerns efficient gene delivery techniques into pancreatic islets to ultimately treat patients with Type 1 diabetes.

PP-483
Comparison of acute phase reactant levels in cases with metabolic syndrome

F. Karakoyunlu[1], **N. Eren**[1], Ş. Ciğerli[1], B. Aslan[1], A. Yıldız[1], L. Aydın[2] and Y. Altuntaş[3]
[1]*Biochemistry Department, Şişli Etfal Education and Research Hospital, İstanbul, Turkey,* [2]*Hypertension and Lipid Outpatient Clinic, Şişli Etfal Education and Research Hospital, İstanbul, Turkey,* [3]*II.Internal Medicine Clinic, Şişli Etfal Education and Research Hospital, İstanbul, Turkey.*
E-mail: nezaketeren@yahoo.co.uk

Aim: We investigated inflammatory markers; hs-CRP (high sensitivity-CRP), fibrinogen, and erythrocyte sedimentation rate (ESR) and insulin levels in Metabolic Syndrome (MetS).
Design and Methods: We obtained fasting glucose, insulin, lipid levels, hs-CRP (Latex immunoassay), fibrinogen (Fib), and ESR. Patients were stratified by the number of possessed MetS criteria into groups; 0 to 5/5 positive as group 0 to 5. Insulin resistance calculated by HOMA-IR.
Results: We enrolled 86 cases (M/F: 29/57); 18 healthy, 68 patients with at least one to all criteria of MetS (53) possessed. The groups were similar in respect of age, gender ($P > 0.05$). Differences among control and first four group were insignificant ($P > 0.05$), whereas differences in all parameters between Gr 5 and control group were statistically significant ($P < 0.05$). Besides, Gr 5 demonstrated a linear significance level in the entire inter-group comparisons in respect of hs-CRP levels (P values were 0.000; 0.01; 0.05; 0.002; 0.025, respectively). An average degree of positive correlation observed in followings; Fib versus ESR1-2 ($P < 0.01$; r: 0.649, r: 0.661) hs-CRP versus HOMA, fibrinogen, ESR2 levels ($P < 0.01$; r: 0.512, r: 0.642, r: 0.686, respectively), nevertheless hs-CRP levels displayed a high degree of positive correlation with ESR1 ($P < 0.01$; r: 0.797).
Conclusion: Our study clarified once more the outcomes of MetS; the more the no of criteria, the higher the levels of inflammatory markers and the worse the patient's outcome can be predicted.

PP-484
Relationship between insulin resistance and IL-6, CRP, fibrinogen in obese patients with type-II diabetes mellitus

E. Koyuer[1], **N. Yücel**[1], F. Turgay[1], Ş. Ciğerli[1], G. Kafadenk[1], N. Eren[1], S. Uçak[2] and Y. Altuntaş[2]
[1]*Biochemistry Department, Şişli Etfal Education and Research Hospital, İstanbul, Turkey,* [2]*Second Internal Medicine Clinic, Şişli Etfal Education and Research Hospital, İstanbul, Turkey.*
E-mail: nezaketeren@yahoo.co.uk

In this study, IL-6, CRP and fibrinogen levels which are the indexes of inflammation with respect to the body mass index (BMI) and the waist/hip ratio and the relationship of these parameters with insulin resistance in obese and non obese Type-II diabetes mellitus patients and non obese healthy cases are investigated. Total 70 cases; 30 obese and 20 non-obese Type-II DM patients and 20 non obese healthy individuals are included in the study. The HOMA value showing the insulin resistance is calculated as 14.6 ± 17.9 in obese group, 4.69 ± 2.91 in non-obese group and 87 ± 1.05 in control group. IL-6 levels in obese group (3.72 ± 2.30 pg/ml) were significantly higher ($P < 0.05$) than those of non-obese (2.49 ± 1.32 pg/ml) and control (2.17 ± 0.38 pg/ml) groups. There was not any significant difference between IL-6 levels of the control and non-obese groups ($P > 0.05$). There was not any significant difference between CRP levels of obese, non-obese and control groups ($P > 0.05$). The fibrinogen levels of obese group were higher than those of control group ($P < 0.05$). In all groups, fibrinogen values were found to have a positive correlation with IL-6 values. In conclusion, in this study, we showed, in concordance with the literature, that in obese patients with BMI > 30 kg/m^2 insulin resistance was developing and related to this, HOMA values and IL-6 and fibrinogen levels were increasing. It is determined that these parameters were in strong relation with BMI and WHR, which is an indicator of android obesity.

PP-485
Study on the physiological role of drosophila muscarinic acetylcholine receptor homologue in insulin-like peptide signalling

H-Y. Lee[1], C-T. Oh[2], K-B. Nam[2], J-H. Ryu[2], W-J. Lee[1] and **S-J. Han**[2]
[1]*Division of Molecular Life Science and Center for Cell Signalling Research, Ewha Woman's University, Seoul, South Korea,* [2]*Innate Immunology Laboratory, Institute of Pasteur Korea, Seoul, South Korea. E-mail: han@pasteur.or.kr*

Muscarinic acetylcholine receptor (mAChR) is a prototypical class I (Rhodopsin-like) G protein-coupled receptor. Until now, five subtypes (M1–M5) have been found in mammals and reported to play many important roles in central and peripheral nervous system. It was known that there are two mAChR homologues in Drosophila (dmAChR), but no study on the physiological function of these genes has been reported yet. In mammalian pancreatic beta-cell studies, it was revealed that mAChR agonist (i.e. Oxotremorine-M) could stimulate glucose induced insulin secretion. and recent results showed that Drosophila has insulin-like peptide producing cell (IPC) which is partially corresponding to mammalian pancreatic beta-cell. Based on above reports, we examined whether dmAChRs are involved in insulin-like peptide signalling in Drosophila. It is well known that the decreasing of insulin receptor signalling potential causes improved dFoxo gene activity resulting in increased mRNA level of d4E-BP and dPepck genes in Drosophila. Surprisingly, in the dmAChR knock-down flies, the expression level of d4E-BP and dPepck genes were significantly increased. Furthermore, the dmAChR knock-down flies showed longer lifespan, and this is consistent to the phenotype which has been observed in dFoxo over-expressed flies. Our results suggest strongly that dmAChR function is closely involved in insulin signalling in Drosophila.

PP-486
The effects of thyroid hormones on glucokinase enzyme activities in diabetic rat liver tissues

D. Sahin[1], **A. Sepici Dincel**[1], A. Engin[2] and N. Altan[1]
[1]*Department of Medical Biochemistry, Gazi University, Faculty of Medicine, Ankara, Turkey,* [2]*Department of Surgery, Gazi University, Faculty of Medicine, Ankara, Turkey.*
E-mail: asepici@yahoo.com

The enzyme glucokinase (GK) (E.C. 2.7.1.2) has a low affinity for glucose and largely expressed in the liver and pancreatic beta-cells, playing a key 'glucose sensing' role to regulate hepatic glucose balance and insulin secretion. The expression of GK in rat liver tissue is under multi-hormonal control. After food withdrawal and subsequent re-feeding a maximal response in GK

activity is only achieved in the presence of glucocorticoids and thyroid hormones that can contribute to the regulation of blood sugar by accelerating the turnover of glucose. The aim of our study was to evaluate the effects of insulin and thyroid hormone treatments on GK enzyme activities in diabetes and hypothyroidism. Sprague-Dawley rats were assigned to eight groups: Group1; control, Group2; diabetes (DM) (intraperitoneally with stz 55 mg/kg), Group 3; DM + insulin (5 weeks after the stz, 7–10 kg/day, s.c.), Group4, surgically thyroidectomized control, Group5; thyroidectomized + DM (3 weeks after the operation), Group6; thyroidectomized + DM + insulin, Group7; thyroidectomized + DM + insulin + thyroid hormone (TH) (5 mg/kg), Group8; thyroidectomized + diabetes + insulin + TH (2.5 mg/kg). Glucose levels were determined by glucose oxidase enzymatic assay (Glucometer, Ames). Free and total T3, T4 levels were measured in serum samples by TOSOH otoanalyzer and GK enzyme activities were measured in liver tissue samples according to the method of Walker and Parry. GK enzyme activities were significantly decreased ($P < 0.05$) in Group 2, Group 6 and Group 7 compared to Group 1, increased ($P < 0.05$) in all groups compared to Group 2 and increased ($P < 0.05$) in Group 6, Group 7 and Group 8 compared to group 5.The possible contribution of thyroid hormones to insulin effect to normalize diabetic induced changes in liver tissue has been seen.

PP-487
Effects of beta-endorphin on plasma insulin, glucagon and glucose levels in streptozotocin-induced diabetic rats

P. Peker Akalın and N. Başpınar
Departments of Biochemistry, Selcuk University Veterinary faculty, Konya, Turkie. E-mail: pinarpekakalin@hotmail.com

In this study we aimed to determine the effects of beta-endorphin on plasma glucose, insulin and glucagon levels in streptozotocin (stz) induced diabetic rats and healthy rats. Both the diabetic and healthy groups were divided into control and experimental groups. Control groups were divided into three groups and two groups were given 0.85% NaCl solution intraperitoneally (ip) and blood samples were taken at 15 and 30 min after injection ($n = 5$ for each). Five animals were only given anaesthetics and blood samples were taken at 0 min for sham group. Experimental group was divided into two groups and injected 50 μg/kg beta-endorphin i.p. ($n = 5$ for each group) and blood was taken at 15th and 30th-min. In stz-rats, beta-endorphin reduced plasma glucose levels 30 min after injection compared to control group. Beta-endorphin reduced glucagon levels at 15 and 30 min after injection whereas insulin was increased at 15 min and decreased at 30 min. Reduced glucagon and increased insulin levels at 15 min seems to decrease glucose at 30 min. In healthy group, beta-endorphin increased insulin levels and decreased glucagon and glucose levels at both times and increased insulin and decreased glucagon levels seems to decrease glucose levels at 30 min like the diabetic group. The data was not statistically significant. In stz-diabetic rats, plasma beta-endorphin levels were lower 15 min after injection and were increased at 30 min on the contrary in healthy group beta-endorphin levels were higher at 15 min than 30 min after injection because of the plasma degradation. This data may suggest that in stz-group beta-endorphin absorption is delayed which the reason is not known exactly. As a conclusion i.p. beta-endorphin has slight effects on plasma glucose levels in diabetic and healthy individuals.

PP-488
Investigation of microalbuminuria in non-diabetic, normotensive obese women

T. Y. Erdem[1], **H. Balci**[2], S. Ugurlu[3], E. Caglar[3], A. Ucgul[3], O. Acbay[1] and S. Gundogdu[1]
[1]Department of Endocrinology, Cerrahpasa School of Medicine, Istanbul University, Istanbul, Turkey, [2]Cerrahpasa School of Medicine, Fikret Biyal Central Research Laboratory, Istanbul University, Istanbul, Turkey, [3]Department of Medicine, Cerrahpasa School of Medicine, Istanbul University, Istanbul, Turkey. E-mail: huriyebalci@yahoo.com

Objectives: Obesity is generally accepted as a very important risk factor for atherosclerosis and diabetes. In diabetic patients, hyperglycaemia may be considered as the leading cause of microalbuminuria. To investigate if obesity not accompanied by diabetes and/or hypertension is associated with microalbuminuria in female patients.
Methods: Seventy-three obese female patients were enrolled into the study. Patients with accompanying diabetes mellitus, hypertension, obesity associated with any endocrine abnormality, hepatic or renal disease, fever, infectious disease, malignancy were excluded. Weight, height, body-mass index (BMI), waist circumference, waist/hip ratio (WHR) and blood pressures were recorded. Albumin excretion in 24 h urine samples (UAE) were measured using SYNCHRON LX20 System with Microalbumin kit in 24 h urine sample.
Results: The mean albumin excretion in 24 h urine sample was 12.01 ± 10.69 mg. This value is under the lower limit of the range, albumin excretion of 30–300 mg/day, which is defined as microalbuminuria. There were no correlations between the albumin excretion in 24 h urine samples and BMI, waist circumference and WHR.
Conclusions: Diabetes mellitus and hypertension are known to be associated with microalbuminuria. However few authors investigated a possible relationship between obesity, which is a risk factor for both diabetes and hypertension, and microalbuminuria. In our study, microalbuminuria was not detected in obese women without diabetes and/or hypertension.

PP-489
Thyroid hormones-mediated effects of insulin on antioxidant enzymes from diabetic rat kidneys

C. Ozturk
Department of Medical Biochemistry, Gazi University, Faculty of Medicine, Ankara, Turkey. E-mail: asepici@yahoo.com

Alterations in antioxidant enzyme activities have been reported in diabetes, and thus the tissue antioxidant status seems to emerge as an important factor in the aetiology of diabetic complications such as cardiomyopathy, retinopathy and nephropathy. In our present study we investigated superoxide dismutase (SOD) and glutathione peroxidase (GPX) for antioxidant enzyme activities and for lipid peroxidation products, MDA and lipid hydroperoxides FOX, which can be effectively quantified in animal tissue extracts using an assay based on the formation of Fe (III) xylenol orange complex in kidney tissues of stz diabetic rats before and after thyroidectomy depending on insulin and thyroid hormone treatment. Sprague-Dawley rats were assigned to eight groups: GroupI; control, Group2; diabetes (intraperitoneally with stz 55 mg/kg), Group3; diabetes + insulin (5 weeks after the stz, 7–10 kg/day, s.c.), Group4, surgically thyroidectomized control, Group5; thyroidectomized + diabetes (3 weeks after the operation), Group6; thyroidectomized + diabetes + insulin,

Group7; thyroidectomized + diabetes + insulin + thyroid hormone (5 mg/kg), Group8; thyroidectomized + diabetes + insulin + thyroid hormone (2.5 mg/kg). Glucose levels were determined by glucose oxidase enzymatic assay (Glucometer, Ames). Free and total T3 and T4 levels were measured in serum samples by TOSOH otoanalyzer. SOD and GPX enzyme activities were significantly increased ($P < 0.05$) in Group 2 compared to Group1 and SOD levels were significantly decreased ($P < 0.05$) and GPX levels were not significantly changed in other groups compared to Group1. MDA and FOX levels were significantly increased ($P < 0.05$) in Group2 and 4 compared to Group1 and MDA levels were significantly decreased ($P < 0.05$) in Group3, 5, 6, 7 and 8 compared to Group1. Our results suggest that diabetes-induced hypothyroidism and treatments with insulin and thyroid hormones may contribute to the antioxidant enzyme alterations depending on the duration of the diseases in diabetic rats.

PP-490
Effects of ginkgo biloba extract on lipid profile and leptin levels in diet induced obese rats

Zn. Dogruer[1], B. Ercan[1], S. Kul[2], M. Y. B. Cimen[1], G. Eskandari[1], L. Tamer[1], G. Polat[1] and U. Atik[1]
[1]*Department of Biochemistry, Medical Faculty, Mersin University, Mersin, Turkey,* [2]*Department of Biostatistics, Medical Faculty, Mersin University, Mersin, Turkey. E-mail: nilzeynep@yahoo.com*

Obesity is defined as accumulation of excess body fat. Leptin, an adipocyte-derived hormone plays an important role in the energy homeostasis. We aimed to investigate possible effects of ginkgo biloba extract (EGb761) on lipid profile and leptin levels in obese rats. The study consisted of four groups. Group 1 was fed with chow diet (control). The others were divided into three groups according to diet and EGb761 therapy. After 7 weeks high-fat diet consumption groups became 20% heavier relative to control. Then Group 2 was used as obese control group and fed only with high-fat diet. The rest of groups were treated with oral EGb761 in 20 mg/kg (Group 3) and 100 mg/kg doses (Group 4) respectively. After 23 days treatment, serum triglyceride levels were 52.5 ± 9.07 mg/dl in Group 1; 109.54 ± 6.8 in Group 2; 62.31 ± 8.8 in Group 3 and 65.62 ± 8.8 in Group 4. The difference between the obese control group and EGb761 treatment groups was statistically significant ($P < 0.05$). Serum leptin levels were 1.65 ± 1.42 ng/ml in Group 1; 4.13 ± 1.83 in Group 2; 3.68 ± 1.41 in Group 3, and 3.74 ± 2.18 in Group 4 and glucose levels were 103.9 ± 17.2 mg/dl in Group 1; 171.91 ± 8.3 in Group 2; 176.51 ± 4.1 in Group 3 and 172.0 ± 28.5 in Group 4. There were a statistically significant increase in both parameters in all obese groups ($P < 0.05$). Our study showed that EGb761 might have an effect in reduction of triglyceride levels while there was no effect on leptin and glucose levels in diet induced obesity.

PP-491
Advanced glycation end products in type 2 diabetes patients with and without coronary artery disease

B. Cimen[1], R. Gelisgen[1], G. Andican[1], V. Yumuk[2], M. Bolayirli[3], M. Hacibekiroglu[3] and G. Burcak[1]
[1]*Department of Biochemistry, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey,* [2]*Department of Internal Medicine, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey,* [3]*Central Research Laboratory, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey.*
E-mail: burakcimen@gmail.com, remisagelisgen@hotmail.com

We aimed in this study to investigate firstly whether circulating advanced glycation end products (AGEs) is increased in patients with type 2 diabetes mellitus compared with control subjects and secondly whether it differs between diabetic patients with and without coronary artery disease (CAD). We studied 60 type 2 diabetic patients and 27 non-diabetic control subjects. We divided the patients according to the presence of CAD. CAD was present in 29 of the diabetic patients. As serum levels of AGEs are influenced by renal function we also evaluated the patients based on their urinary albumin excretion. We assessed AGEs, HbA1c, glucose, creatinine, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol, triglycerides, total protein and albumin in serum. We measured microalbuminuria and glomerular filtration rate (GFR). Plasma AGE peptides were measured with spectrofluorometric and low molecular peptides with spectrophotometric detectors connected online. The serum AGE-peptide concentrations were significantly higher in the diabetic patients than control subjects ($P = 0.000$). AGE-peptide concentrations did not display significant differences between diabetic patients with and without CAD. In both of these groups serum AGE peptides were significantly higher than control group ($P = 0.001$, $P = 0.05$). Between CAD patients with and without microalbuminuria. no difference was found in serum AGE-peptide concentrations.

PP-492
Gliclazide or metformin decrease lipid peroxidation and increase some antioxidants in diabetes mellitus

R. Memişoğullari[1], M. Türkeli[2], E. Bakan[3] and F. Akçay[3]
[1]*Department of Biochemistry, Duzce School of Medicine, Abant Izzet Baysal University, Duzce, Turkey,* [2]*Department of Internal Medicine, School of Medicine, Atatürk University, Erzurum, Turkey,* [3]*Department of Biochemistry, School of Medicine, Atatürk University, Erzurum, Turkey.*
E-mail: rmemisogullari@hotmail.com

The aim of this study was to investigate the antioxidant effects of sulfonylurea gliclazide and the biguanide metformin which are the antihyperglycemic agents. Gliclazide and metformin have different mechanisms to reduce glycaemia. Gliclazide, a second-generation sulfonylurea that possesses a unique azabicyclo-octyl ring, is reported to have an act as a general free radical scavenger *in vitro*. It is also reported that metformin has antioxidant activity. In this study, we evaluated 46 patients with Type 2 DM. The patients were divided into three groups: group I: treated with gliclazide, group II: with metformin, group III: not received any drug except diet treatment. Their mean durations of diabetes were 12.2, 13.4 and 11.3 years, respectively. Erythrocyte glutathione peroxidase, catalase activity, malondialdehyde, as a lipid peroxidation marker, and glutathione levels were measured in this study. The erythrocyte glutathione peroxidase and catalase activities significantly increased; contrarily malondialdehyde levels also significantly decreased in both gliclazide and metformin groups when compared with group III. There was not any significant difference between gliclazide and metformin groups in terms of activities of antioxidant enzymes and levels of malondialdehyde. In addition, glutathione levels were not different among the groups. Thus, the data obtained in this study show that gliclazide or metformin administration may decrease oxidative stress.

PP-493
Regulation of glucose 6-phosphatase gene expression by PPARalpha and RXRalpha in the fasting liver

S. K. Kwon, **S. S. Im**, T. H. Kim, J. M. Park and Y.H. Ahn
[1]*Brain Korea 21 Project for Medical Sciences, Yonsei University College of Medicine, Seoul, South Korea,* [2]*Department of Biochemistry and Molecular Biology, Yonsei University College of Medicine, Seoul, South Korea,* [3]*Center for Chronic Metabolic Disease Research, Yonsei University College of Medicine, Seoul, South Korea. E-mail: ssoonim@yumc.yonsei.ac.kr*

Glucose-6-phosphatase (G6Pase) is known to play a key role of gluconeogenesis in the fasting liver. Peroxisome proliferators activated receptors (PPARs) are transcription factors involved in the regulation of numerous metabolic processes. The PPARα isotype is abundant in liver and activated by fasting. However, it is not very clear that the expression of G6Pase gene was related to PPARa. In this study, we demonstrate that PPARα/RXRα complex also plays a role in carbohydrate metabolism via regulation of glucose 6-phosphatase gene expression. We have attempted to localize and characterize PPARα responsive element (PPRE) in the promoter regions of rat G6Pase, which plays the most important role in the glucose homeostasis in fasting liver. Treatment of Alexander or HepG2 rat hepatoma cell lines as well as primary rat hepatocytes with PPARα/RXRα agonists led to stimulation of G6Pase mRNA expression. The overexpression of PPARα by transfection in liver cell lines activated the promoter activity. Serial deletion of the construct revealed that the promoter activity was dramatically decreased when –231 region was deleted. These data suggest that the putative PPRE may be located in the rat G6Pase promoter between –268 and –256. PPARα agonist Wy14,643 is a direct transcriptional mediator of G6Pase gene.

PP-494
Pharmacokinetics and hypoglycaemic effect of 3 alpha, 7 alpha-dihydroxy-12-oxo-5beta-cholanate (MKC) in diabetic rat

M. Mikov[1], N. S. Boni[1], H. Al-Salami[1], K. Kuhajda[2], S. Kevresan[3], J.P. Fawcett[1]
[1]*School of Pharmacy, University of Otago, Dunedin, New Zealand,* [2]*Department of Biochemistry, Faculty of Sciences, University of Novi Sad,* [3]*Department of Chemistry, Faculty of Agriculture, University of Novi Sad, Serbia and Montenegro. E-mail: momir.mikov@stonebow.otago.ac.nz*

Our previous studies have shown that the semisynthetic bile acid derivative, sodium MKC (US patent No 6,060,465), has hypoglycaemic activity. The aim of this study was to investigate the relationship between the pharmacokinetics (PK) and hypoglycaemic activity of MKC in healthy and diabetic rats. Groups of healthy and alloxan-induced diabetic rats were dosed i.v. or orally with MKC (4 mg/kg). Blood samples were taken before the dose and at 20, 40, 60, 80, 120, 150, 180, 210 and 240 min post-dose. MKC serum concentrations were measured by a newly developed HPLC method. PK parameters were determined using the WinNonlin program. Absolute bioavailability of MKC was low in both healthy and diabetic rats (29 and 23% respectively) and there was no significant difference in relative bioavailability between the groups. MRT, Vd and t1/2 of MKC after oral application were significantly smaller in diabetic than in control rats (21%, 31% and 29% respectively). After the i.v. dose, the change in blood glucose concentration was not significant in either control or diabetic rats. After the oral dose, the decrease in blood glucose concentration was significant reaching a maximum decrease from baseline of 24% in control and 15% in diabetic rats. The results suggest a first pass effect is crucial for the hypoglycaemic activity of MKC indicating a metabolite of MKC and/or interference with metabolism and transport of glucose is responsible.

Acknowledgment: Supported by University of Otago Research Grant.

PP-495
Effects of simvastatin on cardiac nitric oxide synthase and nitrite levels in streptozotocine-induced diabetic rats

U. Yazgan[1], **Ö. Yıldırım**[2] and D. Dinçer[2]
[1]*Department of Pharmacology, Faculty of Pharmacy, Ankara University, Ankara, Turkey,* [2]*Department of Biology, Faculty of Science, Ankara University, Ankara, Turkey. E-mail: yildirim@science.ankara.edu.tr- ozlemesn@mail.com*

Cardiovascular complications are most important cause of death for diabetic patients. Simvastatin, HMG-CoA reductase inhibitor is widely prescribed to lower high serum cholesterol levels also in diabetic patients. Recent publications stated that it has demonstrated pleotropic effects along with hypolipidemic effects by increasing NOS levels. We studied the cardiac effects of simvastatin by examining the nitric oxide synthase expressions in Type 1 diabetic animals, which we have constituted experimentally. For this, we treated Sprauge-Dawley rats with 1 mg/kg i.p. simvastatin for 6 weeks, 8 weeks after we had established Type I diabetes with a single shot of 45 mg/kg streptozotocine. The total cholesterol, triglyceride and VLDL levels, increasing with diabetes, recessed to control levels. With the measurements of cardiac nitric oxide synthase expressions, no statistical differences were found between control animals, diabetic animals and the ones receiving simvastatin treatment in both eNOS and iNOS levels. Though no difference was found in gene expressions, nitrite levels, by-products of NO cleavage, was also measured and we tried to determine whether a difference in the final product was present or not. The measurement demonstrated a decrease due to diabetes and values exceeding control levels after the treatment. But these deviations have no statistical importance. As a consequence, the lowness of nitrite levels measured in diabetes can be due to the decrease in NO effect.

PP-496
The effect of leptin secretion in fat distribution of women

A. Abusafa[1], G. Dikmen[2], H. Cantel[1], S. Onder[3], **I. Unsal**[4], P. Dogan[4] and Y. Erk[1]
[1]*Department of Plastic Surgery, University of Hacettepe, Sihhiye, Ankara, Turkey,* [2]*Department of Biochemistry, University of Hacettepe, Sihhiye, Ankara, Turkey,* [3]*Department of Pathology, University of Hacettepe, Sihhiye, Ankara, Turkey,* [4]*Department of Biochemistry, Acibadem Health Group, LABMED, Uskudar, Istanbul, Turkey, E-mail: iunsal@asg.com.tr*

Leptin, the product of Ob gene, is a recently isolated circulating peptide hormone that is primarily synthesized and secreted by adipocytes. Although plenty of obesity related studies with leptin secretion levels have already been conducted, there was not any study for demonstration of relation between specific body fat distribution and leptin secretion. The objective of the study was to compare the levels of leptin secretion from three different anatomic parts of jenoid and android women based on their body mass index (BMI, kg/m^2) and waist/hip ratio.

Adipose tissue samples were collected from breast, abdomen and medial part of thigh of 23 premenoposal women (11 jenoid, 12 android) under general anaesthesia. Tissue samples were incubated with Krebs Ringer Phosphate (pH: 7.4) at 370C for 2 h and leptin secretion was measured by Leptin Elisa kit (Diagnostic Biochem Canada). In the jenoid group, the leptin secretion from the waist area was found to be significantly higher than the leptin secretion from the hip area ($P < 0.05$). On the other hand, there was no such a difference between the anatomic locations in the android group ($P < 0.05$). Additionally, histopathologic examination of fat tissue obtained from the waist area of the jenoid group demonstrated increased vascularity in this specific area. Leptin may be the reason of increased vascularity and metabolism in the waist area of jenoid group and therefore may be responsible from the jenoid type fat distribution in women.

Lipid Related Disorders and Atherosclerosis

PP-497
The effects of CETP Taq1B polymorphism in renal transplant patients

U. Zeybek[1], H. A. Ergen[1], H. Yilmaz[1], N. Bozkurt[1], E. Akoglu[2] and **T. Isbir**[1]
[1]Department of Molecular Medicine, DETAE, Istanbul University, Istanbul, Turkey, [2]Department of Internal Medicine, Marmara University, Istanbul, Turkey. E-mail: tisbir@superonline.com

Background: Dyslipidemia is an important complication in renal transplant patients. Cholesterol ester transfer protein (CETP) mediates the exchange of cholesteryl ester between high density lipoprotein (HDL) and low density lipoprotein (LDL). The aim of this study was to investigate CETP Taq1B gene mutations and lipid abnormalities in renal transplant patients.
Methods: We studied 29 renal transplant patients and 29 healthy controls. Gene polymorphism of CETP Taq1B was determined by PCR (polymerase chain reaction) and RFLP techniques. Serum lipid levels were measured enzymatically. Statistical analyses were performed by SPSS for windows version 10.0.
Results: The frequencies of CETP Taq1B B1B1, B1B2 and B2B2 genotypes among the patients 44.8%, 34.5%, 20.7% and in control subjects were 37.9%, 37.9%, 24.2% respectively. The patients with B1B1 genotype had the highest levels of TC, TG, LDL-cholesterol and VLDL-cholesterol ($P < 0.05$). And also carrying B1 allel in patients had higher levels of TC, LDL-cholesterol, VLDL-cholesterol ($P < 0.05$) compared to healthy controls.
Conclusions: We observed that CETP Taq1B B1 allele and B1B1genotype have effected on the serum lipid profile in renal transplant patients.

PP-498
Interleukin-1 receptor antagonist 2/2 genotype is associated with single vessel coronary artery disease in Turkish population

A. Coker[1], A. Arman[2], O. Soylu[3], A. Yıldırım[3], N. Ercelen[4], A. Aydogan[3] and T. Tezel[3]
[1]The Department of Biology, Art and Sciences, Marmara University, Istanbul, Turkey, [2]The Faculty of Engineering, Marmara University, Istanbul, Turkey, [3]The Department of Cardiology, Dr. Siyami Ersek Hospital, Istanbul, Turkey, [4]The Department of Medical Genetics, American Hospital, Istanbul, Turkey. E-mail: ajda_coker@yahoo.com

Atherosclerosis is an inflammatory disease that affects the arteries. Coronary Artery Disease (CAD) is a multifactorial heart disease caused by atherosclerosis of coronary arteries. Cytokine gene variations such as IL-1 family are involved in the pathogenesis of atherosclerosis. The purpose of this study was to determine the relationship between polymorphism of IL-1 gene family and risk factor to CAD in Turkish population. 266 patients participated for coronary angiography and they were grouped: 112 patient control subjects, and 154 CAD; and then CAD group were divided into two subgroups: 58 SVD, and 96 Multiple Vessel Disease (MVD). Polymerase chain reaction (PCR) was used to determine the genotype of the IL-1RN. Genotyping of IL-1B polymorphisms at positions −511 and +3953 was detected by PCR followed restriction fragment length analysis. The significant association was seen in IL-1 RN 2/2 genotype between SVD and normal subjects ($P = 0.011$). Also, no statistically significant differences were found in IL-1RN allele frequency between CAD and control subjects, or MVD and control subjects, or SVD and control subjects. In addition, no significant association was observed in genotype distribution and the allelic frequency of IL-1B promoter (−511) and IL-B (+3953) between SVD and the controls or MVD and the control or CAD and the control. All these results imply that IL-1R N 2/2 genotype may be risk factor for SVD of coronary artery disease in Turkish population

PP-499
Effects of smoking cessation on antioxidant status and paraoxonase activity in cigarette smokers

I. Erguder[1], T. Erguder[2], C. Ozkan[3], N. Bozkurt[3], K. Soylu[4], E. Devrim[1] and I. Durak[1]
[1]Department of Biochemistry, Ankara University School of Medicine, Ankara, Turkey, [2]Substance Dependence Department of Primary Health Care General Directorate, Ministry of Health, Ankara, Turkey, [3]Company Doctor, MAN Ankara, Turkey, [4]Biochemistry Laboratory, Cebeci Hospital, Ankara University School of Medicine, Ankara, Turkey.
E-mail: imgeerguder@yahoo.com

Aim: The aim of this study was to compare short term effects of smoking cessation on blood oxidant/antioxidant status, cholesterol levels and paraoxonase activity.
Methods: Sixteen healthy, asymptomatic long-term cigarette smokers (mean age: 35 ± 9 years) participated in the study in the smoking cessation program. After and before smoking cessation, subjects were examined for oxidant/antioxidant status, cholesterol level, paraoxonase activity, breath carbon monoxide levels and blood carboxyhemoglobin values.
Results: When compared to previous values, subjects were revealed statistically significant decreases in malondialdehyde and carbon monoxide levels 4 weeks after the smoking cessation. HDL (High density lipoprotein)/LDL (Low density lipoprotein) cholesterol ratio was found to be increased. Significantly increased paraoxonase activity was also observed in the blood samples obtained after cigarette cessation period.

Conclusions: It has been concluded that all these changes observed after smoking cessation might be of importance in the reduction of cardiovascular risk parameters in the smokers.

PP-500
Soluble costimulatory molecules as markers for atherosclerosis

P. Sakthivel[1], **V. Shively**[2], M. Kakoulidou[1], W. H. Pearce[2] and A. K. Lefvert[1]
[1]Karolinska Institute, CMM (L8:00), Immunology Research Unit, Stockholm, Sweden, [2]Department of vascular surgery, Feinberg School of medicine, Northwestern University, Chicago, USA.
E-mail: priya.sakthivel@cmm.ki.se

The importance of T cell costimulatory molecules such as CD28 and CTLA-4 and their ligands CD80 and CD86 in immune activation is highlighted, these days. These molecules exist as membrane bound and also as soluble forms. We, hence, determined the circulating levels of soluble forms of these costimulatory molecules in the patients with abdominal aortic aneurysm (AAA) and in elderly causacians and correlated with the other biomarkers for inflammation and atherosclerosis in these conditions. AAA is a localized dilatation in the wall of the abdominal aorta. We found significantly elevated levels of soluble CD28 and CD86 and significantly decreased levels of soluble CTLA-4 in the patients when compared with healthy controls. These soluble costimulatory molecules were also correlated with other biomarkers such as Matrix metalloproteinase (MMP) 9, C-reactive protein and also with size of the aorta. We found a significant inverse relationship between the soluble CD80 and CTLA-4 with MMP-9.We also analysed the soluble T cell costimulatory molecules and levels of adipocyte secreted hormones such as adiponectin and leptin in the elderly Caucasians. We found that the soluble CD86 has significant inverse correlation with the levels of adiponectin. These data shows the importance of soluble costimulatory molecules in the diagnosis. Therefore, we conclude that the soluble costimulatory molecules could serve as functional biomarkers in the AAA patients and also in elderly causacians.

PP-501
Test-system for inhibitors of inflammation at initial stages of ischemia and atherosclerosis

D. A. Aleksandrov, M. A. Pushkareva, S. I. Galkin and G. F. Sudina
A. N. Belozersky Institute for Physico-Chemical Biology, Moscow State University, Moscow, Russia.
E-mail: alex-1-a-rus@yandex.ru

As known, cholesterol deposition, macrophage invasion, lipoprotein oxidation and leukocyte adhesion lead to endothelium lesions and restriction of blood flow. 5-lipoxygenase in activated polymorphonuclear leukocytes (PMNL) and monocytes starts to synthesize leukotrienes (LT), witch are inflammatory chemoattractants. Here we present a novel test-system for inhibitors of LT synthesis, reactive oxygen intermediates production and PMNL/endothelial cells (human umbilical vein endothelial cells) and PMNL/collagen adhesion. Such a wide scope provides us with a possibility to estimate the effect of potential medicine compounds on many cellular functions, which is important for evaluation of side effects of tested compounds. Importantly, using this way we can get a lot of different parameters not only in complicated multifactor system but as well in several simple single-cell type assays, what is dramatically important for optimization of already existent inhibitors. All in all presented approach allows us to take into account the levels of endogenous pro-inflammatory agents and to evaluate the effects of newly synthesized molecular inhibitors. The results obtained using such system have confirmed its advances and revealed unknown functions of the natural substances like cholesterol phosphate, sulfate and sulfated galactocerebrosides. We propose this test-system to be a promising approach in further investigations of atherogenesis and drug development.

PP-502
Low density lipoprotein (LDL) subtypes of a group of patients undergoing coronary angiography

Y. Oztas[1], C. Ozdol[2] and L. Karaca[3]
[1]Biochemistry, Medical Faculty, Hacettepe University, Ankara, Turkey, [2]Cardiology, Ibni Sina Hospital, Ankara University, Ankara, Turkey, [3]Biochemistry, Medical Faculty, Ufuk University, Ankara, Turkey. E-mail: yesimo55@yahoo.com

Although small, dense LDL is a proposed risk factor for coronary artery disease, large LDL can cause atherosclerosis by different metabolic pathways. This study has been conducted to assess the association between LDL subtypes and coronary stenosis in a group of patients with and without stenosis according to angiography performed in the same month. LDLs were separated according to size by polyacrylamide gradient gel electrophoresis method. LDL phenotype was designated as small, medium, and large. The cases with at least one vessel stenosed according to the angiography were assigned to the stenosis group ($n = 36$) and the patients without stenosis were included in the nonstenosis group ($n = 20$). Of the 56 cases, 28 had large LDL, 12 had medium LDL and 16 had small LDL phenotypes. Nonstenosis group had 45% large LDL and 25% small LDL whereas stenosis group had 52.7% large LDL and 30.5% small LDL. The difference in LDL phenotypes between stenosis and nonstenosis groups were not significant statistically. Mean serum triglyceride levels were different between patients with small, medium and large LDL phenotypes ($P < 0.005$), being highest in the patients with small LDL phenotype. It was concluded that detection of LDL subclasses does not make an extra contribution to the information obtained by measuring the serum levels of LDL and triglycerides for the assessment of dyslipidemia in atherosclerosis in our patient group.

PP-503
Apolipoprotein (A) polymorphisms in a healthy Macedonian population

K. Tosheska, D. Labudovic, S. Alabakovska and B. Todorova
Department of medical and experimental biochemistry, Medical faculty, Skopje, R. Macedonia.
E-mail: tosheskatrajkovska@yahoo.com

Elevated lipoprotein (a) [Lp (a)] concentrations are positively correlated with premature coronary heart disease (CHD) and are thought to reflect allelic variation in apolipoprotein (a) [apo (a)], the protein unique to Lp (a). Apo (a) exists in polymorphic forms that exhibit different apparent molecular masses. We used a 3–15% gradient polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS-PAGE) method followed by immunoblotting to separate and visualize apo(a) forms present in plasma from 180 healthy Macedonian blood donors. One or two isoforms (relative molecular mass from 417 000 to 785 000) were present in each individual. On the hypothesis that apo (a) polymorphism is controlled by different alleles at a single locus, the frequency of the six alleles determined from the observed phenotypes was: LpB = 0.022, LpS1 = 0.028, LpS3 = 0.201,

LpS4 = 0.397, Lp > S4 = 0.110, LpO = 0.242. These fit the expectations of the Hardy-Weinberg equilibrium in this population. A significant inverse correlation was found between plasma Lp(a) levels and the size of Apo (a) isoforms (Pearson's correlation coefficient (r) = -0.3477, $P < 0.001$). A highly skewed distribution of Lp (a) toward lower levels in the Macedonian population may be explained by the high frequencies of alleles for large apo (a) isoforms and the null allele.

PP-504
Serum paraoxonase activity in patients with coronary artery disease: a preliminary study

T. Bayrak[1], A. Bayrak[1], B. Yavuz[2], Ö. Düzgünçınar[1], A. Deniz[2], L. Tokgözoğlu[2] and E. Demirpençe[1]
[1]*Departments of Biochemistry, Faculty of Medicine, Hacettepe University, Ankara, Turkey,* [2]*Departments of Cardiology, Faculty of Medicine, Hacettepe University, Ankara, Turkey.*
E-mail: bayrakt@hacettepe.edu.tr

The protective effect of HDL against atherosclerosis is believed to reside in its enzymes, particularly paraoxonase. Human serum paraoxonase (PON1) is closely associated with a specific HDL subfraction also containing apoA1 and its major physiological function appears to be the hydrolysis of oxidized lipids. The aim of this study is to evaluate the relationship between serum PON1 activity and the severity of coronary artery disease (CAD). Thirty control subjects (Group I) with normal coronary artery and 47 patients with CAD (Group II) documented by coronary angiography were included in this study. The coronary artery lesions score was recorded according to Gensini scoring system. In addition to routine blood chemistry, PON1 activity was measured through a spectrophotometric method using paraoxon as substrate. In this preliminary study, we found that there was no significant difference between serum PON1 activities of patients with CAD and control subjects (median 76.0 [(Range 15–229) U/ml versus median 46.5 (Range 15–298) U/ml]. Serum PON1 activity was not correlated with the severity of the disease either. We concluded that a broader subject group is needed to assess the role of PON1 activity as a negative factor in CAD. We are currently involved in such a study evaluating paraoxonase gene polymorphisms and PON1 activity in both healthy and atherosclerotic subjects in Turkish population.

Key words: atherosclerosis, paraoxonase, independent risk factor.

PP-505
Plasma myeloperoxidase level correlates with the severity of coronary artery disease

O. Düzgünçınar and E. Demirpençe
Biochemistry Department, Medicine Faculty, Hacettepe University, Ankara, Turkey. E-mail: drodc@yahoo.com

Neutrophil myeloperoxidase (MPO) is thought to be involved in the pathophysiology of atherosclerosis through the oxidation of apolipoproteins. It has been shown that the main apolipoprotein of HDL, apoA1, is subjected to nitration by MPO and this oxidative modification renders HDL proatherogenic. The aim of this study is to evaluate the relationship between MPO level and severity of coronary artery disease (CAD). Forty-eight patients with CAD and 30 control subjects with normal coronary circulation documented by coronary angiography were included in this study. The coronary artery lesions score was evaluated using Gensini scoring system. In addition to routine blood chemistry, plasma MPO levels were measured using an enzyme immunoassay for human MPO. MPO levels were significantly higher in patients with CAD than control subjects [median 4.27 (Range 1.60–42.43) U/mg versus median 2.93 (Range, 1.00 to 9.25) U/mg, $P = 0.002$]. A positive relationship was found between MPO levels and Gensini scores ($P = 0.044$, r = 0.228). Our study showed that increased MPO levels were found in patients with CAD and this increase was correlated with the severity of the disease. We suggest that elevated plasma MPO might be an independent risk factor for atherosclerosis. Thus it may be considered as a putative prognostic marker in patients with CAD.

PP-506
The effect of hormone replacement therapy on the levels of serum lipids, apolipoprotein AI, apolipoprotein B and lipoprotein (A) in Turkish postmenopausal women

A. Bayrak[1], D. Aldemir[2], T. Bayrak[1], P. Dursun[3] and A. Çorakçı[4]
[1]*Departments of Biochemistry, Faculty of Medicine, Hacettepe University, Turkey,* [2]*Biochemistry Department, Faculty of Medicine, Başkent University, Ankara, Turkey,* [3]*Departments of Obstetrics and Gynecology, Faculty of Medicine, Hacettepe University, Turkey,* [4]*Departments of Obstetrics and Gynecology, Faculty of Medicine, Kocaeli University, Turkey.*
E-mail: ahmetb@hacettepe.edu.tr

Objectives: Estrogen replacement therapy (ERT) alters the lipid profiles favorably for delaying atherosclerosis in postmenopausal women. The effects of estrogen plus progesterone combination therapy on lipids are controversial. This study was designed to evaluate the effect of female sex hormones on lipids and lipoproteins and to clarify the influence of progesterone on the effect of estrogen in postmenopausal women.
Methods: Of the 60 postmenopausal women admitted to our menopause clinic, 40 had intact uterus and received continuous 0.625 mg conjugated equine estrogen (CEE) plus 2.5 mg medroxyprogesterone acetate (MPA).Lipid and lipoprotein levels were assessed in each subject at baseline and at the 6th and 18th months of therapy.
Results: Following 18 months of treatment, both regimens reduced total cholesterol (TC) levels as compared with the baseline (6.4% versus 6.9% in the CEE/MPA and CEE groups, respectively). The CEE group had a more pronounced increase in high-density lipoprotein (HDL) cholesterol than the CEE/MPA group (10.3% versus 8.8% respectively). Both groups displayed reduced TC, low-density lipoprotein (LDL) cholesterol and apolipoprotein-B (ApoB) concentrations, whereas triglycerides (TG) increased, with a greater tendency to increase in the CEE/MPA group at the end of the trial.
Conclusion: Both treatment regimens caused positive alterations in the lipid and lipoprotein profiles.
Key words: estrogen replacement therapy (ERT), HRT, lipids.

PP-507
Effects of androgen replacement therapy on cardiac risk markers

S. Aksit[1], **F. B. Aksungar**[2], A. Eroglu[1], A. E. Topkaya[3] and L. Saporta[1]
[1]*Department of Urology, School of Medicine, Maltepe University, Istanbul, Turkey,* [2]*Department of Biochemistry, School of Medicine, Maltepe University, Istanbul, Turkey,* [3]*Department of Microbiology, School of Medicine, Maltepe University, Istanbul. Turkey. E-mail: fehimebenli@aksungar*

Although male sex is a well documented risk factor for cardiovascular diseases, pharmacological doses of testosterone or its

potent metabolite dihydrotestosterone is shown to produce vasodilation. Data from clinical studies indicate that, in men, androgen replacement may provide beneficial effects when coronary artery disease is present. In this study we have evaluated the effects of androgen replacement therapy on cardiac risk markers. Thirteen male hypogonadal patients aged between 46 and 69 (mean 58) were treated with two doses of 250 mg IM testosterone preparations. Serum total cholesterol (Chol), HDL, LDL, triglicerides, lipoprotein (a) and plasma homocysteine, D-dimer and fibrinogen levels were measured before and 4 weeks after treatment. Serum HDL ($P = 0.77$), trigliceride ($P = 0.87$) levels, Chol/HDL ($P = 0.06$) ratio and plasma fibrinogen ($P = 0.97$) levels did not change significantly while total cholesterol ($P = 0.041$), LDL ($P = 0.046$) and Homocysteine ($P = 0.049$), levels were minimally decreased after treatment. Lipoprotein (a) ($P = 0.001$) and D-dimer ($P = 0.017$) levels were significantly decreased after testosterone replacement. Our results show that, androgen replacement therapy has positive effects on independent cardiac risk markers such as D-dimer, homocysteine and lipoprotein (a) and with these findings we conclude, androgens may have protective effects on the cardiovascular system through either their metabolic and direct effects on vascular system.

PP-508
Changes in sialylation of LDL in coronary artery disease

M. T. Goodarzi[1], M. Rezaei[1], E. Moadel[1], M. R. Safari[1] and **H. Homayoonfar**[2]
[1]Department of Biochemistry, Medical School, Hamadan University of Medical Sciences, Hamadan, Iran, [2]Internal Medicine, Hamadan University of Medical Sciences, Hamadan, Iran. E-mail: mtgoodarzi@yahoo.com

Reduction of LDL sialylation may correlate with CAD, but the details of this modification and its effect on the CAD are not studied well. This study was aimed to show desialylation of LDL and to reveal more details of this modification. Blood sample was collected from 16 patients with CAD and 25 healthy individuals. Total serum sialic acid (TSA) was measured and LDL was extracted from all samples. Interaction of extracted LDL with lectins (MAA, SNA, DSA) was studied using lectin-blotting method. Serum total sialic acid (TSA) in CAD patients and healthy individuals were 71.9 ± 2.66 and 60.76 ± 2.34 mg/dl respectively, and the difference between two groups was statistically significant ($P < 0.001$). The intensity of interaction between extracted LDL and SNA and MAA lectins were lower in CAD patients compared to those of normal subjects ($P < 0.001$). The intensity of LDL with DSA was higher in CAD patients ($P < 0.001$). There was a negative correlation between TSA and intensity of LDL interaction with SNA and MAA in both groups, but in case of DSA this correlation was direct and positive. These finding showed increase in desialylation of LDL in CAD. LDL interaction with different lectins indicated increase in desialylated form of LDL in patients with higher level of sialic acid. In CAD patients with higher level of TSA, the desialylated form of LDL was also higher. It was concluded that LDL subjected to glycosylation changes in CAD and there is positive correlation between TSA and desialylated form of LDL.

PP-509
Eicosapentaenoic acid modulates prostaglandin synthesis in RAW 264.7 macrophages stimulated by fetal bovine serum

D. Nieves and **J. Moreno**
Physiology, University of Barcelona, Barcelona, Spain. E-mail: jjmoreno@ub.edu

Eicosapentaenoic acid (EPA) is a polyunsaturated fatty contained in fish oil. It has been reported that EPA in addition to antithrombotic and anti-atherosclerotic effects, also exhibits anti-inflammatory effects. To evaluate the possible mechanism of these effects, we examined the action of EPA on arachidonic acid (AA) incorporation to membrane phospholipids as well as the effect of EPA on AA release and AA metabolism. Our results show that [^{3}H]AA and [^{14}C]EPA were similar incorporated into RAW 264.7 macrophage membranes and the pattern of redistribution between phospholipids was also similar. [^{3}H]AA or [^{14}C]EPA release were induced by fetal bovine serum (FBS) in a similar form and AA was metabolised 3-fold more than EPA. In this way, we observed that AA may be metabolized by cyclooxygenase (COX)-1, COX-2 and 5-lipoxygenase (5-LOX) whereas EPA was metabolised by COX-2 and 5-LOX pathways. Moreover, both fatty acids were able to induce COX-2 expression. When we incubated [^{3}H]AA labelled cells with exogenous EPA, we observed that EPA did not modify [^{3}H]AA release induced by FBS, but EPA presence decreased [^{3}H]AA metabolism and consequently prostaglandin E2 synthesis. These data suggest that the effects of EPA consumption on inflammation and or atherosclerotic processes might be attributable, at least in part, to the marked decrease of eicosanoid release as consequence of the impairment of AA levels in phospholipid membranes, a PUFA easier metabolised than EPA.

PP-510
Plasma leptin concentrations 3 and 6 weeks after cessation of cigarette smoking

A. B. Erbağcı[1], **N. Bayram**[2], R. Deveci[1], A. Topçu[2], M. Tarakçıoğlu[1] and A. Filiz[2]
[1]Department of Biochemistry and Clinical Biochemistry, Faculty of Medicine, University of Gaziantep, Gaziantep, Turkey, [2]Department of Pulmonary Diseases, Faculty of Medicine, University of Gaziantep, Gaziantep, Turkey. E-mail: berbagci@hotmail.com

Cessation of smoking often induces hyperphagia and weight gain associated with decreased energy expenditure as well. Plasma leptin levels and adipose tissue mRNA correlates with mass of adipose tissue, and represent important signals for registration of total body energy status. In this study 19 smokers (13 male, 6 female) referring to the smoking cessation clinic were randomly assigned to control group ($n = 6$), nicotine patch group ($n = 6$), nicotine patch + fluoxetine group ($n = 7$). Plasma leptin levels and, body mass indices (BMI) were determined before and 3, 6 weeks after cessation of smoking. A significant increase ($P < 0.02$) in plasma concentration of leptin was found in the 3rd and 6th weeks compared to basal leptin levels, whereas 3rd and 6th week leptin concentrations were comparable. The univariate analysis of variance which explained 0.541% variances ($P < 0.01$) in leptin levels between 0 and 3 weeks has indicated BMI as the only significant contributor, controlling for BMI, gender, exhaled carbon monoxide levels and study groups. Our

results suggest that cessation of cigarette smoking increased plasma leptin concentration *in vivo*, especially within first 3 weeks associated with increases in BMI. However our model explained about 50% of variation in leptin concentrations suggesting contribution of other factors. Previously acute reduction of plasma leptin concentration upon cigarette smoking was reported which might be due to indirect effects involving catecholamines.

PP-511
Sialic acid levels in dialysis patients

G. Yılmaz[1], F. M. Yılmaz[1], M. Duranay[2], **H. Parpucu**[2] and N. Tekeli[2]
[1]*Biochemistry, Ministry of Health Ankara Hospital, Ankara, Turkey,* [2]*Dialysis Unit, Ministry of Health Ankara Hospital, Ankara, Turkey. E-mail: drgulsenyilmaz@hotmail.com*

Atherosclerosis is a major problem in patients on dialysis and elevated sialic acid (SA) levels has been reported as a strong predictor of cardiovascular mortality. In this study, we aimed to determine SA levels of chronic hemodialysis and peritoneal dialysis patients (CAPD) and investigate its relationship with coronary artery disease (CAD). A total number of 76 subjects (27 HD, 27 CAPD, 22 control) were included in the study and their SA and hs-CRP levels were measured. SA levels of HD and CAPD groups were significantly higher than control group ($P < 0.001$, $P < 0.001$, respectively). There was not a significant difference between HD and CAPD groups ($P = 0.749$). SA and hs-CRP showed a positive correlation with CAD which was diagnosed by previous admission with documented myocardial infarction or symptoms consistent with angina confirmed by exercise test or angiography ($r = 0.238$, $r = 0.422$, respectively). There was a strong correlation between SA and hs-CRP ($r = 0.608$). SA correlated with age but not duration of dialysis ($r = 0.260$, $r = 0.862$, respectively). This study shows raised inflammation markers (SA and hs-CRP) and their correlation with cardiovascular disease. Finally, we consider that there is a need for a complicated study including large number of patients with more certain CAD diagnosis criteria and this will bring more suitable sight in deciding which marker is superior: SA or hs-CRP.

PP-512
Effect of alveolar surfactant protein SP-C on the surface behavior of phospholipid monolayers at different phase state

A. Jordanova[1], R. Valtcheva-Sarker[2], E. Stoimenova[2], G. A. Georgiev[2], D. Petkova[1], R. Todorov[3] and Z. Lalchev[2]
[1]*Bulgarian Academy of Sciences, Institute of Biophysics, 1113 Sofia, Bulgaria,* [2]*Biological Faculty, Sofia University "St. Kliment Ohridski", 1164 Sofia, Bulgaria,* [3]*Bulgarian Academy of Sciences, Institute of Physical Chemistry, 1113 Sofia, Bulgaria. E-mail: albena@biofac.uni-sofia.bg*

The alveolar surfactant (AS) is a lipoprotein film, lining the alveoli and lowering the surface tension at the air/liquid interface thus preserving the lung from collapse during expiration, i.e. from respiratory distress syndrome (RDS). RDS, induced by inactivation of AS, is lipid related disorder connected with phospholipid deficiency and/or change of lining film structure. The phosphatidylethanolamines (PE) are minor lipid class in AS that may form both lamellar and non-lamellar structures under physiological conditions and thus the PE polymorphic phase behavior may have a crucial role in the arrangement and effective functioning of AS. The aim of the present study was to investigate the interaction between the specific hydrophobic surfactant protein SP-C and insoluble PE monolayers formed from dipalmitoleoyl-PE (DPoPE) in different liquid-crystalline lipid phases. The dependences of DPoPE monolayer surface tension versus time for lamellar and non-lamellar DPoPE phases at the air/water interface were investigated in presence and absence of SP-C. The comparative analysis of the results for the surface interaction showed that the incorporation of SP-C into the DPoPE monolayer changes its equilibrium and dynamic surface tension in different degree. The SP-C altered the surface reorganization of the lipid-protein monolayer in different extent, depending on the lipid phase state.

Acknowledgements: This work was supported by grant VU-B-2/05 from BG Ministry of Education and Science.

PP-513
Effect of pectin and amidated pectin on cholesterol homeostasis in rats fed a high-cholesterol diet

M. Marounek[1], Z. Volek[2] and E. Skřivanová[3]
[1]*Laboratory of Physiology of Nutrition, Research Institute of Animal Production and Institute of Animal Physiology and Genetics, Prague, Czech Republic,* [2]*Laboratory of Physiology of Nutrition, Research Institute of Animal Production, Prague, Czech Republic,* [3]*Laboratory of Physiology of Nutrition, Research Institute of Animal Production. E-mail: marounek@iapg.cas.cz*

Experiments were performed on male young rats. Control rats were fed a diet supplied with protected palm fat and cholesterol at 50 and 10 g/kg, respectively. Rats of other groups were fed the same diet containing citrus pectin or its octadecylamidated derivatives at 60 g/kg. There were no differences in the feed intake, but all pectins decreased body weight gain by 11.9–16.7%. Diets were fed for 4 weeks, then rats were sacrified. Pectinamide of lower degree of amidation (30%) increased serum HDL cholesterol at expense of other cholesterol fractions. Pectinamide of higher degree of amidation (53%) significantly decreased total serum cholesterol by 19.7%. Amidated pectins significantly decreased hepatic concentrations of cholesterol and fat by 56.1 and 62.5%, and 17.5 and 23.1%, respectively. Pectinamide of lower and higher degree of substitution increased faecal content of cholesterol by 71.2 and 62.3%, respectively. Faecal concentrations of coprostanol were decreased. Effects of citrus pectin on cholesterol homeostasis were absent or marginal. Histological examination revealed that hepatic tissue of control and pectin-fed rats was infiltrated with lipids. The Sudan-black positive material was absent in liver of rats fed pectinamides. No pathological changes of liver tissue were apparent. In conclusion, hydrophobic amidated pectins altered significantly cholesterol homeostasis in rats. No beneficial effects of intact pectin with the same carbohydrate backbone were observed.

PP-514
The effect of angiotensin-converting enzyme inhibition on endothelial dysfunction and DNA damage in patients with coronary artery disease

S. Arkaya[1], S. Yurdakul[2], A. K. Bilge[2], Y. Nisanci[2] and U. Mutlu-Turkoglu[1]
[1]*Department of Biochemistry, Istanbul Medical Faculty, Istanbul University, 34093 Istanbul, Turkey,* [2]*Department of Cardiology, Istanbul Medical Faculty, Istanbul University, 34093 Istanbul, Turkey. E-mail: Alper.Arkaya@alcatel.com.tr*

Endothelial dysfunction is an important factor in the pathogenesis of atherosclerosis. Angiotensin converting enzyme (ACE)

inhibitors are widely used in the treatment of cardiovascular disease and may preserve endothelial function. The mechanism of their effect on endothelia is believed to derive from their effects on nitric oxide production and antioxidant effects, possibly independent of blood pressure reduction. Reactive oxygen species (ROS)-induced DNA damage has recently been identified in both human and experimental atherosclerosis. The aim of the study was to determine the endothelial dysfunction and to investigate its correlation with DNA damage. Forty patients (20 patients were taking ACEI and 20 were taking medication other than ACEI) with angiographically documented coronary artery disease (CAD) and twenty age and sex matched healthy subjects were included in the study. Endothelial function was analysed by flow-mediated dilatation (FMD) of the brachial artery using Doppler USG. Oxidative DNA damage was evaluated as single strand breaks (SSBs), formamidopyrimidine glycosylase (Fpg) sensitive sites by the comet assay in DNA isolated from peripheral blood lymphocytes. The FMD values were similar among patients taking ACEI and not taking ACEI and control groups. Although there were no significant differences in basal DNA damage scores between the patients and the healthy subjects, the DNA damage score after incubation with Fpg were significantly higher in patients ($P < 0.05$). There was a significant inverse correlation between DNA damage after incubation with Fpg and FMD. Our data support presence of oxidative DNA damage and its relation with endothelial dysfunction assessed by FMD in CAD patients.

PP-515
The association of paraoxonase polymorphisms PON1-L55M and PON1-R192Q with coronary artery disease in Turkish population

S. Tanrikulu, U. Mutlu-Turkoglu, K. Ozcan and E. Ademoglu
Department of Biochemistry, Istanbul Medical Faculty, Istanbul University, 34093 Istanbul, Turkey. E-mail: sev_t80@yahoo.com

Human paraoxonase (PON) enzyme has been implicated in the pathogenesis of atherosclerosis. Several studies have suggested that genetic variations of the PON gene associated with plasma HDL levels and coronary artery disease (CAD). The aim of this study was to determine the frequency of two common polymorphisms of PON1 (L/M 55 and R/Q 192) in Turkish subjects and to investigate its relationship with plasma lipid variables and CAD. One-hundred and thirty four subjects with significant coronary stenosis and 100 controls were screened using a combination of polymerase chain reaction and restriction enzyme digestion. In the all population, the genotype frequencies of PON-55 LL, LM, MM and PON-192 QQ, QR, RR were 84 (36.8%), 96 (42.1%), 48 (21.1%) and 89 (41.3%), 93 (44.7%), 26 (13.95%) respectively. CAD subjects did not show any significant differences in the distribution of PON1-55 or PON1 Q192R genotypes as compared to controls. Moreover, either the L55M or Q192R polymorphisms did not show any interaction with lipid variables in CAD subjects. However, the PON-55 LM genotype was associated with significantly higher total-cholesterol ($P < 0.05$) and LDL-cholesterol levels ($P < 0.05$) in the pool subjects. No mutation was associated with the number of major coronary artery vessels with a >50% reduction in lumen diameter in CAD patients. In conclusion, our data suggest that there is no association between PON1 L55M or Q192R polymorphisms of the human PON1 gene and CAD in Turkish patients.

PP-516
ILE405VAL and TAQ IB polymorphisms of the cholesteryl ester transfer protein (CETP) in Turkish population

S. Doğru-Abbasoğlu, H. Parıldar-Karpuzoğlu, B. Depboylu, M. Uysal and G. Aykaç-Toker
Department of Biochemistry, Istanbul Medical Faculty, Istanbul University, Turkey. E-mail: sdabbasoglu@yahoo.com

Cholesteryl ester transfer protein (CETP) is a key protein involved in HDL metabolism. It promotes the net transfer of lipids among lipoproteins. There has been an ongoing debate as to whether CETP is pro- or anti-atherogenic as it provides a mechanism for transfer of cholesterol from the cardioprotective HDL subfraction to potentially atherogenic LDL subfraction. Several CETP mutations have been identified that lead to alteration in CETP activity. TaqIB polymorphism has been one of the most widely studied, which results from a silent mutation in nucleotide 277, in intron 1 of the gene. The B2 allele of the TaqIB polymorphism has been associated with decreased CETP levels and high HDL-cholesterol levels and with coronary heart disease risk. Position 405 of CETP is also polymorphic, being either an isoleucine or a valine, due to an A-to-G substitution in exon 14 of the CETP gene. This polymorphism (Ile405Val) has been reported to be significantly associated with HDL concentration in two European populations. Therefore, we selected these two polymorphisms for our analysis. A total of 150 healthy subjects were enrolled in this study. These polymorphisms were analysed by PCR-RFLP method. According to our results, the incidence of II, IV, and VV genotype of the Ile405Val polymorphism was 34.7, 47.3, and 18%, respectively. The distribution of B1B1, B1B2, and B2B2 genotypes was 29.3, 46.7, and 24%, respectively. These observations are similar to the other European populations.

PP-517
Coenzyme Q10 in heart failure and healthy subjects.

S. L. Molyneux[1], C. M. Florkowski[1], P. M. George[1], M. Lever[1] and A. M. Richards[2]
[1]*Biochemistry Unit, Canterbury Health Laboratories, Christchurch, New Zealand,* [2]*Christchurch School of Medicine and Health Sciences, Cardioendocrine Research Group, Christchurch, New Zealand. E-mail: sarah.molyneux@cdhb.govt.nz*

Coenzyme Q10 (CoQ) is a cofactor in the mitochondrial respiratory chain, and the reduced form has antioxidant activity. CoQ deficiency has been associated with heart failure and statin therapy, due to the common biosynthetic pathway of cholesterol and CoQ. We determined the reference interval for plasma CoQ in the New Zealand population to be 0.46–1.78 μmol/l, median 0.90 μmol/l ($n = 205$). We also determined the biological variation of CoQ in healthy subjects ($n = 10$). We found that CoQ concentrations in different individuals are tightly distributed around different individual homeostatic set-points, and that significant changes in CoQ can occur within the reference interval. For plasma CoQ, inter-individual variation is greater than intra-individual variation. Analytical, intra-, and inter -individual variation were 3.3%, 12%, and 29%, respectively. The calculated reference change value for a 95% significant change was 35%. CoQ is not stable in plasma stored at -13 °C for 12 months, but is stable for at least 18 months when stored at -80 °C. In plasma from a cohort of heart failure patients ($n = 337$), the median CoQ concentration was 0.58 μmol/l, significantly lower than that in normal healthy people ($P < 0.001$). In the 91 patients with plasma CoQ <0.45 μmol/l at presentation the odds ratio for death during follow up over 4–6 years was 1.54. The results

confirm the requirement for well designed clinical trials investigating the role of CoQ in heart failure.

PP-518
Role of vitamin E in homocysteine induced atherosclerosis

D. Yat[1,2], Y. Negis[1], N. Aytan[1], L. Binnetoglu[1] and N. K. Özer[1]
[1]*Department of Biochemistry, Faculty of Medicine, Marmara University, 34668, Istanbul, Turkey,* [2]*Department of Biochemistry, Faculty of Medicine, Yeditepe University, 34755, Istanbul, Turkey. E-mail: dyat@yeditepe.edu.tr*

Elevated plasma levels of homocysteine have been identified as an important and independent risk factor for cerebral, coronary and peripheral atherosclerosis. *In vitro* studies have suggested that homocysteine stimulates the proliferation of smooth muscle cells, linked to the development of atherosclerosis. Antioxidant vitamins and in particular vitamin E have been found to retard the development of atherosclerosis. Here have shown that homocysteine induces DNA synthesis and proliferation of vascular smooth muscle cells by interfering with mitogen activated protein kinase kinase (MAPKK) pathway. We also evaluated the effects of hyperhomocysteinemia on rat aortic wall. Rats were rendered hyperhomocysteinemic upon administration of methionine in the drinking water for 4 weeks. Vitamin E administered as i.m. Homocysteine has increased arteriosclerosis-like lesion area in this *in vivo* model. We have detected by microscopy, hyperhomocysteinemia causes endothelial damage, degenerative alterations in the media and increased collagen formation in the intracellular space in the aorta. Collagen amount in aortic samples were also found high. Vitamin E has been shown that it prevents atherosclerosis like changes and inhibits collagen increase in thoracic aorta. Our data suggest that homocysteine induces smooth muscle cell growth through the activation of MAPKK pathway, increases collagen accumulation and accelerates the progression of atherosclerosis which is protected by vitamin E.

PP-519
Metabolic syndrome may lead to coronary artery disease by increasing circulating asymmetric dimethylarginine levels

E. Cakir[1], H. Yaman[3], **O. Ozcan**[2], S. Demirkol[4], Y. G. Kurt[3], E. O. Akgul[3], C. Bilgi[1], M. K. Erbil[3] and E. Isik[4]
[1]*Emergency Medicine, Gulhane Military Medical Academy, Ankara, Turkey,* [2]*Laboratory of Biochemistry, Haydarpasa Training Hospital, Gulhane Military Medical Academy, Istanbul, Turkey,* [3]*Clinical Biochemistry, Gulhane Military Medical Academy, Ankara, Turkey,* [4]*Cardiology, Gulhane Military Medical Academy, Ankara. Turkey E-mail: erdcakir@yahoo.com*

Metabolic syndrome is a frequent disorder in patients with coronary artery disease (CAD) and known as an important state for CAD progression. Endothelial dysfunction is one of the most significant changes in the development of arteriosclerosis. Asymmetric dimethylarginine (ADMA), endogenous inhibitor of endothelial nitric oxide synthase (eNOS) may lead to endothelial dysfunction and reported as an independent risk factor for CAD progression. We aimed to investigate the effect of metabolic syndrome on ADMA levels and to find the relationship between the extent of CAD and ADMA levels in patients with metabolic syndrome. Blood ADMA levels of 83 patients with metabolic syndrome (34 patients without CAD, 16 patients with CAD in one-vessel, 21 patients with CAD in two-vessels, and 12 patients with CAD in three vessels) were determined by high performance liquid chromatography (HPLC). There were a positive and significant correlation between ADMA levels and the extent of CAD in patients with metabolic syndrome ($r = 0.735$, $P = 0.0001$). ADMA levels were highest in patients with CAD in three-vessels (1.82 ± 0.75 mmol/l) and lowest in patients without CAD (0.73 ± 0.17 mmol/l). The strong relationship between the extent of CAD and blood ADMA levels in patients with metabolic syndrome was firstly shown in this study. Thus metabolic syndrome might cause endothelial dysfunction by elevating circulating ADMA levels during CAD progression.

PP-520
Infrared studies of pH effect on human low density lipoproteins

J. L. Arrondo[1], J. A. Fernandez Higuero[1], J. C. Milicua[1], F. Goñi[1] and G. Pifat[2]
[1]*Unidad de Biofisica and Departamento de Bioquimica, Universidad del Pais Vasco, Bilbao, Spain,* [2]*Rudjer Boškovic Institute, Zagreb, Croatia. E-mail: joseluis.arrondo@ehu.es*

Low density lipoprotein (LDL) is the principal carrier of cholesterol and play an important role in the development of atherosclerotic lesions. It is described as a spherical particle containing a hydrophobic core of cholesteryl esters and triglycerides surrounded by an amphipatic monolayer of phospholipid and cholesterol in which a single molecule of protein (apoB) is located. ApoB is one of the largest proteins known and is extremely insoluble in aqueous media. This has hindered the understanding of the structural basis of apoB function. Infrared spectroscopy has been applied to characterize the secondary structure of apoB and its topology in the LDL particle. Structural analysis usually implies a mathematical approach in order to extract the information contained in the composite band, known as amide I, obtained from proteins. Changes in the protein components of LDL play a substantial role in the initiation and progression of atherosclerosis. In the atheroma, pH is low, far from the physiological values, being a factor that can induce conformational changes in apoB-100 related with the atherogenic properties of the particle. We have measured the amide I band at pH 4.5 proving that a change characterized by a stronger intensity at 1650 cm^{-1} is produced by acidification. Restoration of pH at 7.4 recovers the original shape. The changes and its reversibility have been corroborated by studies of thermal profiles of amide I components, and 2D-IR spectroscopy.

PP-521
Albumin cobalt binding assay and fatty acid-binding protein in early detection of myocardial infarction

K. Sönmez[1], G. Saydam[1], H. Şimşek[1], M. Balk[1], A. Türkmen[1] and **Y. Balbay**[2]
[1]*Clinical Biochemistry Laboratory, T. Yükses İhtisas Research and Training Hospital, Ankara, Turkey,* [2]*Cardiology Clinic, T. Yükses İhtisas Research and Training Hospital, Ankara, Turkey. E-mail: gsaydam@yahoo.com*

Diagnosis of acute myocardial infarction (AMI) in patients attending emergency departments with acute chest pain is often difficult. Cardiac Troponin I and T (cTnI, cTnT), CK-MB are sensitive and specific for detection of myocardial damage, but they may not rise during early stage of myocardial infarction. The release of currently used myocardial markers into the circulation are believed to require tissue necrosis, whereas the assessment of cardiac ischemia before or in the absence of cell death is frequently an important component of clinical decision-making in the suspected AMI patient. Albumin Cobalt Binding Assay

(ACB) and heart type Fatty Acid-Binding Protein (HFABP) have recently been shown to be sensitive and early biochemical markers of ischemia. The aim of our study was to compare H FABP and ACB tests with cTnI, cTnT and CK-MB tests in the first 3 h in the patients with the chest pain who have been diagnosed as AMI according to EKG, biochemical markers and family history. In our study, H-FABP was measured with the CardioDetect test kit based on qualitative immunocromotographic cassette method and ACB was measured with Bar-Or method based on indirect colorimetric principle in 30 patients with chest pain which started 2 h before. The sensitivity and specificity of the H FABP and ACB were higher than that of CK-MB and cTnT, cTnI. We conclude that H FABP and ACB are more sensitive and specific markers than cTnT, cTnI and CK-MB in the early diagnosis of AMI.

PP-522
Lipid peroxidation and homocysteine levels in Behcet's disease

G. Mungan[1], M. Can[1], S. Acikgoz[1], E. Esturk[2], C. Altinyazar[2] and C. Altinyazar[2]
[1]*Department of Biochemistry, Faculty of Medicine, Karaelmas University, Zonguldak, Turkey,* [2]*Department of Dermatology, Faculty of Medicine, Karaelmas University, Zonguldak, Turkey. E-mail: drcanmurat@yahoo.com*

Behcet's disease (BD), first described by Hulusi Behcet in 1937. Increased output of reactive oxygen species by activated neutrophils, which may be responsible for oxidative tissue damage seen in BD and high levels of homocysteine have been considered to be correlated to the etiopathogenesis of BD. The aim of this study was to investigate serum paraoxonase (PON1) activity in relation to homocysteine, malondialdehyde (MDA) and lipid parameters in active and inactive state of BD. A total of 46 consecutive BD patients (24 male aged 34.9 ± 11.0 and 22 female 30.3 ± 10.7) and 25 healthy control subjects (10 men and 15 women; mean age 36.9 ± 12.2 and 30.7 ± 10.9 years) included in the present study. Serum paraoxonase activity in both active and inactive BD was found to decrease significantly as compared with healthy subjects ($P < 0.05$). When compared to control groups, serum MDA levels were significantly higher in both active and inactive BD ($P < 0.05$). Serum CRP and homocysteine concentrations were significantly higher in active BD than those in inactive BD and control subjects ($P < 0.05$). In addition, there was significant negative correlation between serum paraoxonase and MDA levels ($r = -0.697$, $P < 0.05$) and serum paraoxonase activity was also found to be negatively correlated with homocysteine levels ($r = -0.428$, $P < 0.05$) in BD. In conclusion, decreased PON1 could explain the increased lipid peroxidation and oxidative stress observed in BD. Also according to our results, we suggest that homocysteine may be contributed in decreased serum PON1 activity.

PP-523
Effects of native, oxidized LDL and HDL on platelet activation, apoptotic activity and membrane lipid peroxidation

D. Özsavcı[1], **A. Şener**[1], R. Oba[1], B. Göker[1], E. Yurtsever[1], G. Yanıkkaya Demirel[2] and K. T. Yardımcı[1]
[1]*Biochemistry Department, Faculty of Pharmacy, Marmara University, İstanbul, Turkey,* [2]*Centro Laboratory, İstanbul, Turkey. E-mail: azizesener@hotmail.com*

Background: LDL and especially oxidized form (ox-LDL) are established risk factors for atherosclerosis and contribute to prothrombotic risk via enhanced platelet reactivity. The aim of this study was to investigate the effects of LDL, ox-LDL and HDL on platelet activation, apoptosis response and membrane lipid peroxidation.

Methods: Venous blood was collected from seven healthy volunteers and platelets were washed. Commercial LDL was oxidized with $CuSO_4$ for 24 h at 37 °C. LDL, HDL and ox-LDL were treated at 100 μg/ml with ADP induced washed platelets. Expressions of GPIIb/IIIa (CD41a), antifibrinogen, P-selectin (CD62-P) and membrane phosphatdlyserine (Annexin-V) were measured by flow cytometry. Platelet MDA levels were also determined.

Results: CD41a, CD62-P, antifibrinogen and annexin levels increased significantly after ADP activation ($P < 0.05$, $P < 0.05$, $P < 0.01$, $P < 0.001$ respectively).After treatment with LDL/ox-LDL, CD62-P, antifibrinogen and annexin-V expressions increased significantly(LDL: $P < 0.05$; ox-LDL: $P < 0.001$) whereas CD41a levels decreased significantly ($P < 0.001$). Platelet MDA levels increased significantly with ox-LDL ($P < 0.05$). Addition of HDL to LDL/ox-LDL caused significant decrease in antifibrinogen, CD62-P and MDA levels.

Conclusion: We have concluded that LDL especially ox-LDL may contribute to the atherothrombotic process by enhancing not only agonist induced platelet activation but also platelet apoptosis response and platelet membrane lipid peroxidation; nevertheless HDL has a protective role by reducing the platelet activation and oxidative stress triggering effects of LDL/0x-LDL.

PP-524
The comparison of the effect of red grape juice and its wine on LDL oxidation *in vitro*

H. A. Uydu, M. Bilici, G. Rumeli, D. A. Ataç, A. Özen, A. Ekinci and **N. Aktaş**
Department of Chemistry, Faculty of Science and Art, 53100, Rize, Turkey. E-mail: isiin@yahoo.com

It is known that moderate red wine consumption can reduce the risk of cardiovascular disease. The protective effects of wine have been attributed to phenolic compounds Flavonoids are plant products, derivatives of 2-phenyl-1-benzopyran. The aim of our study was to compare the effects of grape juice (GJ, *Vitis vinifera*) and its wine and, their different concentrations on the low-density lipoprotein (LDL) oxidation *in vitro*. Therefore, the dien conjugation was monitored to examine the effects of these antioxidant products on the resistance of LDL oxidation. It was observed that both GJ and its wine compared to the control group had similar effect significantly in decreasing the lipoprotein oxidation and increased the resistance of LDL oxidation. The GJ had higher total antioxidant capacity than the its wine, decreased the lipoprotein oxidation (tg-lag 255 ± 12 min, tw-lag 245 ± 13.7 min, $P < 0.0001$) but not significantly. In beverages with different polyphenol concentrations, it was observed the prolongation of the lag-time of metal ion dependent LDL oxidation. At high concentrations red grape juice and its wine extended lag-time (at high flavonoid; 1.6 nM, tg-lag 315 ± 22.8 min, tw-lag 295 ± 28.1 min. and at low flavonoid; 0.89 nM, tg-lag 160.2 ± 13.2 min., tw-lag 157.6 ± 15.2 min, $P < 0.0001$) compared to the control group (t-lag 70 ± 28.1 min). In conclusion, our results showed that polyphenolic compounds in red grape and its wine may have inhibitory activity on the lipoprotein oxidation.

PP-525
The relation between serum high sensitive CRP levels and angiographically documented coronary artery disease

E. Albayrak[1], M. Yavuz[2], M. Yazici[1], H. Ozhan[1], E. Erbilen[1], O. Yavuz[3] and A. Kaya[1]
[1]*Department of Cardiology, AIBU Duzce Medical Faculty, Duzce, Turkey,* [2]*Department of Clinical Microbiology, AIBU Duzce Medical Faculty, Duzce, Turkey,* [3]*Department of Clinical Biochemistry, AIBU Duzce Medical Faculty, Duzce, Turkey. E-mail: sinansnn@yahoo.com*

Aim: To evaluate the relation between the serum Hs-CRP levels and the presence of angiographically documented coronary artery disease (CAD).
Methods: Eighty one patients (39 female, 42 male with a mean age of 59 ± 10) were recruited fort the study. Besides routine blood chemistry, homosistein, ferritine, TNF-α, IL-1, IL-6, sICAM-1, E-selectin and oxidized LDL levels were analysed. Patients who had ≥50% occlusion in at least one major coronary artery were classified as having CAD. Clinical, biochemical, echocardiographic data of the two groups were compared.
Results: Coronary artery disease was diagnosed in 33 patients. Demographic, clinical and routine biochemical variables were not different between two groups. Only Hs-CRP levels differed between the two groups, which was significantly higher among patients with CAD (0.57 ± 0.5 mg/l versus 1.7 ± 3.4 mg/l; $P < 0.023$). ROC analysis demonstrated that Hs-CRP levels of >1.23 mg/l can predict angiographically significant CAD with a sensitivity of 33% and specificity of 89%. Further multivariate logistic regression analysis revealed that Hs-CRP was found to be an independent predictor of CAD ($P = 0.001$) with hypertension ($P = 0.002$), smoking status ($P = 0.007$) and HDL cholesterol ($P = 0.001$).
Conclusion: Serum level of Hs-CRP was increased in patients with CAD and correlated well with the significance of CAD. Hs-CRP can predict CAD; therefore further prospective studies should be designed for their clinical utility as predictors of silent CAD in the general population.

PP-526
Interaction between *Chlamydia pneumoniae,* inflammation and risk factors in patients with severe coronary stenosis

M. T. Yavuz[1], **O. Yavuz**[2], M. Yazici[3], S. Guler[2], H. Ozhan[3], S. Albayrak[3] and A. Coskun[2]
[1]*Clinical Microbiology, AIBU, Duzce School of Medicine, Duzce, Turkey,* [2]*Clinical Biochemistry, AIBU, Duzce School of Medicine, Duzce, Turkey,* [3]*Cardiology, AIBU, Duzce School of Medicine, Duzce, Turkey. E-mail: tyavuz@superonline.com*

Atherosclerosis is a multifactorial disease with an important inflammatory component. *Chlamydia pneumoniae* (*CPn*) infection plays a causal role in atherosclerosis, perhaps by affecting other known risk factors of coronary artery disease (CAD) and/or by triggering the inflammation that may result in vascular lesions. We investigated whether *CPn* seropositivity in patients with CAD was associated with severity of coronary atherosclerosis. The relationship of traditional risk factors for atherosclerosis and *CPn* seropositivity was also examined. To evaluate the inflammation and endothelial dysfunction, we included high sensitive C-reactive protein (hs-CRP), proinflammatory cytokines, adhesion molecules and oxidized LDL (oxLDL). *Cpn* IgA and IgG seropositivities were significantly associated with the presence of CAD and these were independent predictive factors for severity of coronary atherosclerosis. The elevated levels of IL-6, triglyceride and low level of HDL-C were significantly predicted by *CPn* IgA and IgG seropositivity. We concluded that higher seropositivity to *CPn* IgA and IgG was a risk factor for patients with CAD. Furthermore the risk associated with *Cpn* was strongly interacted with dyslipidemia and IL-6 levels, which are risk factors for CAD. These observations may indicate that *CPn* infection may be one of the entry points in the contributory or causal pathway that leads to atherosclerosis and its clinical manifestations.

PP-527
Influences of new risk markers on the severity of coronary atherosclerosis in patients with different risk profiles

S. Guler[1], **O. Yavuz**[1], M. T. Yavuz[2], M. Yazici[3], H. Ozhan[3], A. Coskun[1] and S. Albayrak[3]
[1]*Clinical Biochemistry, AIBU, Duzce School of Medicine, Duzce, Turkey,* [2]*Clinical Microbiology, AIBU, Duzce School of Medicine, Duzce, Turkey,* [3]*Cardiology, AIBU, Duzce School of Medicine, Duzce, Turkey. E-mail: o_yavuz@yahoo.com*

Recent evidence has demonstrated that a number of novel markers besides well-characterized classical factors are associated with increased risk for coronary artery disease (CAD). We investigated the influences of homocysteine, high sensitivity C- reactive protein (hsCRP), tumor necrosis factor-alpha (TNF-alpha), interleukin -1beta and 6 (IL-1beta, IL-6), soluble intercellular adhesion molecule-1 (sICAM-1), E selectin and oxidized LDL (oxLDL) levels on the severity of coronary atherosclerosis among patients with different risk profiles. Subjects were divided into high and low risk groups according to Framingham risk score based on major conventional risk factors. The severity of coronary atherosclerosis was evaluated by Gensini score. Patients who had ≥50% occlusion in at least one major coronary artery were classified as having CAD. E selectin levels were significantly higher in high risk patients. IL-6 levels were significantly higher among patients with significant CAD. Stepwise multiple regression analysis revealed that E selectin, hsCRP and oxLDL levels of patients in the low risk group and male gender characteristic of patients in the high risk group were independent predictors of severity of coronary atherosclerosis. We concluded that the levels of hsCRP, E selectin and oxLDL may help to predict the degree of severity of coronary atherosclerosis. IL-6 may be useful to ameliorate risk stratification in high risk patients with silent CAD.

PP-528
Congenital hypothyroidism causes altered lipogenic response to thyroid hormones in adult rat liver

L. Fernández-Pérez[1], L. Henríquez-Hernández[1], R. Santana-Farré[1], C. Mateo-Díaz[1], P. Parini[2] and A. Flores-Morales[3]
[1]*Clinical Sciences, University of Las Palmas de GC, Las Palmas de GC, Spain,* [2]*Center for Metabolism and Endocrinology, Karolinska University Hospital, Huddinge, Sweden,* [3]*Molecular Medicine, Karolinska Institute, Stockholm, Sweden. E-mail: lfernandez@dcc.ulpgc.es*

Thyroid Hormone (T3) affects development of tissues at risk of postnatal pathophysiology when T3 is impaired by congenital hypothyroidism (TXC). In the present study, we have utilized qRTPCR and lipid serum measurements to explore the reactivity of adult liver from TXC+ rats to T3 in comparison with that of adult-acquired hypothyroid animals (TXC-). In both TXC+ and TXC-, hypothyroidism (TX) resulted in an over 2-fold increase in

serum cholesterol (C) and a 2-fold reduction in triglyceride (Tg) concentrations. The increased C was due to a large increase in LDL-c and T3 treatment decreased its concentration level. In TXC+, TX resulted in an over 3–4-fold reduction in serum free fatty acids (FFA) and T3 treatment restored values to intact rat levels. In TXC-, however, neither TX nor T3 treatment modified serum FFA. In TXC-, TX decreased and T3 treatment induced mRNA expression levels of malic enzyme (ME) 12-fold. However, TX itself did not modify the expression level of ME in TXC+ rats. However, T3 administration to TXC+ rats caused 30-fold induction of ME mRNA. TX did not modify SREBP-1c mRNA expression level in TXC- rats whereas it was induced 4–5 fold in TXC+ and T3 treatment restored its level. These findings suggest that there is exists an altered lipogenic response to T3 in TXC+ suggesting an epigenetic effect of TXC on liver.

Acknowledgment: Grants FIS 1/1000, PETRI1995-0711, Pfizer CN-78/02-05045, MCYT SAF2003-02117.

PP-529
Effects of melatonin on lipid parameters of diet-induced hypercholesterolemic rats

İ. Bütün[1], H. Sönmez[1], M. Caner[2], Ö. Çiftçi[1], **T. Altuğ**[3] and E. Kökoğlu[1]

[1]Department of Biochemistry, Medical Faculty of Cerrahpasa, University of Istanbul, Istanbul, Turkey, [2]Department of Surgery, Cerrahpaşa Medical School, Istanbul University, Istanbul, Turkey, [3]Department of Experimental Animal Breeding and Research Laboratory, Medical Faculty of Cerrahpaşa, University of Istanbul, İstanbul, Turkey. E-mail: islerbutun@yahoo.com

It is well known that hypercholesterolemia, especially high levels of LDL cholesterol in serum are important factors in atherosclerosis. In recent studies melatonin has been suggested to have effects on cholesterol metabolism. In this study our aim was to investigate the effects of low and high doses of melatonin in rats fed with a high cholesterol diet, on lipid parameters. 30 of the 42 rats in our experiment were fed with high cholesterol diet; while the 12 rats were identified as control group so fed with normal diet. 10 of the experiment group received daily intraperitoneal injections of 1 mg/kg/d melatonin, 10 of them received 10 mg/kg/d melatonin and the last received nothing. 6 of the control rats received 0.9% NaCl i.p. injections and the last 10 received 4% ethanol in 0.9% NaCl. The blood collection and lipid analysis of the rats were done at the start and the 12th week. Total and LDL cholesterol levels of the group that receiving only cholesterol were increased while the groups receiving melatonin had decreased levels of total and LDL cholesterol ($P = 0.00$). At the 12th week LDL cholesterol levels and the LDL/HDL ratio of the group receiving high doses of melatonin were significantly lower than the group that received low doses of melatonin ($P = 0.00$). Results demonstrated that melatonin can be effective on cholesterol metabolism. Because it has specially effects on LDL cholesterol; melatonin can be thought as to play an important role in atherosclerosis.

PP-530
Cathepsin K and survivin in a collar-induced early atherosclerosis model

H. Islekel[1], B. Reel[2], G. Oktay[1], A. Ozkaya[1], G. Ozsarlak Sozer[2] and Z. Kerry[2]

[1]Biochemistry, School of Medicine, Dokuz Eylul University, Izmir, Turkey, [2]Pharmacology, School of Pharmacy, Ege University, Izmir, Turkey. E-mail: huray.islekel@deu.edu.tr

Intimal thickening, an early and essential step of atherosclerotic process, results from migration and proliferation of the vascular smooth muscle cells (SMC). Cathepsin K, a member of lysosomal cysteine protease family, is considered to play a role in arterial extracellular matrix (ECM) degradation, which may facilitate SMC migration leading to intimal thickening. The dual function of survivin in the regulation of vascular cell proliferation and death has also been demonstrated. We aimed to investigate the potential roles of cathepsin K and survivin in development of collar-induced intimal thickening which is an early atherosclerosis model. A flexible silicon collar was placed around the left carotid artery of white rabbits ($n = 9$). The right carotid artery was sham operated. On day 22, the rabbits were sacrificed and both carotid arteries were dissected then stored at -80 °C. Cathepsin K and survivin levels were determined by ELISA. Cathepsin K levels were significantly decreased in collared arterial tissues compared to those of sham-operated tissues (sham 45.50 ± 10.52; collared 21.88 ± 6.50 nmol/mg protein, mean ± SEM, $P < 0.05$). Survivin levels did not differ between collared and sham-operated tissues (sham 400.50 ± 80.59; collared 334.75 ± 61.01 pg/mg protein). These results may suggest that the contribution of cathepsin K and survivin is not particularly prominent in collar-induced early atherosclerosis model.

PP-531
Comparison of inflammatory mediators, cardiac risk factors and functional-structural changes of left ventricle

G. Toprak[1], S. Alan[2], K. Cevik[2] and Y. Celik[3]

[1]Biochemistry, Dicle University, Diyarbakir, Turkey, [2]Cardiology, Dicle University, Diyarbakir, Turkey, [3]Biostatistics, Dicle University, Diyarbakir, Turkey. E-mail: gtoprak@dicle.edu.tr

Chronic inflammatory process is an essential factor in atherosclerosis. The aim of this study was to investigate the relationship between inflammatory mediators [hsCRP and Interleukins (IL)] and cardiovascular risk factors, functional-structural changes of left ventricle. Angiographically diagnosed 50 patients (aged 58.88 ± 9.99) with coronary heart disease and 25 healthy people (45 ± 8) as a control group were investigated. In all patients IL and hsCRP levels were measured, and the functional-structural changes were evaluated by echocardiography. In the patients group hsCRP, IL-6 and IL-8 levels were significantly higher (5.7 ± 2, 3.0 ± 1 to 25 ± 6.10 ± 2 $P < 0.05$) whereas ejection fraction (EF %) was considerably lower (47 ± 12, 59 ± 15, $P < 0.05$). The positive correlation between hsCRP levels and triglyceride ($r = 12$, $P < 0.05$) and total-C/HDL-C levels was demonstrated ($r = 13$, $P < 0.05$) as well as the positive correlation between hsCRP and left ventricle posterior wall thickness($r = 0.14$, $P < 0.05$), and the positive correlation between IL-1 and total-C/HDL-C ($r = 8$, $P < 0.05$); between IL-10 and EF were also determined($r = 6$, $P < 0.05$). As a conclusion, in the presence of cardiovascular risk factors we observed an enhancement in chronic inflammatory process. Negative effects of chronic inflammatory markers on left ventricle functions and its structure were also demonstrated. Thus, the observations suggest that chronic inflammatory markers could be used in determination of prognoses of cardiovascular diseases.

PP-532
Identification of second-generation peptide mimetics for pathogenic auto phospholipid antibody

S. Yüksel and P. P. Chen
Medicine/Rheumatology, University of California, Los Angeles, CA, 90095-1670, USA. E-mail: sahru.yuksel@boun.edu.tr

Antiphospholipid syndrome (APS) is an autoimmune disease in which the presence of antiphospholipid antibodies (aPL) are associated with the development of thrombosis and/or fetal loss. Current understanding of the molecular mechanism of APS is limited and patients cannot be diagnosed accurately before the first clinical symptom appears. Better predictive tests are needed to treat the patients who are at high risk for clotting since the effective treatments are available but potentially hazardous. CL15 is a patient derived IgG monoclonal anticardiolipin antibody (aCL) that has been shown to be prothrombotic *in vivo*. CL15 can bind and inhibit the anti-coagulant activity of activated protein C and fibrinolytic activity of plasmin. Previously, a peptide mimetic called CL154C, has been identified when 17 phage display peptide libraries were screened with CL15. This peptide has been shown to have good diagnostic power. The aim of this study is to construct a CL154C-based evolutionary phage display peptide library and identify peptide mimetics that could detect CL15 with improved sensitivity and specificity. Once identified, such mimetics could be used to develop better diagnostic tests and may also be used as therapeutic agents for pathogenic aPL. In this study, an evolutionary library of CL154C has been generated. Affinity selection with CL15 resulted in the identification of clones that bind to CL15 with at least ten times higher affinity than the original clone.

PP-533
Serum nitric oxide levels in coronary artery patients

A. Celik[1], S. Soydinc[3], S. Demiryurek[2], At. Demiryurek[2] and M. Tarakcioglu[1]
[1]Department of Biochemistry, Medical Faculty, Gaziantep University, Gaziantep, Turkey, [2]Department of Pharmacology, Medical Faculty, Gaziantep University, Gaziantep, Turkey, [3]Department of Cardiology Medical Faculty, Gaziantep University, Gaziantep, Turkey. E-mail: ahmetcelikdr@hotmail.com

The risk factors involving in CAD like dyslipidemia and hypertension have importance in etiopathogenesis. Therefore, drugs like ACE inhibitor and statin group are wide in used. In CAD, reduced NO levels are expected because of increase in oxidative stress and deterioration of endothelial dysfunction. In CAD patients, the potential relation between the degree of vessel lesion and serum NO levels; the effect of risk factors like hypercholesterolemia, hypertension, obesity, dyslipidemia, and age on this potential relation; this determinants' CAD degree should be important in terms of treatment and observation of the disease. Among the patients applied to our hospital's angiography unit of Cardiology department consisting 90 people and 49 healthy subjects. The biochemical parameters involving NO, total cholesterol, triglyceride, LDL, and HDL were assessed. In CAD patients, comparing with control group interestingly, serum NO levels were higher in CAD group, but this result was not statistically significant. In earlier studies, dyslipidemia and hypertension were shown to increase the pathophysiological effect of CAD. However, in our study the most of the patients were using ACE inhibitors or/and statins which directed us to evaluate the results on this basis. As a result, these drugs increased the synthesis of NO but was not enough capable of balancing the deteriorated oxidant/antioxidant level, the antioxidant defense was to be made strong.

PP-534
Coronary angiography and serum malondialdehyde levels

A. Celik[1], **H. Cicek**[1], S. Bayil[1], I. Geyikli[1], S. Soydinc[2] and M. Tarakcioglu[1]
[1]Department of Biochemistry, Gaziantep University Medical Faculty, Gaziantep, Turkey, [2]Department of Cardiology, Gaziantep University Medical Faculty, Gaziantep, Turkey. E-mail: ahmetcelikdr@hotmail.com

Coronary artery disease (CAD) has importance on community health regarding life quality and health costs. In CAD patients, the potential relation between the degree of vessel lesion and serum MDA (malondialdehyde) levels; the effect of risk factors like hypercholesterolemia, hypertension, obesity, dyslipidemia, and age on this potential relation; this determinants' CAD degree should be important in terms of treatment and observation of the disease. In earlier studies, dyslipidemia and hypertension were shown to increase the pathophysiological effect of CAD. Among the patients applied to our hospital's angiography unit of Cardiology department consisting 90 people and 49 healthy subjects. The biochemical parameters involving MDA, total cholesterol, triglyceride, LDL, and HDL were assessed. In CAD patients, comparing with control group MDA levels were statistically significantly higher. The patients were sub-grouped in terms of coronary artery retention. Below 20% wall narrowness were attributed as 0 vessel disease; of the rest, vessel disease were attributed in terms of the number of narrowed vessels. There was no significant difference of MDA levels among these subgroups. But there was serious difference in every subgroup comparing with control groupAs a result MDA levels are significantly higher in CAD patients but not correlated with degree of narrowness coronary vessels when we compare with healthy subjects.

PP-535
Exercise induced change of mean platelet volume (MPV) in myocardial ischemia

A. Olgun[1], A. Ö. Karaçalıoğlu[2], M. A. Serdar[1], Ş. Akman[1] and M. K. Erbil[1]
[1]Department of Biochemistry and Clinical Biochemistry, Gulhane School of Medicine, 06018-Etlik, Ankara, Turkey, [2]Department of Nuclear Medicine, Gulhane School of Medicine, 06018-Etlik, Ankara, Turkey. E-mail: aolgun@yahoo.com

Mean platelet volume (MPV) reflects platelet activation. We analysed the change in MPV at rest and after Bruce protocol exercise in patients who underwent myocardial perfusion scintigraphy with Tc-99m MIBI. The patients were divided into three groups as normal, ischemia, and infarction according to the scintigraphy results. In ischemia and infarction groups, resting MPV values were found significantly higher than normal group. This finding is in accordance with the published studies. In control and infarction groups, the increase of MPV values just after the exercise was ~1 fL, in the ischemia group it was ~0.5 fL. While, in control and infarction groups, MPV values measured at 90 min after the exercise were returned to resting values; they did not return to and remained above the resting levels in ischemia group. We suggest that the lower increase of exercise induced MPV in myocardial ishemia patients compared with control and infarction groups can provide new insight into the role of platelets in myocardial ischemia; and also be used as a new parameter in the evaluation of patients with cardiovascular pathologies.

PP-536
Comparison of inflammatory mediators, cardiac risk factors and functional-structural changes of left ventricle

G. Toprak[1], **S. Alan**[2], K. Cevik[2], B. Yokus[3] and Y. Celik[4]
[1]Department of Biochemistry, Faculty of Medicine, University of Dicle, Diyarbakir, Turkey, [2]Department of Cardiology, Faculty of Medicine, University of Dicle, Diyarbakir, Turkey, [3]Department of Biochemistry, Faculty of Veterinary, University of Dicle, Diyarbakir, Turkey, [4]Department of Biostatistics, Faculty of Medicine, University of Dicle, Diyarbakir, Turkey.
E-mail: gtoprak@dicle.edu.tr

Chronic inflammatory process is an essential factor in atherosclerosis. The aim of this study was to investigate the relationship between inflammatory mediators [hsCRP and Interleukins (IL)] and cardiovascular risk factors, functional-structural changes of left ventricle. Angiographically diagnosed 50 patients (aged 58.88 ± 9.99) with coronary heart disease and 25 healthy people (45 ± 8) as a control group were investigated. In all patients IL and hsCRP levels were measured, and the functional-structural changes were evaluated by echocardiography. In the patients group hsCRP, IL-6 and IL-8 levels were significantly higher (5.76 ± 4.61, 25.78 ± 27.1, 50.5 ± 5.85 respectively, $P < 0.05$) whereas ejection fraction (EF %) was considerably lower (47.7 ± 9.6, $P < 0.05$). The positive correlation between triglyceride and total-C/HDL-C levels was demonstrated ($P < 0.05$) as well as the positive correlation between hsCRP and left ventricle posterior wall thickness ($P < 0.05$), and the positive correlation between IL-1 and total-C/HDL-C ($P < 0.05$); between IL-10 and EF were also determined ($P < 0.05$). As a conclusion, in the presence of cardiovascular risk factors we observed an enhancement in chronic inflammatory process. Negative effects of chronic inflammatory markers on left ventricle functions and its structure were also demonstrated. Thus, the observations suggest that chronic inflammatory markers could be used in determination of prognoses of cardiovascular diseases.

Oncogenes and Tumor Supressors

PP-537
Nitrolinoleate activates PPAR gamma signaling in THP-1 cells: the importance of the p21Ras-MAP kinases signaling pathway

S. Ferderbar[1], H. P. Monteiro[2] and **D. S. P. Abdalla**[1]
[1]Department of Clinical and Toxicological Analysis, University of São Paulo, São Paulo, Brasil, [2]Department of Biochemistry and Molecular Biology, UNIFESP, São Paulo, Brasil.
E-mail: dspa@usp.br

Background: The peroxisome proliferator-activated receptor gamma (PPAR gamma) can be activated by a nitroalkene derivative of linoleic acid (18 : 2), nitrolinoleic acid (LNO2). LNO2 is formed via nitric oxide (NO)–dependent oxidative reactions and can be decomposed, yielding NO and linoleic acid. As NO activates the p21Ras-MAP kinases signaling pathway, we investigated the participation of the p21Ras-MAP kinases signaling pathway in the activation of PPAR gamma by LNO2.
Methods: Nitrolinoleic acid was synthesized from linoleic acid and NO2BF4 and characterized using HPLC and LC-ESI/MS/MS-based techniques. The eletrophoretic mobility shift assay was used to evaluate the LNO2-mediated activation of PPAR gamma in THP-1 cells, a human monocytic cell line. Western blot analysis was used to evaluate the participation of the MAP kinases ERK 1/2 and p38 on PPAR gamma activation by LNO2.
Results: Nitrolinoleic acid (10 nM) activated PPAR gamma at much lower concentrations as compared to linoleic acid (10 μM). LNO2 (10 nM) activated the ERK1/2 MAP kinases while had no effect upon p38 MAP kinase. In addition, the MEK inhibitor PD98059 blocked PPAR gamma activation by LNO2, suggesting a connection between ERK1/2 MAP kinases and the activation of this transcription factor.
Conclusion: Our observations suggest a new mechanism involving the participation of the ERK1/2 MAP kinases in the activation of PPAR gamma by LNO2.
Acknowledgment: Financial support by FAPESP/CNPQ, Instituto do Milênio Redoxoma.

PP-538
MSI-H in bilateral breast tumors: treatment-related origin of some contralateral malignancies?

E. N. Suspitsin[1], E. Sh. Kuligina[1], K. G. Buslov[1], Yu. R. Lazareva[2], A. V. Togo[1] and E. N. Imyanitov[1]
[1]Group of Molecular Diagnostics, N. N. Petrov Institute of Oncology, St.Petersburg, Russia, [2]Department of Pathomorphology, N. N. Petrov Institute of Oncology, St. Petersburg, Russia

Background: High-frequency microsatellite instability (MSI-H) occurs frequently in colorectal cancers and some other tumor types, but is very uncommon for breast cancer. The authors previously detected several MSI-H tumors in patients with the bilateral form of breast cancer (biBC). The present study was designed to examine this phenomenon in more detail.
Methods: All DNA samples were tested by microsatellite markers BAT25, BAT26, BAT40, D5S346 and D17S250. If the tumor was unstable for at least one marker, or PCR amplification was not successful for any of the listed above loci, the analysis of additional five dinucleotide markers (D1S225, D11S4167, D22S272, D22S1166, D3S3527) was performed. Tumors showing instability in ≥30% loci were classified as MSI-H.
Results: In biBC group, MSI-H status was detected in 6/60 (10%) contralateral tumors, but in 0/50 (0%) first malignancies ($P = 0.021$) and only in 1/22 (5%) synchronous biBC ($P = 0.434$). None of 52 unilateral breast cancers showed MSI-H status. Shifts of mononucleotide markers were revealed in 4 s carcinomas from biBC patients but in none of breast tumors from other categories.
Conclusion: MSI-H is detected with a noticeable frequency in bilateral but not in unilateral breast cancers. Preferable occurrence of MSI-H in second metachronous tumors from biBC patients allows to hypothesize that the development of some contralateral breast neoplasms is casually related to the treatment of the initial malignancy.

PP-539
Alpha-fetoprotein gene expression and glutathione S-transferase activity of hepatocytes during hepatocarcinogenesis in rats treated with *Berberis vulgaris* fruit extract

G. Motalleb[1], P. Hanachi[2], A. Rahmat[3] and **F. Othman**[1]
[1]Department of Biological Science, Faculty of Medicine and health Sciences, University Putra Malaysia, 43400, UPM, Serdang, selangor, Malaysia, [2]Department of Biomedical Science, Women Research Center, Alzahra University, Tehran, Iran, [3]Department of Health and Nutritional Sciences, Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400, UPM, Serdang, Selangor, Malaysia. E-mail: reza_motalleb@yahoo.com

The chemopreventive activity of *Berberis vulgaris* fruit extract has been investigated against Solt Farber protocol of hepatocarcinogenesis in the liver of female Sprague dawley rats. Forty-eight Sprague dawley rats (weighing 150–250 g) were divided into two groups, normal and cancerous. Each group was divided into four groups. The first group of normal group act as normal control while the others were treated with different doses i.e. 25, 50 and 100 mg/kg of *Berberis vulgaris* extract and respectively considered as NC, NC25, NC50 and NC100. The first group of cancerous rats act as cancer control while the others were treated with 25, 50 and 100 mg/kg of *Berberis vulgaris* extract and considered as C, C25, C50 and C100. The glutathione S-transferase activity and α-fetoprotein gene expression in hepatocytes were investigated as the biomarker of extract's effect on the liver during hepatocarcinogenesis in rats. Treatment with *Berberis vulgaris* fruit extract (25, 50, 100 mg/kg/bodyweight) decreased GST activities in the liver cytosol of rats. GST activity in cancer control group was significantly higher ($P < 0.05$) compared with other groups. RT-PCR analysis showed AFP gene expression in cancer groups treated with *Berberis vulgaris* has been blocked, as a sign of anticancer property.

PP-540
Transcriptional downregulation of EphB receptors during colorectal cancer progression

J. L. Fernández-Masip, C. Cortina, E. Sancho and E. Batlle
Colorectal Cancer and Intestinal Epithelium Biology, Institute for Research in Biomedicine, Barcelona Science Park, Barcelona, Spain. E-mail: jlfernandez@pcb.ub.es

EphB receptors are targets of beta-catenin/Tcf activity and play a crucial role in the positioning of precursor cells along the crypts of the intestinal mucosa. As a result of the constitutive activation of the beta-catenin/Tcf complex in colorectal cancer (CRC), these receptors are over-expressed in early colorectal adenomas. However, EphB expression is shut down around the adenoma-carcinoma transition, despite the constitutive activity of the beta-catenin/TCF complex. Loss of EphB2 expression correlates with poor prognosis in CRC and it has been demonstrated that down-regulation of EphB activity (particularly of EphB3) in animal models of intestinal cancer accelerates dramatically tumour progression. Downregulation of EphB expression appears to occur at the transcriptional level. We have undertaken the study of the regulation of EphB receptors expression in CRC. We have demonstrated that downregulation of EphB3 mRNA strongly correlates with the lack of EphB3 gene promoter activity. By comprehensive analysis of EphB3 promoter, we have identified an enhancer upstream of the transcription initiation site that exhibits differential activity amongst colorectal cancer cell lines that express or have lost EphB3 mRNA expression. Exhaustive analysis of this 600 bp sequence has revealed a small subset of transcription factors and signalling pathways that critically regulate EphB expression and silencing in CRC. We will discuss these findings in the context of CRC progression.

PP-541
EphB receptors control intestinal crypt cell positioning and suppress colorectal cancer progression

C. Cortina, J. L. Fernández-Masip, E. Sancho and E. Batlle
Colorectal Cancer and Intestinal Epithelium Biology, Institute for Research in Biomedicine, Barcelona Science Park, Barcelona, Spain. E-mail: ccortina@pcb.ub.es

Receptor Tyrosine kinases of the Eph family are activated at the sites of cell-to-cell contacts by transmembrane ligands known as ephrins. Their activation frequently results in cellular repulsion, a phenomenon required for the formation of boundaries between adjacent compartments or axon pathfinding during embryonic development. Our group has demonstrated that beta-catenin/Tcf inversely controls the expression of EphB receptors and ephrinB ligands in the intestinal epithelium. The counter expression of EphB and ephrinB in the crypt-villus axis is required for the accurate compartmentalization and migration of cell types in the epithelium. In addition, EphB activity suppresses colorectal cancer progression at the onset tumorigenesis. Here, we will show a recently established *in vitro* model that mimics eph-mediated cell sorting in epithelial cells. We will provide evidences that EphB-ephrinB interactions reorganize cell-cell adhesion at the boundary of adjacent epithelial compartments. Besides, we will show new data on how EphB-ephrinB interaction suppresses colorectal cancer progression.

PP-542
FGF-2 stimulatory effects on human prostate cancer cells are mediated by HARP/pleiotrophin: implication of hydrogen peroxide

M. Hatziapostolou[1], C. Polytarchou[2] and E. Papadimitriou[2]
[1]Laboratory of Cell Biology, Department of Biology, University of Patras, Greece, [2]Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, Greece. E-mail: hatziap@upatras.gr, cpolyt@upatras.gr,epapad@upatras.gr

Fibroblast growth factor 2 (FGF-2) has been implicated in prostate cancer (PC) progression. We found that FGF-2 significantly increased human prostate cancer LNCaP cell proliferation and migration. Heparin affin regulatory peptide (HARP) seems to be an important mediator of FGF-2 stimulatory effects, since the latter had no effect on stably transfected LNCaP cells that did not express HARP. FGF-2 significantly induced HARP expression and secretion by LNCaP cells and increased luciferase activity of the 5'-flanking region of the HARP gene introduced in a reporter gene vector. FGFR-signaling inhibition blocked the FGF2-increased HARP gene activation and the consequent protein release, leading to impairment of LNCaP cell proliferation. FGF2-stimulatory effects depended on hydrogen peroxide (HP) generation. Low concentrations of HP had similar stimulatory effects on HARP expression and secretion and LNCaP cell proliferation and migration. Activator protein-1 (AP-1) seems to be involved in FGF2-stimulated HARP expression and secretion by LNCaP cells, as revealed using AP-1 decoy oligonucleotides and point mutation analyses, as was the case for HP. The effect of FGF-2/HP seems to be due to binding of Fra-1, JunD and phospho-c-Jun to the HARP promoter. These results establish the role and mode of activity of FGF2 in LNCaP cells, extend the notion that HARP is important for PC cell biology and

emphasize on the requirement of HP production in FGF2 downstream effects on LNCaP cells.

PP-543
In vitro influence of tyrosin kinase inhibitors on WT1 expression in primary CML cells

E. Otahalova, V. Ullmannova, J. Moravcova, H. Klamova and C. Haskovec
Department of Molecular Genetics, Institute of Hematology and Blood Transfusion, Prague, Czech Republic.
E-mail: eva.otahalova@uhkt.cz

Wilms tumor suppressor gene (WT1) is a possible prognostic marker of chronic myeloid leukemia (CML) and can serve as an indicator of response versus resistance to Glivec treatment. The resistance can be caused by mutations in the ATP-binding site, BCR-ABL amplification, or involvement of other protein kinases. To evaluate the resistance we implemented an *in vitro* short term cultivation of the primary cells of 40 CML patients with Glivec and other inhibitors of protein kinases (SRC, JAK, MAP). The effect of inhibitors was characterized by expression of BCR-ABL, WT1 and the proliferative factor Ki67 by qRT-PCR. In the case of 33 patients (83%), Glivec induction led to cell vitality deterioration and gene down-regulation (to 30% of expression in non-affected cells). All of these patients responded to Glivec therapy well and reached complete cytogenetic remission. However, in the case of the seven remnant patients (17%) only a slight WT1 down-regulation (to 90%) was observed. In this case, the phenomenon of cell vitality deterioration and monitored gene down-regulation was attained after cultivation with inhibitors of SRC family. These patients did not respond to Glivec therapy. Our results confirmed the usefulness of WT1 expression monitoring to evaluate the upfront Glivec resistance. The results also suggest that in some cases of Glivec resistant patients the role of BCR-ABL in the CML pathogenesis could be supplanted by SRC kinases.

Acknowledgment: Support by VZ MZCR 023736.

PP-544
Expression profiling of BRCA1-induced genes in primary breast tumors

B. Gur[1], B. Bozkurt[2], O. Konu[1], **S. Seckin**[3] and I. Yulug[1]
[1]Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey, [2]General Surgery, Ankara Numune Research and Teaching Hospital, Ankara, Turkey, [3]Department of Pathology, Ankara Numune Research and Teaching Hospital, Ankara, Turkey. E-mail: bala@fen.bilkent.edu.tr

BRCA1 regulates the expression of one or more downstream genes and it is important to determine which genes are transcriptionally influenced by BRCA1 *in vivo* to explain its role in tumor suppression and cancer development. In our previous study, a BRCA1 over-expression system enabled us to define the genes whose expression levels were induced in MCF-7 breast cancer cells by using the PCR-based SSH technique. The expression profiles of selected genes were analysed by using real-time qRT-PCR in normal-matched primary breast tumors. Expression profiles of all the genes, except MAC30, displayed significant correlation with BRCA1 expression (Pearson correlation, Minitab; $n = 60$; $P < 0.02$). There is a strong relationship between expression profiles of the tumors and their grade status. Grade III tumors have low level of expression in general while grade I tumors have higher level of expression of target genes (Discriminan Analysis, Minitab; $n = 30$). Although each gene is contributing to grade prediction, SMG1 and RAD21 are the genes which are significantly increasing the power of the prediction (Stepwise regression, Minitab). Our findings support the view that association of the patient's clinical and pathological parameters with the gene expression profiles of breast tumor samples carries great importance in the classification of tumor subtypes.

PP-545
Role of the C-terminal part of forkhead domain of transcription factor FoxO4 in DNA binding

E. Boura and T. Obsil
Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, Prague, Czech Republic.
E-mail: eugenee@seznam.cz

The protein FoxO4 belongs to the forkhead box (Fox) family of transcription factors. These structurally related transcriptional activators have been found in a variety of species ranging from yeast to human. The common feature of the Fox family transcription factors is the presence of the forkhead box DNA binding domain (DBD). This domain comprises about 100 amino acid residues and is characterized by three a-helices packed against each other and a small three stranded b-sheet from which two characteristic large wings protrude. Our main goal was to investigate the role of the C-terminal part of the forkhead box DBD. The reason we believed the C-terminal part of the DBD might be important is the presence of 14-3-3 protein binding site, and the fact that the corresponding sequences of structurally related proteins Genesis and HNF-3g interact directly with DNA. Several FoxO4-DBD mutants were constructed and various techniques of fluorescence spectroscopy as well as computer modeling were used to investigate interactions between FoxO4-DBD and DNA. Our data suggest that the C-terminal part of FoxO4-DBD in the region of Arg188 and Arg189 directly interacts with DNA. This conclusion is also supported by the fact that the interaction with the 14-3-3 protein completely abolishes DNA binding ability of FoxO4.

Acknowledgment: Grant 204/06/0565 of the Grant Agency of the Czech Republic, Grant B/CH/331/2005 of the Grant Agency of the Charles University, and Centre of Neurosciences LC554.

PP-546
Induction of replicative senescence in hepatocellular carcinoma cells

N. Ozturk[1], E. Erdal[1], M. Mumcuoglu[1], K. C. Akcali[1], O. Yalcin[1], S. Senturk[1], A. Arslan-Ergul[1], M. Tez[2] and **M. Ozturk**[1]
[1]Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey, [2]Department of 5th Surgery, Numune Training and Research Hospital, Ankara, Turkey.
E-mail: nurio@bilkent.edu.tr

Replicative immortality is a common acquired feature of cancer. Cancer cell immortality contrasts with the intrinsic control of the number of cell divisions in human somatic cells by a mechanism called replicative senescence. Replicative immortality is acquired by inactivation of p53 and $p16^{INK4a}$ genes and reactivation of telomerase reverse transcriptase gene expression. As the inactivation of p53 and $p16^{INK4a}$ genes is often an irreversible event, it is assumed that cancer cell immortality is also irreversible. However, it is presently unknown whether cancer cells can be reprogrammed for replicative senescence. Using human hepatocellular carcinoma as a model system, we show the spontaneous induction of replicative senescence in p53- and $p16^{INK4a}$-deficient cancer cells. This phenomenon is characterized with the repression of telomerase reverse transcriptase expression, telomere shortening,

senescence arrest, and tumor suppression. We also show that the SIP1 gene encoding a transcriptional repressor protein is partly responsible for replicative senescence. shRNA-mediated SIP1 inactivation released hTERT repression and rescued clonal HCC cells from senescence arrest. These observations provide experimental evidence for reprogramming replicative senescence in cancer cells. Thus, the replicative immortality appears to be reversible characteristics of cancer cells and this could be used as a novel approach for cancer therapy.

PP-547
Butyric acid downregulates the activity of p53 in human T lymphocytes in multiple ways

I. Kalousek, P. Otevrelova and P. Roselova
Institute of Hematology, Prague, Czech Republic.
E-mail: kal@uhkt.cz

We examined the cellular content of the tumor suppressor p53 in relation to events induced by butyrate in PHA (phytohemagglutinin) stimulated lymphocytes. Contrary to genotoxic inhibitors, upregulating the expression of p53, butyrate induced a p53 decrease.

Results: (a) Butyrate caused an attenuation of the rRNA level dependent dispersion of nucleolar fibrillar centers in PHA stimulated G1 cells. (b) Despite G0 status of expression of the majority of examined proteins in PHA/butyrate treated cells, levels of p21waf1 exceeded its G1 amounts. (c) The diminution of rRNA synthesis caused by butyrate paralleled the diminution of expression of factors engaged in rRNA generation. (d) Upregulation of p53 in cells treated with PHA and actinomycin D was partially inhibited by butyrate. (e) Butyrate treatment caused the additional downregulation of the primarily low level of p53 in G0 phase inversely correlating with the expression of hdm2 (human double minute oncogene).

Conclusions: (a) Butyrate arrests lymphocytes stimulated with PHA at G0/G1 boundary. (b) Butyrate stimulated and p53 independent translation of p21waf1 runs in G0/G1 state. (c) The translation of p21waf1 stimulated with butyrate precedes and diminishes the translation of p53 induced by genotoxic stress caused by actinomycin D. (d) The downregulation of rRNA synthesis is implicated in p53 diminution. (e) Butyrate upregulates low levels of hdm2 in G0 cells and ubiquitin dependent p53 proteolysis would occur.

Acknowledgment: Grant is from MHCR NR7998-3.

PP-548
Wnt/-β-catenin signalling is repressed in poorly differentiated hepatocellular carcinoma cells

K. Benhaj, N. Ozturk, H. Yuzugullu, C. Akcali and **M. Ozturk**
Molecular Biology and Genetics, Bilkent University, Ankara, Turkey. E-mail: halukyu@bilkent.edu.tr

The β-catenin gene is mutated in about 20% of hepatocellular carcinomas (HCCs). High frequencies of β-catenin mutation and nuclear β-catenin staining have been detected in well differentiated HCCs, but both aberrations decline in less differentiated tumours. We hypothesized that canonical Wnt/β-catenin signaling activity is associated with the differentiation status of HCC cells. We classified human hepatoma cell lines into well differentiated (WD) and poorly differentiated (PD) groups using hepatocyte-specific biomarkers. Canonical Wnt/β-catenin signaling was active in 80% of WD and 14% of PD cell lines, respectively, as tested by TCF4-luciferase assay. Furthermore, transient expression of mutant S33Y β-catenin resulted in strong canonical Wnt/β-catenin activity in WD, but not in PD HCC cells. We also established a tetracyclin-responsive clone expressing N-terminally truncated oncogenic β-catenin from PD SNU449 cell line. Strong induction of oncogenic β-catenin in this cell line resulted in a weak response (< 5-fold) of TCF4 reporter assay, supporting the hypothesis that canonical-wnt-β-catenin signaling is repressed in PD HCC cells. We also observed an inverse correlation between the canonical Wnt/β-catenin activity and the expression of noncanonical Wnt5A and Wnt5B ligands in HCC cells. Thus, canonical Wnt/β-catenin signaling may play a dual role in HCC, being active in WD, but repressed in PD cells. We are currently investigating molecular mechanisms of this dual regulation.

PP-549
Role of TGF-β signaling in hepatocellular senescence

S. Senturk[1], A. Arslan-Ergul[1], N. Ozturk[1], H. Akkiz[2] and **M. Ozturk**[1]
[1]Bilkent University, Ankara, Turkey, [2]Cukurova University, Adana, Turkey. E-mail: serif@bilkent.edu.tr

Hepatocellular senescence is closely implicated in the development of liver cirrhosis. On the other hand, cellular immortality is a hallmark of cancer, including hepatocellular carcinoma (HCC). Thus, cellular senescence appears to be a major barrier against liver cancer development. We first analysed liver tissue samples for senescence-associated β-galactosidase (SABG) activity. Samples from different benign non-cirrhotic liver diseases displayed negative SABG staining. In contrast, all cirrhosis samples as well as some HCCs were positive, suggesting that hepatocytes both in cirrhotic liver and HCC undergo senescence arrest *in vivo* .Cellular senescence is known to occur in telomere-dependent and -independent mechanisms. TGF-β has been implicated in telomere-independent senescence arrest and in liver disease processes. Therefore, we asked whether TGF-β can be involved in senescence arrest in HCC cells. We screened the response of 14 HCC cell lines to TGF-β1 treatment.Hep40 cells displayed a progressive senescence response with nearly complete arrest at day 10 characterized by SABG staining and flat cell morphology, as well as resistance to BrdU incorporation. TGF-β1-induced senescence in Hep40 cells was associated with p21cip1 mRNA induction and reciprocal decrease in c-myc RNA levels. Hep40 cells express a mutant p53 protein and lack pRb suggesting that senescence arrest was p53- and pRb-independent. We are currently analysing mechanisms of this phenomenon.

PP-550
MDM2 T309G polymorphism is associated with bladder cancer

O. E. Onat[1], M. Tez[2], T. Ozcelik[1], **G. A. Toruner**[3]
[1]Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey, [2]Department of Surgery, Ankara Numune Research and Teaching Hospital, Ankara, Turkey, [3]Center for Human and Molecular Genetics, UMDNJ–New Jersey Medical School, Newark, NJ, USA. E-mail: emre@fen.bilkent.edu.tr

Recently a functional T to G polymorphism at nucleotide 309 in the promoter region of MDM2 gene (rs: 2279744, SNP 309) was identified. This polymorphism has an impact on the expression of the MDM2 gene, which is a key negative regulator of the tumor suppressor molecule p53. We hypothesized that this gene polymorphism might be a critical predisposition factor for bladder cancer, as MDM2 molecule is an important player in bladder cancer pathogenesis evidenced by its over-expression in 30% of urothelial carcinomas. Bladder cancer is a major cause of morbidity

and mortality. In the Turkish population it is the third most common cancer in men and eighth in women. We studied the effect of T309G polymorphism of the MDM2 gene on bladder cancer susceptibility in a case control study of 75 bladder cancer patients and 103 controls of the Turkish population. Genomic DNA was isolated from 200 μl blood by standard phenol-chloroform extraction. MDM2 T309G polymorphism was determined by polymerase chain reaction (PCR) and restriction digestion. The G/G genotype exhibits an increased risk of 2.68 (95% CI, 1.34–5.40) for bladder cancer compared with the combination of low-risk genotypes T/T and T/G at this locus. These results show an association between MDM2 T309G polymorphism and bladder cancer in our study group. To the best of our knowledge, this is the first study reporting that MDM2 T309G polymorphism could be a potential genetic susceptibility factor for bladder cancer.

PP-551
Src is a potent modulator of the Akt/mTOR signalling pathway

Z. Tuhackova, M. Vojtechova, J. Tureckova, D. Kucerova, E. Sloncova and H. Handrkova
Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic. E-mail: tuhack@img.cas.cz

Tyrosine kinase activity of Src is elevated in several types of human cancer, and perturbation of Src signalling results in alterations of Src activity and malignant transformation. In cells transformed by the v-Src oncogene, inhibition of Src protein kinase activity by the Src selective inhibitor, SU6656, is accompanied with the downregulation of the Akt/mTOR-dependent signalling pathway. Phosphorylation and activity of mTOR and its downstream targets, p70 S6K and 4E-BP1, decreased approximately to the same extent as that obtained by rapamycin, the specific inhibitor of mTOR. Treatment of the cells with either of these inhibitors reduced protein synthesis and suppressed the oncogenicity of v-src-transformed cells as measured by colony formation in soft agar. The constitutively active form of Akt over expressed in the cells in which Src protein was inactivated did not restore the phosphorylation and activity of mTOR, 4E-BP1 and p70 S6K present endogenously and neither did it activate ectopic p70 S6K transfected into the cells. Since the mTOR signalling pathway was found upregulated during malignant transformation and cancer progression in many human cancers, mTOR is emerging as a selective target for cancer therapy. Our data show that not only rapamycin and its analogs, which are examined as potent antitumor agents, but also inhibitors of Src activity may display therapeutical efficiency against a variety of human tumors in which the Akt/mTOR signalling pathway is activated.

PP-552
Regulation of the Ras-GAP activity of the grd of neurofibromin by the sec14 domain

G. Leondaritis, **G. Vallianatos**, Z. Daifoti, E. Tsirimonaki and D. Mangoura
Foundation for Biomedical Research of the Academy of Athens, Athens, Greece. E-mail: mangoura@bioacademy.gr

Neurofibromatosis type 1 (NF1) affects the function of many tissues, and it is manifested primarily as uncontrolled growth. The NF1 gene encodes neurofibromin, a Ras-GAP protein. The Ras-GAP activity is due to the presence of central 360 aa domain (GRD), and has been thoroughly characterized *in vitro*. It is largely unknown however whether other regions/domains of neurofibromin regulate GRD function *in vivo* or *in vitro*. In this study we examined whether SEC14, a C-terminal adjacent domain, has a functional role on GRD. Transient expression of GFP-SEC14 in several mammalian cells and fluorescence imaging of fixed or live cells showed a distinct pattern of perinuclear tubulovesicular localization. Interestingly, GFP-SEC14 localization was sensitive to nocodazole treatment indicating a functional interaction with the microtubule cytoskeleton as has been observed for endogenous neurofibromin in several cell lines including neurons. When SEC14 was included in a GFP-GRD + SEC14 fusion protein it drastically changed the diffuse localization pattern of GRD towards that of SEC14 alone. When these constructs were tested for their Ras-GAP activities *in vivo* towards EGF or TPA-induced Ras-GTP, SEC14 enhanced significantly the ability of over expressed GRD to inhibit Ras activation and downstream MEK/ERK signaling. Taken together, these data provide evidence that SEC14 may function as a specific targeting domain of neurofibromin and regulates the RasGAP activity of GRD.

PP-553
Evaluating the expression of survivin, a regulator of cell proliferation and death, in bladder and bone tumors

Y. Atlasi[1], E. Babaei[1], M. Emadi Baygi[1], S. A. M. Ziaee[2], S. Shariat Torbaghan[3] and S. J. Mowla[1]
[1]*Department of genetics, Faculty of Basic Sciences, Tarbiat Modares University, Tehran, Iran,* [2]*Labbafi-nejad Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran,* [3]*Cancer Institute, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran. E-mail: yaser_atlasi@hotmail.com*

Survivin, an inhibitor of apoptosis protein (IAP) capable of regulating both cell proliferation and cellular death has been recently defined as a universal tumor antigen and as the fourth most significant transcript expressed in human tumors. Since there is a correlation between expression of survivin and unfavorable outcome of cancer, it may be used as a specific molecular marker for diagnosis, prognosis, and molecular classification of tumors. Recently, five splicing variants of survivin have been further characterized with different anti-apoptotic activity, but their different functions in carcinogenesis are largely unknown. Using RT-PCR and immunostaining techniques, we have evaluated the expression of survivin in: (a) 33 fresh tumoral (Transitional Cell Carcinoma) and 28 non-tumoral bladder tissues as well as the urine samples obtained from 13 patients and 13 healthy volunteer controls (b) 28 formalin-fixed, paraffin-embedded (FFPE) specimens of high-grade osteosarcoma as well as eight non-tumoral bone tissues. Levels of survivin were significantly higher in the tumoral compared to non-tumoral bladder and osteosarcoma samples ($P < 0.0001$). Immunohistochemical study has further confirmed the restricted expression of survivin protein in tumoral samples. Also, the differential expression of two alternatively spliced variants of survivin (survivin-2B and survivin-ΔEx3) in tumoral and non-tumoral bladder tissues and urine samples is being investigated. All together, our data revealed that determining survivin gene expression could have a potential usefulness in diagnosis and prognosis of bladder and bone tumors.

PP-554
The proteomic signature of NPM-ALK reveals deregulation of multiple cellular pathways

M. Lim, M. Carlson, D. Crockett and K. Elenitoba-Johnson
Department of Pathology, University of Utah, UT, USA. E-mail: megan.lim@path.utah.edu

Constitutive expression of the chimeric NPM-ALK fusion protein is a key oncogenic event in anaplastic large cell lymphomas

(ALCL) harboring the t (2; 5) (p23; q35). In this study, we used a functional quantitative proteomic approach to determine the global effects of NPM-ALK expression. Quantitative analyses of differentially expressed proteins in Jurkat cells transfected with a plasmid containing the NPM-ALK gene versus the vector control was performed by isotope-coded affinity tagging followed by liquid chromatography and tandem mass spectrometry. 124 proteins showed a 1.5 fold or greater change in the NPM-ALK positive cells as compared to the vector control cells. Analysis of functional groups of proteins demonstrated upregulation of protein kinases, cytoskeletal proteins and proteins associated with proliferation and translation. Differential expression of selected proteins was validated by western blot analyses in transfected cells as well as in t (2;5) + ALCL cell lines. In addition, upregulation of proteins reported to be important mediators of the ALK signaling pathway including, PLCã1, Ki-67, GRB2, Jak2, and PI3-K were observed. Notably, proteins within the Ras, MAPK, mTOR and NF-kB pathways were differentially expressed. This study reveals the global proteomic consequences of NPM-ALK overexpression as a singular molecular abnormality and provides novel insight into the diverse signal transduction pathways induced transformation of NPM-ALK.

PP-555
Aberrant promoter hypermethylation of *p16* and *MGMT* genes among Greek NSCLC patients and heavy smokers

E. Georgiou[1], C. H. Karageorgopoulos[1], G. Tzimagiorgis[1], R. Valeri[2], J. Angel[3], T. Agidou[1], Th. Katopodi[1], D. Krikelis[1], Ch. Tsilikas[3], G. Sarikos[4], Ch. Destouni[2], P. Spanos[5] and S. Kouidou[1]

[1]Laboratory of Biological Chemistry, Medical School, Aristotle University, Greece, [2]Department of Cytopathology, Theagenion Cancer Hospital, Thessaloniki, Greece, [3]Department of Pneumonology, Theagenion Cancer Hospital, Thessaloniki, Greece, [4]Department of Thoracic Surgery, Theagenion Anticancer Hospital, Thessaloniki, Greece, [5]Thoracic and Cardiovascular Surgery Clinic, Medical School, Aristotle University, Greece. E-mail: tzimagio@med.auth.gr

Aberrant promoter hypermethylation, an epigenetic change associated with gene inactivation, has been demonstrated to occur in a variety of human cancers including lung cancer. In this study, we determined the frequency of aberrant promoter methylation of *p16* and *MGMT* genes, two of the most promising epigenetic markers for lung cancer, in 34 resected primary NSCLC specimens and corresponding non malignant lung tissues as well as in 40 sputum samples from heavy smokers (cancer-free individuals) using a two-stage methylation specific PCR. Our results reveal that aberrant methylation is detected in the promoter regions of *p16* (27/33, 81.8%) and *MGMT* (23/29, 79.3%) genes. In non-malignant lung tissues the observed frequency for *p16* methylation was 15/33, 45.5%, while for the *MGMT* it was 21/28 (75.0%). All cancer tissue specimens tested exhibited *p16* and/or *MGMT* methylation. Sputum analysis revealed that aberrant methylation was detected in the promoter regions of *p16* (9/40, 22.5%) and *MGMT* (8/36, 22.2%) genes. Interestingly, in all sputum samples tested, methylation was observed in either *p16* or *MGMT* promoter region. Correlation of the *p16* and/or *MGMT* methylation positive individuals with elevated cigarette consumption reveals that only *p16* methylation was observed in the heaviest smokers (7/13, 53.84%, $P < 0.001$). In summary, evaluation of *p16* and /or *MGMT* aberrant methylation could be of particular importance for the early detection of human lung cancer in high-risk populations.

PP-556
Anticancer drug-treated of breast and ovarian cancer cells reveals distinct modulations in expression of apoptotic genes

H. Thomadaki[1], A. Ardavanis[2], M. Talieri[3] and **A. Scorilas**[1]

[1]Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Athens, Panepistimioupolis, Zografou, 15 701, Athens, Greece. Email: ascorilas@biol.uoa.gr, [2]1st Department of Medical Oncology, St Savvas Anticancer-Oncologic Hospital, Greece, [3]Department of Biochemistry and Molecular Biology, Papanikolaou Cancer Research Center, St Savas Hospital, 171 Alexandras Avenue, Athens 11522, Greece. E-mail: ascorilas@biol.uoa.gr

Most apoptosis-related genes regulate cellular fate as a response to anticancer drugs. The drugs cisplatin and carboplatin are commonly utilized in the treatment of both breast and ovarian cancer. Recently, BCL2L12, a new member of the BCL2 family, was cloned by our group. Here we studied the possible alterations in the mRNA expression profile of various apoptosis-related genes including BCL2, BAX, FAS and BCL2L12 after cell treatment with cisplatin or carboplatin, in breast and ovarian cancer cell lines (BT-20 and OVCAR-3, respectively). The cytotoxic effect of each drug was evaluated by the MTT method and trypan blue staining, whereas the expression levels of distinct apoptosis-related genes were analysed by RT-PCR, using gene specific primers. The percentage of non-viable cells was up regulated with increasing concentrations and cell exposure time to the different anticancer drugs. Distinct modulations of apoptosis-related genes, at the mRNA level, were also observed. Further work is ongoing in order to ascertain whether the mRNA expression levels of the distinct apoptosis-related genes may present a significant relationship with chemotherapy outcome prediction in breast and ovarian cancer.

Acknowledgements: The present study was funded through the program EPEAEK II in the framework of the project 'Pythagoras – Support of University Research Groups' with 75% from European Social Funds and 25% from National Funds.

PP-557
p16 epigenetic alterations during cervical carcinogenesis

G. Anton[1], M. Anton[2], A. Plesa[1], C. Bleotu[1] and **E. Antipa**[1]

[1]Stefan S Nicolau Institute of Virology, Bucharest, Romania, [2]Bucharest University of Medicine and Pharmacy, Bucharest, Romania. E-mail: gabi_anton2000@yahoo.com

Cervical cancer is one of the most important causes of death in women worldwide. A number of epigenetic alterations occur during cervical carcinogenesis in both viral (human papillomavirus) and host cellular genomes. These alterations are reversible and include histone modification and hypo or hypermethylation of key tumor suppressor genes, such as p16, a cell cycle regulating gene. HPV (human papillomavirus)-infected invasive cervical squamos cell carcinomas tend to express higher immunoreactivity for p16 protein or methylation status for p16 than HPV-negative cervical carcinomas. Therefore, p16 is now considered a surrogate biomarker for HPV infection. In this context we tested the p16 promoter methylation status in DNAs isolated from tumors and corresponding sera harvested from 58 patients with LSIL, HSIL and cervical cancer (all presenting HPV genital infection). Our results suggested that aberrant promoter methylation is present in advanced stages of the disease. Similar electrophoretic patterns for p16 methylation in tumors and sera were observed only in nine cases (CIN III and squamous carcinoma). Two cases with squamous carcinoma presented methylation prophyle for DNA

isolated from sera, but no methylation for tumor DNA. In conclusion, detection of epigenetic changes in tissues and serum DNA are important for early detection and prognosis of disease and might become a new biomarker.

PP-558
Adipokine regulate proliferation and metastasis of breast cancer cells

K. Kim, H. Y. Kim, J. Y. Kim and Y. Yang
Biological Science, Sookmyung Women's University, Seoul, Korea. E-mail: yyang@sm.ac.kr

The interactions of tumor cells with the surrounding stromal cells play key roles in the tumor progression and metastasis. The relevance of adipocyte-derived factors to breast cancer cell survival and growth is well established. However, it remains unknown which specific adipocyte-derived factors are most critical in this process. Therefore we treated a breast cancer cell line with adipocytes culture supernatant, in order to investigate the multiple ways adipocytes and adipokines can uniquely affect the characteristics of malignant breast ductal epithelial cells. Microarray analysis and luciferase reporter assays indicate that adipokines specifically induce several transcriptional programs involved in promoting tumorigenesis, invasion, survival, and angiogenesis. Effects of adipokines on MCF-7 cells were compared with that of MDA-MB-231. Among adipokines, differentially regulated genes in both cell lines was firstly investigated to know functions associated with tumor progression. Taken together, adipocytes play a key role in making the ECM environment for normal and tumor-derived ductal epithelial cells as well as in tumor progression.

Acknowledgment: This work was supported by the SRC program of KOSEF (R11-2005-017-01002-0) and Grant R01-2004-000-10648-0 from the Basic Research Program of the Korea Science and Engineering Foundation.

PP-559
Experimental model to study the role of connexin 43 on glial cell proliferation

S. Herrero-González, R. Sánchez-Álvarez, **J. M. Medina** and A. Tabernero
Bioquímica y Biología Molecular, Universidad de Salamanca, Salamanca, Spain. E-mail: Sandra81@usal.es

Astrocytes are extensively coupled through gap junctions, which allow the cell-to-cell passage of small molecules such as ions, second messengers and metabolites. In tumours derived from astrocytes, the expression of connexin 43 (Cx43), the main connexin in astrocytes, is inversely correlated with the degree of malignancy. Moreover, the transfection of Cx43 in gliomas decreases their rate of growth, indicating that the expression of Cx43, and hence the establishment of gap junction communication, are critical events for the control of proliferation. In order to study the molecular mechanism by which Cx43 regulates cell proliferation, C6 glioma cells were transfected with a construction designated GFPCx43 which results in the expression of Cx43 as a fusion to the C-terminus of the green fluorescent protein (GFP). Our results show that whereas GFP does not exhibit a specific subcellular location, GFPCx43 is mainly located in the plasmatic membrane, indicating a correct subcellular location. In addition, the rate of proliferation decrease in glioma cells transfected with GFPCx43 as compare to glioma cells transfected with GFP or as compare to non transfected glioma cells. In conclusion this is a good experimental model of study the mechanism by which Cx43 regulates glioma cell proliferation.

Intracellular Trafficking in Health and Disease

PP-560
Effects of TGF-β and chondroitinsulfate on p38 and ERK 1/2 in human articular chondrocytes

J. Holzmann, N. Brandl, A. Zemann and M. Huettinger
Center for Physiology and Pathophysiology, Institut fuer Medizinische Chemie, Vienna, Austria.
E-mail: johann.holzmann@meduniwien.ac.at

Inadequate cellular response of chondrocytes to stress frequently terminates in Osteoarthritis. Adequate response is fundamentally modulated by concerted cytokine signalling events, directing degradation and synthesis of cartilage on articular sufaces where and whenever necessary. TGF-β is a prominent mediator in cartilage anabolism. Clearly, before the TGF-β signal gets through to the gene regulatory machinery, cross talk with modulators occurs. We tested the hypothesis whether chondroitinsulfate modulates cell signalling. TGF-β and/or soluble chondroitinsulfate was added to human articular chondrocytes and activation of p38 and ERK 1/2 was determined by immunoblot analysis. Expression levels of mRNA of MMP 2, 3 and 13 were determined by real-time-PCR. Significant effects were observed when cells were stimulated with LPS, invigorating catabolic metabolism in chondrocytes. LPS effects, however, were profoundly modulated by TGF-β, chondroitinsulfate and both applied in combination. Most prominent, the silencing of p38 stress signal by chondroitinsulfate was superimposable to that of TGF-β. Chondroitinsulfate treatment, alone or combined with TGF-β, reduced p38 phosphorylation significantly below LPS induced levels. Soluble chondroitinsulfate modulates signalling events in chondrocytes concurrent with MMP-13 down regulation. The effects observed suggest a feedback signalling mechanism cross talking with TGF-β-signal pathways.

PP-561
Translocon pores in the endoplasmic reticulum are permeable to small anions

B. Lizák[1], I. Czegle[1], M. Csala[1], A. Benedetti[2], J. Mandl[1] and G. Bánhegyi[1]
[1]*Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest, Hungary,* [2]*Dipartimento di Fisiopatologia, Medicina Sperimentale e Sanità Pubblica, Università di Siena, 53100 Siena, Italy.*
E-mail: lizakbea@puskin.sote.hu

Contribution of translocon peptide channel to the permeation of low molecular mass anions was investigated in rat liver microsomes. Puromycin, which purges translocon pores of nascent polypeptides creating additional empty pores raised the microsomal uptake of radiolabeled UDP-glucuronic acid. The role of translocon pore in the transport of small anions was envisaged by measuring the effect of puromycin on the activity of microsomal enzymes with intraluminal active sites. The mannose-6-phosphatase activity of glucose-6-phosphatase (G6Pase) and the

activity of UDP-glucuronosyltransferase (UGT) were elevated upon addition of puromycin, but G6Pase and β-glucuronidase activities were not changed. The increase in enzyme activities was due to a decrease in latency rather than to activation of the enzymes. Antibody against Sec61 translocon component decreased the activity of UGT and antagonized the effect of puromycin. Similarly, the addition of the puromycin antagonist anisomycin or treatments of microsomes resulting in the release of ribosomes prevented the puromycin-dependent increase in the activity. Mannose-6-phosphatase and UGT activities of smooth microsomal vesicles showed higher basal latencies, which were not affected by puromycin. In conclusion, translationally inactive, ribosome-bound translocons allow small anions to cross the endoplasmic reticulum membrane. This pathway can contribute to the non-specific substrate supply of enzymes with intraluminal active center.

PP-562
Organ-specific mammalian Ctr1 gene expression and *in silico* analysis of its putative protein product

S. Samsonov[1], N. Platonova[2], A. Vasin[1], A. Skvortsov[1], N. Tsymbalenko[2] and L. Puchkova[2]
[1]Biophysical Department, State Polytechnical University, Saint-Petersburg, Russia, [2]Molecular Genetics Department, Research Institute for Experimental Medicine, Saint-Petersburg, Russia. E-mail: sas@atom.nw.ru

Both copper excess and deficiency lead to severe neurodegenerative diseases. CTR1 is the main candidate for cell copper import, which crucially affects copper metabolic system (CMS) made up of organ-specific CMSs with distinct genes expression pattern and copper donors. This research aim was to compare Ctr1 gene activity and copper status in rat organs with distinct CMSs in development using semiquantitative RT-PCR, immunological and atomic absorption methods. The found relations between Ctr1 gene expression and copper status were divided into three distinct types, characterized by (a) decrease of CTR1-mRNA level under copper accumulation (liver and choroid plexus in newborns); (b) high Ctr1 gene activity, associated with high level of Cp-mRNA and high rate of secretory Cp synthesis (liver and choroid plexus of adult rats, mammary gland during lactation); (c) Ctr1 gene expression decrease, correlating with Cp gene activity and Cp concentration in milk decrease (lactating mammary gland). We conclude that transcriptional activity of mammalian Ctr1 gene is repressed under high intracellular copper content and is activated/inactivated dependently on physiologically demanded cuproenzymes synthesis level. CTR1 domains sequences were theoretically analysed and obtained data suggest that CTR1 is possible universal both extracellular copper acceptor and intracellular copper donor for Cu-chaperons.

PP-563
Ricin transport from endosomes to the golgi apparatus is regulated by Rab6A and Rab6A'

A. Utskarpen, H. Slagsvold, T.-G. Iversen, S. Wälchli and **K. Sandvig**
Department of Biochemistry, Faculty division The Norwegian Radium Hospital, University of Oslo, Oslo, Norway. E-mail: ksandvig@radium.uio.no

The plant toxin ricin is a protein toxin that is endocytosed and transported through the Golgi apparatus to the endoplasmic reticulum, from where its enzymatic moiety is translocated to the cytosol. We have investigated the possible role of several proteins in the intracellular transport of ricin, among others the GTPases Rab6A and Rab6A', which are splice variants differing in three amino acids. Specific siRNA oligos were designed to deplete the two isoforms both separately and simultaneously. The ricin transport to the *trans*-Golgi network was then measured by using a modified ricin molecule with a sulfation site. An inhibition of ricin transport was observed when at least 40% of Rab6A mRNA was depleted, but when the knockdown of Rab6A exceeded 75% and Rab6A' at the same time was up-regulated, the inhibition of ricin transport was abolished. The up-regulation of Rab6A' therefore seems to compensate for the loss of Rab6A. There was also an inhibition of ricin transport to the Golgi of about 40% when Rab6A' was depleted, but even if Rab6A was up-regulated it did not compensate for the loss of Rab6A'. When both Rab6A and Rab6A' were knocked down a stronger inhibition of ricin transport occurred than when the two isoforms were depleted separately. Altogether, these data indicate that both Rab6A and Rab6A' regulate endosome to Golgi transport of ricin.

PP-564
Loss of ClC-7 or its novel β-subunit Ostm1 leads to osteopetrosis and neurodegeneration

P. F. Lange, L. Wartosch, T. J. Jentsch and J. C. Fuhrmann
Zentrum für Molekulare Neurobiologie Hamburg - ZMNH, Universitat Hamburg, D-20246 Hamburg, Germany. E-mail: plange@zmnh.uni-hamburg.de

Mutations in ClC-7, a lysosomal member of the CLC family of chloride channels and transporters, cause osteopetrosis and lysosomal storage disease in human and mice. Severe osteopetrosis is also observed with mutations in the OSTM1 gene, which encodes a membrane protein of unknown function. Here we show that both ClC-7 and Ostm1 proteins co-localize in late endosomes and lysosomes of various tissues, as well as in the ruffled border of bone-resorbing osteoclasts. Co-immunoprecipitations show that ClC-7 and Ostm1 form a molecular complex and suggest that Ostm1 is a β–subunit of ClC-7. ClC-7 is required for Ostm1 to reach lysosomes, where the highly glycosylated Ostm1 luminal domain is cleaved. Protein, but not RNA levels of ClC-7 are greatly reduced in grey-lethal mice, which lack Ostm1, suggesting that the ClC-7/Ostm1 interaction is important for protein stability. As ClC-7 protein levels in Ostm1-deficient tissues and cells, including osteoclasts, are decreased below 10% of normal levels, Ostm1 mutations probably cause osteopetrosis by impairing the acidification of the osteoclast resorption lacuna, which depends on ClC-7. The finding that grey-lethal mice, just like ClC-7-deficient mice, show lysosomal storage and neurodegeneration in addition to osteopetrosis implies a more general importance for ClC-7/Ostm1 complexes.

Reference:

1. Lange PF, Wartosch L, Jentsch TJ & Fuhrmann JC ClC-7 requires Ostm1 as a b-subunit to support bone resorption and lysosomal function. Nature. (in press).

PP-565
Is there any interplay between P-gp mediated multidrug resistance and metabolism of saccharides?

Z. Sulova[1], A. Kovarova[2], H. Kavcova[1], B. Uhrik[1], D. Mislovicova[2] and A. Breier[1]
[1]Institute of Molecular Physiology and Genetics, SAS, Bratislava, Slovak Republic, [2]Institute of Chemistry SAS, Bratislava, Slovak Republic. E-mail: zdena.sulova@savba.sk

Multidrug resistance of murine leukaemic cell line L1210/VCR (obtained by adaptation of parental drug sensitive L1210 cells to

vincristine) is associated with overexpression of P-glycoprotein (P-gp). 31P-NMR spectra of parental L1210 and L1210/VCR cells revealed a decrease of ATP and UDP-saccharides levels in resistant cells. Recently we have assumed that biosynthesis of oligo and polysaccharides was markedly depressed [1]. Cytochemical staining of negatively charged cell surface sialic acid by ruthenium red (RR) revealed a compact layer of RR bound to the external coat of sensitive cells. In resistant cells RR layer was either reduced or absent. Consistently, resistant cells were found to be less sensitive to Concanavalin A (ConA). This lectin agglutinated resistant cells less potently than parental L1210 cells. This result was also confirmed by enzyme linked lectin binding assay – ELLBA. ConA labeled cell surface of sensitive cells more effectively than resistant cells as documented using lectin cytochemistry. Interestingly, tomato lectin (lycopersicum esculentum agglutinin) was found to show the opposite behaviour. Thus, multidrug resistance of L1210/VCR cells is accompanied by considerable changes of cell surface glycosides, that could be detected specifically by lectins.

Acknowledgment: This work was supported from APVT-51-027404, VEGA-2/4155 and VEGA-2/ 080/26.

Reference:

1. Fiala R *et al*. Biochim Biophys Acta 2003; 1639: 213–224.

PP-566
Measurement of P-glycoprotein function by Calcein/AM and Fluo-3/AM

L. Gibalova[1], I. Dovinova[2], A. Zahradnikova[1], Z. Sulova[1] and A. Breier[1]

[1]Institute of Molecular Physiology and Genetics, SAS, Bratislava, Slovakia, [2]Institute of Virology, SAS, Bratislava, Slovakia. E-mail: lenka.gibalova@savba.sk

We used Calcein/AM and Fluo-3/AM for monitoring of P-glycoprotein (PGP) activity directly in PGP positive L1210/VCR cells for FACS and confocal microscopy measurements. Measurements were carried out in the presence or absence of probenecid, verapamil or vincristine. In the absence of drugs intensive fluorescence was detected in drug sensitive L1210 cells only. Inhibitor of PGP- verapamil was able to induce an elevation in fluorescence of L1210/VCR cells to a similar level as in L1210 cells by concentration dependent manner. Vincristine-induced calcein retention in L1210/VCR cells was much less pronounced than verapamil. Probenecid did not elevate level of fluorescence of L1210/VCR. Probenecid is a known inhibitor of anion transporters including multidrug resistance proteins (MRP) therefore the role of these transporters in mediation of MDR in L1210/VCR cells is negligible. Many calcium antagonists are also known as P-glycoprotein antagonists. For this reason, we have verified the effect of increased calcium concentration on the activity of P-glycoprotein. However, enhancement of extracellular calcium concentration did not alter the transport function of PGP in our experiments.

Acknowledgements: This work was supported APVT-51-027404, VEGA-2/4155 and VEGA-2/3122.

PP-567
Correlation between P-glycoprotein overexpression and calcium homeostasis in L1210/VCR cells

M. Seres, B. Uhrik, A. Breier and Z. Sulova

Institute of Molecular Physiology and Genetics, SAS, Bratislava, Slovak Republic. E-mail: mario.seres@savba.sk

L1210/VCR cells represent a P-glycoprotein (P-gp) positive multidrug resistant (MDR) cell model. We have found a much higher influence of increased extracellular Ca^{2+} concentration on the viability of resistant cells as compared with sensitive cells. Moreover L1210/VCR cells accumulated more $45Ca^{2+}$ as L1210 cells in an experiment with 1.6, 5.0 or 10.0 mmol/l Ca^{2+} applied into the external medium. Ca^{2+} entry blockers as flunarazin and verapamil did not exert a considerable effect on IC50 values for calcium in both sublines of cells. However, both calcium entry blockers were found to be more toxic to resistant than to sensitive cells. Vincristine and some chemosensitizers were influenced uptake of $45Ca^{2+}$ in L1210 and L1210/VCR only slightly. Ultrastructural localization of Ca^{2+} in L1210 and L1210/VCR by a cytochemical precipitation method has demonstrated clusters of precipitate on the surface of plasma membrane and cristae of mitochondria in L1210 cells. The precipitates in L1210/VCR were more numerous and localized mainly along the surface of plasma membrane and within vesicles of ER as well as in cytosol. In sensitive cells higher amounts of calcium binding chaperone protein calnexin than in resistant cells were found. All the above facts indicate that calcium at least indirectly plays a role in regulation of processes involved in MDR phenotype of L1210/VCR cells.

Acknowledgements: This work was supported from APVT-51-027404, VEGA-2/4155 and VEGA- 2/ 080/26.

PP-568
Reversal of P-glycoprotein mediated vincristine resistance by analogues of pentoxifylline

A. Breier[1], I. Kupsakova[2], M. Barancik[2], P. Docolomansky[1] and A. Rybar[3]

[1]Institute of Molecular Physiology and Genetics, SAS, Bratislava, Slovak Republic, [2]Institute for Heart Research, SAS, Brtaislava, Slovak Republic, [3]Institute of Chemistry, SAS, Bratislava, Slovak Republic. E-mail: albert.breier@savba.sk

We have analysed the capability of twenty-five methylxanthines (structurally differ in substituents located in positions N1, N3, N7 and C8) to depress p-glycoprotein (PGP) mediated multidrug resistance (MDR) of L1210/VCR cells. The results indicate that for an effective reversal of P-gp mediated MDR, the existence of a longer polar substituent in the position N1 plays a crucial role. The elongation of the substituent in the positions N3 and N7 increases and in the position C8 decreases the efficacy of xanthines to reverse the vincristine resistance. The multiple linear regression for effectiveness of methylxanthines in reversal of P-gp mediated MDR has been computed, with molar weight – Mw, molar volume – VM, molar refractivity – RM, crystal density – d and partition coefficient n-octanol/water – log P as descriptors. A high intercorrelation of MW, VM and RM was found indicating that only one of these parameters is necessary for testing a potential correlation. The best fit in the multiple linear regression was obtained for RM applied together with d and log P and resulted in a QSAR model given by the following equation:

$$IC50r = -[(32.3 \pm 7.2) \times 10^{-3} \times RM] + [(10.1 \pm 2.3) \times d] + [(0.74 \pm 0.10) \times \log P] - [10.5 \pm 3.2]$$

Model revealed that: (a) the RM influences the effectiveness of xanthine positively; (b) the d and log P influence the MDR reversal effectiveness of xanthine negatively.

Acknowledgements: This work was supported from APVT-51-027404, VEGA-2/4155 and VEGA- 2/ 080/26.

PP-569
Inhibitory effect of osteopontin to calcium oxalate crystal adhesion and deposition in rat kidneys

E. Sogut[1], F. Ustuner[1], H. Postaci[2] and F. Tasli[2]
[1]*Department of Biochemistry and Clinical Biochemistry, Ataturk Training and Research Hospital, Izmir, Turkey,* [2]*Department of Pathology, Izmir Training and Research Hospital, Izmir, Turkey. E-mail: erkanchems@yahoo.com*

Osteopontin (OPN) is a phosphorylated protein of wide tissue distribution. Its production is also related to immunity, infection, inflammation, cell survival, and cancer. It is a strong inhibitor of crystal formation and growth *in vitro*, but there is still debate regarding its effects upon crystal adhesion and deposition to tubular epithelial cells in kidney. This study was conducted to investigate the effects of this protein on stone formation by using an ethylene gylcol (EG)-induced urolithiasis model of rat.
A total of 20 Wistar rats were divided into two groups, namely group 1—control rats, group 2—stone group rats were given special drinking water that contained 1.0% NH4Cl with 0.1% concentrations of EG. The rats were sacrificed 12 days after starting the special water. Bladder urine was also obtained. Blood and urine were tested for calcium, phosphorus, oxalate, citrat, OPN and creatinine. Kidney sections were stained with hematoxylin-eosin, von Kossa stain. Immunohistochemistry stainings were done to localize osteopontin in the kidneys. Enzyme-Linked Immunosorbent Assay was used to evaluate plasma and urine OPN. Osteopontin expression in the kidneys was significantly increased after hyperoxaluria and also after the deposition of calcium oxalate crystals. Urinary excretion of OPN increased concomitantly, associated with oxalate excretion. These results suggest that OPN can inhibit renal calcium oxalate crystal deposition in EG plus NH4Cl-treated experimental rats.

PP-570
Fluctuation of carbonate and citrate transport in healthy and after fracture by the new blood/bone index

K. A. Burda[1], S. M. Kichenko[1], Yu. A. Petrovich[1], R. P. Podorozhnaya[2]and I. M. Dmitriev[2]
[1]*Department of the biochemistry, State University of Medicine and Dentistry, Moscow, Russia,* [2]*Radioisotopic Laboratory, Stomatological Institute Academy of Science of Ukraine, Odessa, Ukraine. E-mail: kichenko@metronet.ru*

We studied the effect of jaw fracture on [^{14}C] carbonate and [3-^{14}C] citrate transport between the blood and bone. Was proposed a new indexes blood/bone relative radioactivity (BBRR), reflecting the ratio between radiocarbon (RC) incorporation into blood and bone, and difference index (DIBBRR) quantity calculated with help of next BBRR subtraction from previous BBRR. DIBBRR designed accordinally to time of their computation. DIBBRR indicated the intensity in surpassing direction of transport between the blood and bone. The percentage of label incorporation, BBRR and DIBBRR were many times measured from the 5th minute to the 192nd hour after intraperitoneal injection of labeled carbonate and citrate to healthy 1-month-old albino rats. We revealed fluctuation of indexes and a biphasic reaction: rapid accumulation and elimination of the RC and slow accumulation followed by slow elimination. After bone fracture at the stage of cellular-and-fibrous callus accumulation of RC dominated over its elimination. At the stage of chondroid callus elimination dominated over accumulation. At the stage of primary osseous callus elimination dominated over accumulation, but did not surpass the control level. After fracture the underwent general changes that were most pronounced in the zone of trauma.

PP-571
Controlling neuronal traffic accompanied by calcium

S. Ahmadian, K. Shahpasand and M. Flahati
Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran. E-mail: ahmadian@ibb.ut.ac.ir

Calcium has been evaluated as a regulating factor in intracellular functions. Regarding the processive cytoskeleton it would be manifested that for the purpose of transport, MTs must move in the radial dimension to make room for a moving vesicle (MV) and after cargo passing, return to the previous position for the maintenance of neuronal plasticity. The preventing factor against this type of movement is steric constraints (SC) due to projection domain of microtubule-associated proteins (MAPs). Here we propose that Ca^{2+} decreases the SC. Hence; the ability of MTs to move radially would be increased. So the convenient circumstances for efficient vesicular transport would be provided. To test this hypothesis, we used viscometer, light scattering, TEM and AFM analysis. The results have shown that the viscosity of MTs solution decreases with increasing Ca^{2+} concentration and the inter-filament spaces in MTs decrease as Ca^{2+}concentration increased as was showed by TEM. MTs solution by increasing Ca^{2+}, increased scatter light by light scattering and the repulsive forces of MAPs decrease as Ca^{2+} increases by using AFM mode. In conclusion, our results show a new effect of Ca^{2+} on cytoskeleton which could be addressed to regulating neuronal traffic.

PP-572
Development and use of the adenovirus dodecahedron for the intracellular delivery of therapeutic agents

E. Szolajska[1], P. Fender[2], A. Naskalska[3], G. Schoehn[4], A. Paca[1], N. Rodnin[1], M. Zochowska[1] and J. Chroboczek[3]
[1]*Department of Protein Biosynthesis, Institute of Biochemistry and Biophysics, Warsaw, Poland,* [2]*Laboratory of Molecular Enzymology, Institut de Biologie Structurale, Grenoble, France,* [3]*Laboratory of Molecular Biophysics, Institut de Biologie Structurale, Grenoble, France,* [4]*Laboratorie de Virologie Moleculaire et Structurale, EMBL, Grenoble, France. E-mail: ewasz@ibb.waw.pl*

Dodecahedron (Dd) is a symmetrical nano-particle spontaneously assembled from 12 Adenovirus (Ad) pentons. The penton is a non-covalent complex composed of two oligomeric viral proteins: penton base and fiber that ensure virus intracellular penetration. The dodecahedra very efficiently enter and transduce cultured animal cells with the efficacy superior to that of Ad, the most efficient vector known now. The recombinant production of the dodecahedra offers a safe way to obtain a highly purified, non-pathogenic pharmaceutical excipient. We propose two applications of Dd in the intracellular delivery. In the first, dodecahedron will be used as a delivery platform for known immunity-conferring proteins of infectious agents for vaccines. In the second, small, non-soluble and labile anti-tumor drug will be encapsulated in virus-like particle with the aim of increasing the bioavailability of the drug and diminishing its non-specific toxicity. For both applications there, we needed to develop approaches that enhance physical and chemical stability of this virus-like particle. We have identified parameters that influence stability of Dd preparations such as buffers, salts, cryoprotectants and appropriate long-term storage conditions. The elaboration of ambient temperature stable liquid preparation of this

remarkable vector should help in establishing the utility of Dd as a vector for vaccines and as well as for direct delivery of therapeutic factors into human and animal cells.

PP-573
The hepatitis E virus ORF3 protein modulates endocytic trafficking and apoptotic pathways to promote survival

S. Jameel[1], A. Kar-Roy[1], S. Moin[1], V. Chandra[1], S. Kumari[2] and S. Mayor[2]
[1]*Virology Group, International Centre for Genetic Engineering and Biotechnology, New Delhi, India,* [2]*National Centre for Biological Sciences, Bangalore, India. E-mail: shahid@icgeb.res.in*

The hepatitis E virus (HEV) is a positive-strand RNA virus and the etiologic agent for hepatitis E. Infections due to HEV are a significant cause of morbidity and mortality, especially among pregnant women living in endemic areas. Its inability to grow in culture has hampered studies on HEV biology and pathogenesis. The open reading frame 3 (ORF3) protein of HEV is a ~13.5 kDa phosphoprotein that we have shown earlier to modulate the cell signaling program (Korkaya H *et al.* J. Biol. Chem. 2001;276:42389; Kar-Roy A *et al.* J. Biol. Chem. 2004;279:28345). In confocal microscopy experiments, the ORF3 protein was found to localize to early and recycling endosomes, but not to late endosomes or lysosomes. In ORF3 expressing cells, the intracellular trafficking of epidermal growth factor receptor (EGFR) from the early/sorting endosome to the late endosome was delayed. This is likely to prolong growth factor signaling from endomembrane sites. The downstream effects of this trafficking modulation were noted on functional regulation of STAT3 and the acute phase response in liver cells. The expression of ORF3 was also found to upregulate expression of the mitochondrial voltage-dependent anion channel (VDAC), to prevent cytochrome c leakage from mitochondria and to reduce oxidative stress in cells. Thus, this viral protein appears to utilize multiple strategies and targets to promote survival of the infected cell. This would benefit viral replication and contribute to pathogenesis.

PP-574
Alterations of liver arginine-NO metabolism in citrate-induced steatohepatitis

O. Unay Demirel[1], E. Ozturan Ozer[1], E. Ogus[2], G. Karabay[3], D. Akaydin Aldemir[1] and S. Turkoglu[1]
[1]*Biochemistry, Baskent University Faculty of Medicine, Ankara, Turkey,* [2]*Biostatistics, Baskent University Faculty of Medicine, Ankara, Turkey,* [3]*Histology and Embryology, Baskent University Faculty of Medicine, Ankara, Turkey.*
E-mail: ounay@baskent.edu.tr

The study aimed to evaluate the importance of arginine-NO metabolism considering tissue repairing in citrate-induced hepatic damage. Rats were randomly assigned into three groups: saline injected control group ($n = 5$), 10 mM (CIT 1; $n = 6$) and 100 mM (CIT 2; $n = 6$) citrate treated groups. All treatments were achieved as a single dose via tail vein. Blood and liver tissue specimens were obtained at the end of fourth week of treatment. Univariate Analysis of Variance coupled with Duncan's Post-Hoc test was performed for statistical evaluation. The significant increase in serum AST/ALT ratio ($P < 0.01$) and existence of steatosis, fibrosis and inflammation, by histopathological examination, confirmed the hepatic damage, developing more severely in CIT 2. Hypertriglyceridemia was also detected in the serum of treated rats ($P < 0.001$). Serum tumor necrosis alpha (TNF-α) levels were significantly elevated in dose-dependent manner ($P < 0.001$), while serum hepatocyte growth factor concentrations were not affected. Liver arginine levels did not alter, whereas the concentrations of agmatine decreased significantly in dose-dependent fashion ($P < 0.001$). Also, putrescine levels significantly decreased in CIT 2 ($P < 0.05$). Liver arginase activity did not alter but nitric oxide synthase (NOS) activity was reduced ($P < 0.01$). Our results have indicated that elevation of TNF-α and modulation of arginine-NO metabolism seem to play crucial role in the pathogenesis of citrate induced hepatic damage.

PP-575
Nitric oxide levels in patients with breast cancer

Ö. Çiftçi[1], İ. Bütün[1], H. Sönmez[1], **V. Çelik**[2] and E. Kökoğlu[1]
[1]*Department of Biochemistry, Medical Faculty of Cerrahpaşa, University of İstanbul, İstanbul, Turkey,* [2]*Department of Surgery, Medical Faculty of Cerrahpaşa, University of İstanbul, İstanbul, Turkey. E-mail: ozlemciftci1@yahoo.com*

In some cancers, particularly those of the breast, two cellular pathways have emerged as being closely associated with angiogenesis and tumor growth. Nitric Oxide (NO) pathway; which oxidizes the guanido group of L-arginine to citrulline and NO, through the activity of Nitric Oxide Synthase (NOS) is one of them. NO production occurs in several steps leading to neovascularisation in breast cancers. It might play an important role in breast cancers depending on its intra-tumoral concentration. We determined NO levels and NOS activity in peritumoral, tumoral tissues and serums of patients with breast cancer ($n = 20$). Serum levels of healthy subjects were used as control ($n = 10$). Determination of the stable end products of NO, nitrite and nitrate, were used as an indirect marker of nitric oxide production. NOS activities were calculated by nitrate concentration by a colorimetric method. Results indicate that; tumoral tissue NO levels and NOS activities are significantly higher than those of peritumoral tissue ($P < 0.05$). Serum NO levels and NOS activities of patients are significantly higher than those of control serums. NO as a product of immune system cells has been implicated in the mechanism of carcinogenesis. Also NOS, has a relationship between angiogenesis in breast cancer. NO pathway has an important role in breast cancers. A more profound understanding of this pathway may improve the knowledge of mechanisms responsible for breast cancers.

PP-576
Myospryn is a novel binding partner for desmin

A. Kouloumenta, M. Mavroidis and Y. Capetanaki
Division of Cell Biology, Center of Basic Research, Foundation of Biomedical Research Academy of Athens, Athens, Greece. E-mail: akouloum@bioacademy.gr

Desmin, the muscle specific intermediate filament protein, surrounds the Z-discs, interconnects them to each other and links the entire contractile apparatus to the sarcolemmal cytoskeleton, cytoplasmic organelles and the nucleus [1]. In an attempt to explore the role of desmin in cardiac function, we have performed a yeast two-hybrid screening of a cardiac cDNA library. Here we show that desmin head domain binds to the C-terminus of Myospryn, muscle specific tripartite motif (TRIM)-related protein. Binding of desmin with myospryn was confirmed: (a) by GST-pull down assay where desmin isolated from heart muscle homogenates was specifically absorbed to GST-myospryn and (b) by co-immunoprecipitation of myospryn and desmin in an *in vitro* transcription/translation system. An antibody against the

C-terminus of myospryn detected its co-localization with desmin at the periphery of the nuclei of mouse primary cardiomyocytes, with a pattern resembling ER structures, which seem altered in the absence of desmin. Myospryn interacts with dysbindin [2], a coiled-coil protein, part of BLOC-1 (the biogenesis of lysosome related organelles complex). Mutations in dysbindin, or other BLOC members associated with it, are linked to defects of lysosome related organelles, thus connecting indirectly desmin to processes, such as vesicle trafficking and organelle biosynthesis.

References:

1 Capetanaki Y. Trends Cardiovasc Med 2002;12 (8): 339–48
2 Benson A. JBC 2004; 279 (11): 10150–10158

PP-577
A functional P-glycoprotein in mitochondria of MDR K562 cells

C. Stenger[1], E. Munteanu[1], F. Grandjean-Forestier[1], J. Robert[2], M. Verdier[1] and Mh. Ratinaud[1]
[1]*Lab Physiologie Mitochondriale, EA 3842 Faculté de Médecine, 87025 Limoges, France,* [2]*Lab Pharmacologie des Agents Anticancéreux, Institut Bergonié, 33076 Bordeaux-cedex, France.*
E-mail: christophe.stenger@etu.unilim.fr

The P-glycoprotein 170 (P-gp) is an ABC family protein overexpressed at the plasma membrane of multidrug resistance (MDR) cells. Previous results suggested that intracellular compartments could accumulate the doxorubicin, and reverting agents may lead to intracellular drug redistributions. We have studied the localization of P-gp in mitochondria of sensitive and doxorubicin resistant K562 lines, as well as its functional properties in this compartment. Using several monoclonal antibodies anti- P-gp (MoAbs), a protein of about 170 kDa was assessed in mitochondria isolated from K562 MDR cells. The functionality of this P-gp has been evaluated by cytometric analyses of doxorubicin accumulation and efflux in whole isolated mitochondria from sensitive and resistant cells. The effects of modulators were studied, using either specific MoAbs, or specific inhibitors. MoAbs did not modify doxorubicin accumulation in the mitochondria from resistant cells, whereas the inhibitors significantly decreased its uptake. We demonstrated that mitochondrial P-gp was involved in doxorubicin accumulation inside the organelle but not in its efflux, suggesting an orientation of P-gp in the mitochondrial membrane inverse to that observed in the plasma membrane. A potential role for mitochondrial P-gp in MDR cells would be to protect the nucleus from doxorubicin. Our results raise different questions about the intracellular trafficking of P-gp and its addressing to mitochondria.

PP-578
Mutations in a solute carrier gene are responsible for pulmonary alveolar and partly for testicular microlithiasis

A. Corut[1], A. Senyigit[2], **S. A. Ugur**[1], S. Altin[3], U. Ozcelik[4], H. Calisir[5], Z. Yildirim[6], A. Gocmen[4] and A. Tolun[1]
[1]*Department of Molecular Biology and Genetics, Bogazici University, Istanbul, Turkey,* [2]*Department of Chest Diseases, Faculty of Medicine, Dicle University, Diyarbakır, Turkey,* [3]*Yedikule Hospital for Pulmonary Diseases, Istanbul, Turkey,* [4]*Division of Pediatric Pulmonary Diseases, Hacettepe University Medical School, Ankara, Turkey,* [5]*Atatürk Pulmonary Disease and Chest Surgery Center, Ankara, Turkey,* [6]*Division of Pulmonary Diseases, Inonu University Medical School, Malatya, Turkey.*
E-mail: tolun@boun.edu.tr

Pulmonary alveolar microlithiasis (PAM) is a rare disease, while testicular microlithiasis (TM) is not rare. By positional-candidate gene approach, we identified loss-of-function mutations in PAM patients and two heterozygous variants in 2 of 15 TM subjects. Our results show that PAM is monogenic with full penetrance, while TM is a complex trait. PAM is characterized by the deposition of calcium phosphate microliths throughout the lungs. The disease leads to slow deterioration of lung functions. We localized the gene responsible for the disease in a consanguineous family with six affected boys and analysed a solute carrier gene at the locus for mutations in PAM patients. We identified homozygous mutations in six PAM patients. Moderate expression of the gene in testis prompted us to search for mutations in men with diffuse bilateral testicular microlithiasis (TM) in order to investigate any role of the gene in the etiology of the condition. We identified two rare variants in the heterozygous state in 2 of the 15 TM subjects studied. The variants could not be assigned as mutations as readily as those detected in the PAM patients. However, they occurred in nucleotide residues conserved in humans and chimpanzee. The data indicates that the gene is most likely responsible at least for some TM cases. The prevalence of TM is estimated to be 0.6–9% in the population. TM is associated with the majority of primary testicular malignancies.

PP-579
Beta-adrenergic and thyroid hormone receptors expression in human failing cardiomyocytes.

M. Marchetta[1], T. Gamberi[1], S. Vanni[2], A. Modesti[1], G. Gensini[2] and P. A. Modesti[2]
[1]*Biochemistry Department, University of Florence, Florence, Italy,* [2]*Dipartimento di Area Critica Medico Chirurgica, University of Florence, Florence, Italy. E-mail: matilde.marchetta@unifi.it*

Beta-adrenergic receptor have been found to be target genes for thyroid hormone and their expression is enhanced by T3 stimulation in rat cardiomyocytes. The gene expression patterns of heart failure resemble those described in hypothyroidism. However, information regarding the status of thyroid hormone receptors in human failing cardiomyocytes are lacking. Therefore we investigated the relationship between the expression levels of thyroid hormone (TRalfa1, TRalfa2 and TRbeta1) and beta-adrenergic receptor (ARB1 and ARB2) in homogenated hearts and in cardiomyocytes isolated from patients undergoing cardiac transplantation for dilated cardiomyopathy (DCM, $n = 5$). Non-failing hearts from tentative donors (NF, $n = 3$), not transplanted for non cardiac reasons, served as controls. Expression of mRNAs was measured by RT-PCR. In isolated myocytes and in homogenated hearts from DCM patients the expression of TRalfa1 isoform (the active form of thyroid hormone receptors) was reduced with unchanged expression of mRNA for the TRbeta1 isoform. Those changes were associated with reduced mRNA expression of both ARB1 and ARB2 receptor subtype in comparison to controls. These findings were confirmed at the protein level by both Western blots and binding studies.
In conclusion the reduced beta adrenergic receptor population in failing cardiomyocytes is closely associated with a reduced expression of thyroid hormone receptors.

PP-580
Identification of new genes involved in endoplasmic reticulum-associated protein degradation in yeast

M. J. Mazón, P. Eraso and F. Portillo
Bioquímica y Genética de Levaduras, Instituto de Investigaciones Biomédicas Alberto Sols CSIC-UAM, Madrid, Spain. E-mail: mjmazon@iib.uam.es

The endoplasmic reticulum quality control system scans the folding process of secretory proteins and retains those species unable to fold correctly. These proteins are eliminated by a process called ER-associated degradation (ERAD), via the ubiquitin proteasome system. We aim for the identification of new genes involved in this process. To achieve this goal we have developed a genetic screening in the yeast *Saccharomyces cerevisiae* that exploits the fact that the *PMA1* gene is an essential gene. Several point mutations in *PMA1* produce malfolded versions of the Pma1 protein that are retained in the ER and are toxic for the yeast cell. These mutations show a dominant-lethal phenotype because the coexpressed wild-type protein is also retained and, as a consequence, cell growth is inhibited. We screened for genes that when overexpressed were able to suppress the dominant-lethal phenotype exhibited by the mutant alleles. Up to now we have isolated several transformants carrying plasmids with large chromosomal fragments that are able to grow under the conditions described. The mechanism of suppression includes an increase in the degradation rate of the Pma1 mutant protein as measured by a chase with cycloheximide. The subcloning and identification of the genes contained in these plasmids will be presented.

PP-581
Pregnancy associated plasma protein-A levels in allergic rhinitis

E. Guclu[1], A. Coskun[2], S. Duran[3], A. Tokmak[1] and O. Ozturk[1]
[1]Department of Otorhinolaryngology, Duzce School of Medicine, Abant Izzet Baysal University, Duzce, Turkey, [2]Department of Clinical Biochemistry, Duzce School of Medicine, Abant Izzet Baysal University, Duzce, Turkey, [3]Department of Cardiology, Duzce School of Medicine, Abant Izzet Baysal University, Duzce, Turkey. E-mail: coskun_a2002@yahoo.com

Pregnancy associated plasma protein A (PAPP-A), known as insulin-like growth factor binding protein-4 (IGFBP-4) protease, has been postulated to amplify local insulin-like growth factor I (IGF-1) activity in wound healing, vascular repair, bone remodeling, and development of the dominant follicle. Although several studies demonstrate the importance of IGFs in the regulation of vascular smooth muscle growth, limited information is available on the pathophysiology of IGFs and related molecules in airway tissues. We aimed to determine the significance of serum PAPP-A levels in allergic rhinitis patients. Serum PAPP-A levels of 31 newly diagnosed allergic rhinitis patients and 30 healthy controls were determined by an ultra sensitive enzyme-linked immunosorbent assay (ELISA). The PAPP-A level was 6.1 ± 2.86 mU/l in the allergic rhinitis group, and 4.54 ± 1.71 mU/l in the control group. The level of the allergic rhinitis group was significantly higher than that of the control group ($P = 0.013$). We concluded that serum PAPP-A levels are elevated in allergic rhinitis patients and increased PAPP-A activity may be involved in inflammation and hypervascularity that occurs in allergic rhinitis.

PP-582
ARF6 is involved in endosome-to-golgi transport of ricin

H. Raa, T. -G. Iversen and K. Sandvig
Department of Biochemistry, Institute for Cancer Research at The Norwegian Radium Hospital, Oslo, Norway. E-mail: hilder-a@radium.uio.no

The plant toxin ricin is endocytosed by the cell and transported retrogradely through the Golgi apparatus to the ER. From the ER, the toxic subunit is translocated into the cytosol where it inhibits protein synthesis. ADP-ribosylation factor 6 (Arf6) is a small GTPase of the Ras-family, thought to be involved in regulating membrane traffic between the plasma membrane and endosomes. Our studies aim to reveal a possible role of ADP-ribosylation factor 6 (Arf6) in transport of the protein toxin ricin into cells. We have established cell lines with inducible overexpression of Arf6 wt and the two mutants T27N and Q67L, which are locked in the GDP- and GTP-bound form, respectively. We have also used the oligobased RNA interference (RNAi) approach to downregulate endogenous expression of Arf6 in HeLa cells. To measure the amount of ricin transported to the Golgi apparatus, we use ricin-sulf-1, a ricin molecule modified to contain a sulfation site. The cells are incubated in the presence of $^{35}SO_4^{2-}$, and the trans Golgi network resident sulfotransferase ensures selective labelling of the ricin molecules that reach the Golgi apparatus. Our results show that downregulation of endogenous Arf6-expression reduces the amount of ricin transported to the trans Golgi network while the endocytosis of ricin remains unaffected, indicating that Arf6 is involved in regulating the transport of ricin from the endosomes to the Golgi apparatus.

PP-583
D-lactate/malate antiporter, in response to the cellular carbonyl stress mediated by methylglyoxal, in yeast mitochondria.

M. Pallotta
Department of Health Sciences, University of Molise, Campobasso, Italy. E-mail: pallotta@unimol.it

Methylglyoxal is a highly reactive dicarbonyl degradation product formed from triose phosphates during glycolysis. Methylglyoxal-derived advanced glycation end-products are involved in neurodegenerative disorders and in the clinical complications of diabetes. Yeast cells are an outstanding cell model for investigating intracellular protein glycation and its implications in cell physiology and aging. In yeast an established pathway for MG detoxification by the action of the glyoxalase system converts MG into D-lactate in the presence of glutathione. Previous studies have already reported the existence of two carriers for D-lactate mitochondrial metabolism: D-lactate/H^+ symporter and D-lactate/pyruvate antiporter. Here, we found that as a result of D-lactate uptake and metabolism by Saccharomyces cerevisiae mitochondria, reducing equivalents were exported from the mitochondrial matrix to the cytosol in the form of malate. The rate of malate efflux, as measured photometrically using $NADP^+$ and malic enzyme, depended on the rate of transport across the mitochondrial membrane. It showed saturation characteristics (Km = 20 μM; Vmax = 6 nmol/min mg of mitochondrial protein) and was inhibited by non-penetrant compounds. These findings demonstrate that reducing equivalent export from mitochondria is due to the occurrence of a putative D-lactate/malate antiporter which differs from D-lactate/pyruvate antiporter as shown by the different inhibitor sensitivity and pH profile.

Neurodegenerative Disorders

PP-584
The substrate specificity of rat lung SSAO and its interaction with some inhibitors

S. Yabanoglu and G. Ucar
Department of Biochemistry, Faculty of Pharmacy, Hacettepe University, 06100 Ankara, Turkey.
E-mail: samiye@hacettepe.edu.tr

Semicarbazide-sensitive amine oxidase (SSAO) was purified from the crude microsomal fractions of rat lung by Cibacron Blue 3GA-agarose and Concanavalin A-Sepharose 4B affinity chromatographies with a specific activity of 5.554 nmol/min/mg of protein. The purified SSAO appeared as a single band with a molecular mass of 184 kDa in PAGE whereas SDS-PAGE under reducing conditions yielded a band of 93 kDa suggesting that the enzyme is a homodimer composed of 93 kDa subunits possibly attached by disulfide bridges. Optimum temperature and pH for the purified enzyme were found as 45 °C and 7.5, respectively. Km and Vmax values for benzylamine and methylamine were determined to be 3.65 μM and 5.6 nmol/min; and 141.5 μM and 4.18 nmol/min, respectively. The velocity of the reaction decreased with increasing substrate concentration in the case of benzylamine indicating that Michaelis-Menten enzyme behaviour was obeyed at only low concentrations for this substrate. Substrate competition studies showed that methylamine, dopamine, phenylethylamine, kynuramine and serotonin inhibited the benzylamine oxidation by SSAO. Semicarbazide and 2-Bromoethylamine (2-BEA) exhibited a suicide type of inhibition on the oxidation of benzylamine by SSAO by possibly interacting first with the enzyme to form a reversible complex with a subsequent reaction and leading this complex to the covalently bound enzyme-inhibitor adduct.

PP-585
TFE-induced aggregation of chymotrypsin: involvement of hydrophobic and electrostatic interactions

N. Rezaei-Ghaleh[1], M. Sabbaghian[1], A. Ebrahim-Habibi[1], A. Ghasemi[1] and M. Nemat-Gorgani[2]
[1]*Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran,* [2]*Stanford Genome Technology Center, Stanford University, Palo Alto, CA, USA. E-mail: nrezaeig@ibb.ut.ac.ir*

It has been demonstrated that alpha-chymotrypsin (CT) can be driven toward amyloid aggregation by addition of TFE at proper concentrations. Herein, this process is investigated in kinetic terms and the effect of solution conditions on protein aggregation (PA) examined. Kinetics of PA was followed by monitoring the relative turbidity at 350 nm. Analysis of kinetic data revealed that PA was 2nd order with respect to protein concentration. Thermal dependence of PA rate showed a positive activation enthalpy, probably related to hydrophobic interactions occurring at the rate-limiting (RL) step of PA. The effect of five salts on the aggregation process was then examined. All salts could make a long lag phase in PA and decrease both the rate and extent of PA. The slowing effect of salts could be explained by Debye-Huckel screening effect, suggesting that the transition state of PA may involve an electrostatic interaction between two oppositely charged groups. Consistently, thermal dependence of aggregation rate indicated negative activation enthalpy. The variation of aggregation rate with pH suggested that specific ionizing groups with pKs around 5 and 10 were involved during the aggregation process. It is concluded that CT aggregation at 32% TFE involves both hydrophobic and electrostatic interactions. Normally, the step involving the hydrophobic interactions is RL, but at higher ionic strengths and extremes of pH, the electrostatic interactions will be slow enough to represent the RL step.

PP-586
Expression of expanded polyglatamine domain in yeast causes death with apoptotic markers

S. Sokolov[1] and F. Severin[2]
[1]*Molecular biology department, Biology faculty, Moscow State University, Moscow, Russian Federation,* [2]*Cellular Machines, BIOTEC, TU, Dresden, Germany. E-mail: sokolss@rambler.ru*

Huntington disease is caused by specific mutations in huntingtin protein. Expansion of a polyglutamine (polyQ) repeat of huntingtin leads to the protein aggregation in neurons followed by cell death with apoptotic markers. The connection between the aggregation and the degeneration of neurons is poorly understood. We show that physiological consequences of the expanded polyQ domain expression in yeast are similar to the ones in neurons. In particular, expanded polyQ in yeast causes apoptotic changes in mitochondria, caspase activation, nuclear DNA fragmentation and cell death. Interestingly, similar to neurons, at the late stages of expression the expanded polyQ accumulates in the nuclei and seem to affect the cell cycle of yeast. We speculate that the disturbance of the cell cycle might contribute to the development of apoptotic process in both systems. Our data show that expression of the polyQ construct in yeast can be used to model patho-physiological effects of polyQ expansion in neurons.

PP-587
Folding of the Sup35 oligopeptide repeat region and [PSI+] variability

I. S. Shkundina[1], V. V. Kushnirov[1], **M. F. Tuite**[2] and M. D. Ter-Avanesyan[1]
[1]*Laboratory of Molecular Genetics, Cardiology Research Center, Moscow, Russia,* [2]*Department of Biosciences, University of Kent, Canterbury, Great Britain. E-mail: irina_shkundina@mail.ru*

S. cerevisiae [PSI+] prion is based on translation termination factor Sup35, able to switch to the self-propagating prion state. Yeast prions serve as a good model for studying the mammalian prion phenomenon. The intriguing property of all prions is the existence of their variants. Prion variants come from the ability of single polypeptide chain to fold into different prion conformations. Sup35 prion-forming domain (PrD) bears a region composed of the six copies of the oligopeptide repeat. In attempt to elucidate the molecular basis of [PSI+] variability the deletion analysis of the Sup35 PrD repeat-containing region was performed for ten [PSI+] isolates. We have shown that the minimal number of PrD repeats required to rescue [PSI+] depends on it's variant. Transmission of prion properties from wild-type Sup35 to Sup35 with the reduced number of PrD repeats weakened [PSI+] suppressor phenotype and reduced it's mitotic stability. The efficiency of [PSI+] transfer depended on [PSI+] variant and on the number of PrD repeats in Sup35 mutant protein. Progressive deletion of the PrD repeats decreased the ability of mutant proteins to co-polymerize with wild-type Sup35. Co-expression of wild-type Sup35 with several Sup35 proteins, which bear the reduced number of the oligopeptide repeats caused variant-specific [PSI+] elimination. Our data suggest that

[PSI+] variability is primarily defined by the differential folding of the Sup35 PrD oligopeptide repeats region.

PP-588
Increasing effect of the inhibitor on glutamate release in low [Na^+] media under extremal conditions

T. Borisova, N. Krisanova and N. Himmelreich
Neurochemistry Department, Palladin Institute of Biochemistry NASU, Kiev, Ukraine. E-mail: tborisov@biochem.kiev.ua

The effect of the competitive nontransportable inhibitor DL-threo-beta-benzyloxyaspartate (DL-TBOA) on the release of glutamate in Ca^{2+}-free Na^+- and NMDG-supplemented media was evaluated after exposure of rats to extremal conditions. 6 min incubation of synaptosomes with 10 μM DL-TBOA in low [Na^+] media resulted in the increase in extracellular L-[14C]glutamate level for control animals by 2.0 ± 0.5% of total accumulated label and 100 μM DL-TBOA - 3.5 ± 0.5%, respectively. The experimental data for animals subjected to centrifuge-induced hypergravity showed 4.0 ± 1.0% and 9.0 ± 2.0% increase in L-[14C]glutamate level for 10 μM and 100 μM DL-TBOA, respectively. The enhancement of the extracellular level of L-[14C]glutamate after application of DL-TBOA would be expected to connect with the inhibition of L-[14C]glutamate uptake process. It appears that DL-TBOA inhibited uptake more potently after hypergravity. The effect of DL-TBOA on depolarization-induced carrier-mediated L-[14C]glutamate release increased after hypergravity loading in Na^+- and low [Na^+] NMDG- supplemented media. 10 μM DL-TBOA-induced decrease in L-[14C]glutamate release in Na^+ – supplemented medium was 15.2 ± 2.2% in the control experiments and 26.2 ± 3.9% after loading and in low [Na^+] medium was 37.0 ± 2.5% and 45.0 ± 3.4%, respectively. DL-TBOA was demonstrated to better inhibit the transporter-mediated release of glutamate under centrifuge-induced hypergravity compared with the control.

PP-589
Transglutaminase 2 – an important molecule in the survival and the demise of the neuronal cells

G. Çolak[1], G. V. W. Johnson[2] and J. Tucholski[2]
[1]Neuroscience Graduate Program, University of Alabama at Birmingham, Birmingham, AL, USA [2]Department of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, Birmingham, AL, USA. E-mail: gcolak@uab.edu

Transglutaminase 2 (TG2) is a multifunctional member of the transglutaminase family, expressed in brain and localized mainly in cell bodies of neurons. TG2 catalyzes a [Ca^{2+}]i dependent transamidation reaction resulting in polyamination, crosslinking, or deamination of substrates. Further, TG2 binds and hydrolyzes GTP. The activities of TG2 are tightly regulated in cells by [Ca^{2+}]i, GTP and by the subcellular localization of TG2. Recent studies show that TG2 plays a major modulatory role in both survival and cell death. To examine the role of TG2 in cell death and survival, we made stable SH-SY5Y cell lines overexpressing different TG2 constructs. In cells that overexpress wild type TG2 (SH/TG2) there was a significant increase in the pro-survival cAMP-PKA-CREB pathway due to either direct activation of adenylyl cyclase and/or inhibition of PDE or by activating adenosine receptors. In contrast, SH/TG2 cells or SH-SY5Y cells transiently infected with TG2 adenovirus were significantly more sensitive to apoptotic stimuli resulting in a significant elevation of [Ca^{2+}]i. However, SH-SY5Y cells constitutively expressing inactive TG2 (C277S-TG2) or transiently infected with C277S adenovirus were significantly less sensitive to the same apoptotic stimuli. Collectively, these data indicate that depending on the stimuli and conditions, TG2 may either play a pro-survival role or facilitate the demise of the neuronal cells.

Acknowledgement: Supported by NIH grants: AG012396 to GVWJ and AG024574 to JT.

PP-590
Determinants of protein aggregation specificity

D. Bak and M. Milewski
Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland. E-mail: dbak@imid.med.pl

A model system was created to research into protein aggregation specificity. Two peptides – N-terminal huntingtin fragment and CFTR C-terminus – with well-characterized determinants of aggregation (poly Q and 9-aa ag region, respectively) were used to prepare fusions. Huntingtin fragments were of different polyQ length (18Q and 75Q). CFTR C-terminal peptides (CFTR C-ter), were modified by deleting the ag region or substituting the crucial His and Arg residues within this region. GFP reporter or HA epitope were used to visualize the constructs. Fusion proteins aggregated at higher levels in comparison to original huntingtin fragments but similarly to CFTR C-ter peptides having the functional ag region. When two different fusion proteins were co-expressed, high specificity of the aggregation was always associated with the presence of the ag region in at least one of them. However, the presence of the GFP reporter in the fusion protein containing the unmodified CFTR C-ter seemed to decrease this specificity. In conclusion, our results suggest that specificity of protein aggregation can depend on short amino acid sequences, although the 'amino acid background' can also play a significant role. Moreover, in our system GFP seems to decrease specificity of protein aggregation. It is also well-known that GFP can influence the propensity of fusion proteins to aggregate. Together these facts infer careful usage of GFP in protein aggregation research.

PP-591
Erythropoietin-conditioned-astrocyte-conditioned medium decreases ethanol neurotoxicity *in vitro*

S. Genc[1], E. Kinik[2], M. Bozdag[2], R. Kilic[2], S. Aykan[1], G. Guner[1] and K. Genc[1]
[1]Learning Resources Center Research Laboratory, Dokuz Eylul University School of Medicine, 35340 Inciralti-Izmir, Turkey, [2]Dokuz Eylul University School of Medicine, 35340 Inciralti-Izmir, Turkey. E-mail: sermingenc@hotmail.com

Epo exerts a remarkable neuroprotection against different insults both *in vitro* and in animal models of nervous system disorders including hypoxic, ischemic and excitotoxic injury. Recently, we have verified a neuroprotective action of Epo against neonatal ethanol neurotoxicity in a murine model. In the present study, we evaluated the possible neuroprotective effect of Epo in human neuroblastoma cells *in vitro*. Undifferentiated and differentiated SH-SY5Y cell cultures were exposed to ethanol in the presence or absence of Epo at various concentrations for 24 h. Cell death was assessed by trypan blue exclusion test. We found that direct exposure of cells to Epo did not decrease ethanol neurotoxicity. Conditioned medium derived from astroglial cell cultures (astrocyte conditioned medium; ACM) after Epo treatment for 24 h significantly decreased cell death induced by ethanol in

differentiated SH-SY5Y cells. We did not observe any effect in undifferentiated cell cultures. These results suggest the presence of astrocyte-derived and Epo-inducible soluble neuroprotective factor(s) in ACM and only differentiated SH-SY5Y cells are responsive to these factors. *In vivo* efficiency of Epo against ethanol neurotoxicity that was previously reported by our group might be the result of some *in vivo* interactions between neurons and astrocytes. Further screening studies are needed to clarify these factors and issues.

PP-592
Erythropoietin upregulates NF-E2 related factor 2 expression in astroglial cells *in vitro*

S. Aykan, M. Egrilmez, S. Genc and G. Guner
Learning Resources Center Research Laboratory, Dokuz Eylul University School of Medicine, 35340 Inciralti-Izmir, Turkey. E-mail: simge.aykan@deu.edu.tr

Background: Erythropoietin (Epo) is a hematopoietic growth factor and cytokine which stimulates erythropoiesis. In recent years, Epo has been shown to have important cytoprotective features in the nervous system. Epo acts in a coordinated fashion at multiple levels in the nervous system, including attenuation of oxidative stress. In this study, we assessed whether Epo regulates NF-E2 related factor (Nrf2) and targets genes expression in cultured human astroglial cells.
Methods and Results: Human astrocytes were treated with Epo (10 U/ml) for 6 h. RT-PCR was performed to measure mRNA levels of Nrf2, Keap1, GCS, NQQ. Our study showed that Epo increased Nrf2 and GSC mRNA expressions and decreased Keap 1 mRNA expression.
Conclusion: The present work demonstrates that Epo can offer novel cytoprotection through direct modulation of Nrf2 signaling.

PP-593
The localization of cellular prion protein in blood platelets

A. Brouckova, H. Glierova and K. Holada
Institute of Immunology and Microbiology, 1st Faculty of Medicine, Charles University in Prague. E-mail: Adela.Brouckova@lf1.cuni.cz

Two recent cases of probable prion disease transmission by transfusion emphasized the necessity of deeper insight into biology of cellular prion protein (PrPc) in blood. The pathological prion protein originates from PrPc, but the site of conversion in the cell is not known. As PrPc is GPI-anchored protein, localization in lipid rafts might play important role in this process. PrPc is expressed by blood platelets and is up-regulated on their surface after activation. Dynamics of PrPc up-regulation followed by flow cytometry correlated with alpha-granular P-selectin. Alpha-granular localization of PrPc was also suggested by analysis of sucrose gradient fractions after centrifugation of sonicated platelets. Surprisingly, using fluorescence microscopy we detected the intracellular PrPc in resting platelets, which did not colocalize with P-selectin. After platelet activation both proteins colocalized in intracellular compartment, but on the cytoplasmic membrane they occupied different areas. The proteinase protection assay on isolated organelles evaluated by Western blotting revealed that part of PrPc is accessible to proteinase K. Our data suggest that a part of PrPc is expressed on the outer side of organelle membrane, whereas the other part and P-selectin are on the inner side. Punctuate staining of PrPc on cytoplasmic membrane may be explained by its clustering in lipid rafts demonstrated by flotation assay on sucrose gradient.
Acknowledgement: GAČR310/04/0419, 310/05/H533.

PP-594
Primate chaperones Hsc70 (constitutive) and Hsp70 (induced) differ functionally in supporting growth and prion propagation in *Saccharomyces cerevisiae*

Y. Tutar[1], Y. Song[2] and D. Masison[3]
[1]Chemistry Department, Cumhuriyet University, Sivas, Turkey, [2]Department of Life Sciences, Liaoning University, Shenyang, China, [3]NIDDK, National Institutes of Health, Bethesda, MD, USA. E-mail: ytutar@yahoo.com

Hsp70s are highly conserved essential protein chaperones that assist protein folding and prevent protein aggregation. They have modular structures consisting of ATPase, substrate-binding and C-terminal domains. Substrate binding and release is regulated by ATP hydrolysis and nucleotide exchange, which in turn are regulated by co-chaperones. Eukaryotes have constitutive (Hsc70) and stress-inducible (iHsp70) isoforms, but their functions have not been systematically compared. Using a yeast system to evaluate heterologous Hsp70s we find primate Hsc70 supported growth but iHsp70 did not. Plant Hsc70 and iHsp70 counterparts behaved similarly, implying evolutionary conservation of this distinction. Swapping yeast and primate Hsp70 domains showed (a) the Hsc70–iHsp70 distinction resided in the ATPase domain, (b) substrate binding domains of Hsp70s within and across species functioned similarly regarding growth, (c) C-terminal domain function was important for growth, and (d) Hsp70 functions important for cell growth and prion propagation were separable. Enzymatic analysis uncovered a correlation between substrate affinity and prion phenotype and showed that ATPase and protein folding activities were generally similar. Our data support a view that intrinsic activities of Hsp70 isoforms are comparable, and functional differences *in vivo* lie mainly in complex interactions of Hsp70 with co-chaperones.

PP-595
Outlining folding nuclei in amyloidogenic proteins

S. O. Garbuzynskiy and O. V. Galzitskaya
Laboratory of Protein Physics, Institute of Protein Research RAS, Pushchino, Russia. E-mail: ogalzit@vega.protres.ru

Our theoretical approach to prediction of folding nuclei in three-dimensional protein structures is based on a search for free energy saddle points on networks of protein folding/unfolding pathways. Under some approximations, this search is performed rapidly by dynamic programming and results in prediction of Φ values, which can be compared with those found experimentally. We compared the theoretically obtained and experimental Φ values (which characterize involvement of residues in folding nuclei) for 17 proteins, where Φ values are now known for many residues. We show that the model provides good Φ value predictions for proteins whose structures have been determined by X-ray analysis, with a more limited success for proteins whose structures have been determined by NMR techniques. We have applied our method for outlining folding nuclei in eight globular proteins which are connected with amyloid diseases and in which amyloidogenic regions are now localized experimentally. Surprisingly, for some of the proteins the amyloidogenic fragments intersect with the regions of the largest predicted Φ values. This can

indicate that the part of the folding nucleus can be involved in amyloid formation.

Acknowledgements: This work was supported by the RAS program 'Physical and Chemical Biology', by the Russian Foundation for Basic Research, by the INTAS grant and by Howard Hughes Medical Institute.

PP-596
Neuronal survivle in β-amyloid induced neurodegeneration

K. Yenkoyan[1], M. Aghajanov[1] and L. Mkrtchyan[2]
[1]*Biochemistry, Yerevan State Medical University after M. Heratsi,* [2]*Pathology, Yerevan State Medical University after M. Heratsi. E-mail: enkoyan@yahoo.com*

The toxicity of Aβ in Alzheimer's disease (AD) leads to the widespread neuronal/neuritic dysfunction and death of brain cells with progressive deficit in neurotransmitters and growth factors such as insulin-like growth factor (IGF) and nerve growth factor (NGF). The goal of our investigation was to show the regulatory role of a new proteoglycan of embrional genesis (PEG) on the level of IGF and NGF in cerebral cortex and hippocampus, as well as to reveal the histological changes in those areas in Aβ-induced neurodegeneration. In Aβ-induced neurodegeneration IGF level significantly increased in cerebral cortex and hippocampus, which is apparently result of neuronal survival. In contrast with IGF, the level of NGF decreased. The preliminary injection of PEG prevents the changes in IGF and NGF levels. The morphological data testify the typical picture of neurodegeneration in cerebral cortex and hippocampus under the effect of Aβ. During the injection of PEG the degree of expression of morphological changes in cerebral cortex and hippocampus was loosely revealed. It should be especially mentioned the great proliferation of the ependimal cells (which are the stem cells for brain) in all experimental groups with administration of PEG. This phenomenon can play a crucial role in the action of PEG. Based on the researched data it can be concluded that a new proteoglican play an effective 'survival' and regulatory effect in Aβ-induced neurodegeneration.

PP-597
Prevention of thermal aggregation of glutamate dehydrogenase by polyamines

M. Sabbaghian[1], N. Rezaei-Ghaleh[1], A. Ebrahim-Habibi[1], A. Ghasemi[1] and M. Nemat-Gorgani[2]
[1]*Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran,* [2]*Stanford Genome Technology Center, Stanford University, Palo Alto, CA, USA. E-mail: sabbaghian@ibb.ut.ac.ir*

Proteins tend to form inactive aggregates at high temperatures. Protein aggregation is a major problem in large scale production of recombinant proteins, as well as in living cells, where it may lead to the appearance of fatal diseases. One of the main approaches used to prevent protein aggregation is the addition of small molecules to the solution. Polyamines such as putrescine, spermidine and spermine are relatively simple structures composed of multivalent amines. It has been previously shown that polyamines effectively prevent thermal aggregation of hen lysozyme while they accelerate the aggregation and fibrillization of alpha-synuclein in the present study, we investigated the effect of polyamines on thermal aggregation of glutamate dehydrogenase, a well known allosteric enzyme. Heat treatment at 50 °C for 40 min resulted in extensive aggregation of the protein. Putrescine used at 50 mM concentration diminished the extent of heat-induced aggregation, whereas spermine and spermidine, added at the same concentration, were clearly more effective and total prevention of aggregation was achieved. Moreover, both spermine and spermidine were effective in solubilizing previously-formed aggregated structures. When these polyamines were added to heated protein solution at various stages of the aggregation process, the extent of aggregation was dramatically reduced. These results confirm that polyamines may be utilized as suitable additives to prevent protein aggregation.

PP-598
Calmyrin1 expression and Ca-independent interaction with Presenilin2 in forebrain

A. Sobczak[1], M. Blazejczyk[1], C. Spilker[2], M. R. Kreutz[2], J. Kuznicki[1] and U. Wojda[1]
[1]*Laboratory of Neurodegeneration, International Institute of Molecular and Cell Biology, Warsaw, Poland,* [2]*Department of Neurochemistry, Leibniz Institute for Neurobiology, Magdeburg, Germany. E-mail: adams@iimcb.gov.pl*

Deregulation of Ca-signaling in brain contributes to pathogenesis of Alzheimer disease (AD). Calmyrin1 (CaMy1, CIB1) is a member of Neuronal Calcium Sensor protein family. Interaction of CaMy1 and Presenilin2 (PS2, a membrane component of gamma-secretase complexes responsible for generation of toxic beta-amyloid peptides) was proposed to represent a potential link between deregulation of Ca-homeostasis and AD. To elucidate this possibility we analysed Ca-dependence of CaMy1/PS2 interaction by affinity chromatography, pull-down and immunoprecipitation methods. CaMy1 proved to bind specifically to PS2 and not PS1 in Ca-independent manner. Since CaMy1 is myristoylated *in vivo*, we also analysed whether a Ca-myristoil switch in CaMy1 might provide another mechanism of its Ca-dependent translocation to cellular membranes for the interaction with PS2. In transfected cells Ca-dependent translocation of a positive control, VILIP-GFP was confirmed, but no translocation was observed in case of CaMy1-GFP. In addition, immunocytochemical stainings of rat brain with antiPS2 and antiCaMy1 antibodies and western blotting of subcellular fractions demonstrated limited overlap of CaMy1 and PS2 in forebrain neurons. We conclude that CaMy1 does not contribute significantly as a Ca-sensor in transduction of Ca-signals to PS2 in forebrain.

Acknowledgment: Supported by KBN PBZ-MIN-001/P05/09, BMBF 612 01GZ0307, and EU LSHM-CT-2003-503330 (APOPIS).

PP-599
Retinoic acid isomers protect hippocampal neurons from amyloid-β induced neurodegeneration

M. Sahin[1], S. Berker Karauzum[2], G. Perry[3], M. A. Smith[3] and Y. Aliciguzel[1]
[1]*Department of Biochemistry, Faculty of Medicine, Akdeniz University, Antalya, Turkey,* [2]*Department of Medical Biology and Genetics, Faculty of Medicine, Akdeniz University, Antalya, Turkey,* [3]*Institute of Pathology, Case Western Reserve University, Cleveland, OH, USA. E-mail: msahin@akdeniz.edu.tr*

Attenuating amyloid-β mediated neurodegeneration is of major therapeutic consideration in the potential treatment of Alzheimer disease. Previously, we found that a high dietary consumption of retinoic acid was associated with a reduced incidence of Alzheimer disease. Therefore, in this study, we investigated whether amyloid-β mediated cell death in primary hippocampal neurons could be prevented by retinoic acid isomers. Our results suggest that retinoic acid isomers, including all-trans retinoic acid, 9-cis

retinoic acid, and 13-cis retinoic acid, may play an important role in protecting neurons from amyloid-β-induced cell death. Retinoic acid may therefore afford a novel therapeutic mechanism for the treatment and prevention of Alzheimer disease.

PP-600
Acylation of LYS residues prevents thermal aggregation of glutamate dehydrogenase

A. Ghasemi[1], M. Sabbaghian[1], N. Rezaei-Ghaleh[1], D. Morshedi[1], A. Ebrahim-Habibi[1] and M. Nemat-Gorgani[2]
[1]*Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran,* [2]*Stanford Genome Technology Center, Stanford University, Palo Alto, CA, USA. E-mail: a.ghasemi@ibb.ut.ac.ir*

In the last decade, protein aggregation has moved beyond being a relatively ignored area of protein chemistry to become a key topic in medical and biological sciences and, consequently, prevention of aggregation has gained much attention. Chemical modification of proteins gives them new properties with regard to specificity and stability. In the present study, modification of lysine residues in glutamate dehydrogenase was carried out using citraconic anhydride and the number of modified residues was determined employing fluorescamine. Due to the presence of 192 lysine residues in this enzyme, we expected to observe extensive changes in its structure-function upon modification. By increasing the amount of citraconic anhydride, modified enzyme preparations were obtained containing 40, 60 and 104 acylated lysine residues with gradual loss of catalytic activity concomitantly. While extensive aggregation was observed by heat treatment of the native structure at 50 °C (pH 8, 30 min), no aggregates could be detected for any of the modified forms. Moreover, changes in the structural properties of the protein were suggested by intrinsic fluorescence studies and some preliminary circular dichroism data. It is clear that acylation of Lysine residues prevents thermal aggregation of glutamate dehydrogenase.

PP-601
Association of apolipoprotein E genotype with late onset Alzheimer's disease in Denizli, Turkey

H. Aybek[1], D. Aslan[1], F. Ercan[1] and T. Şahiner[2]
[1]*Department of Biochemistry, Faculty of Medicine, Pamukkale University, Denizli, Turkey,* [2]*Department of Neurology, Faculty of Medicine, Pamukkale University, Denizli, Turkey. E-mail: hulyaaybek2000@yahoo.com*

Apolipoprotein E (Apo E) polymorphism is the most prevalent genetic risk factor associated with late onset Alzheimer's Disease (AD). The Apo E gene shows a polymorphism with three common alleles (named E2, E3, and E4), which produce three homozygous (E2/E2, E3/E3 and E4/E4) and three heterozygous (E2/E3, E2/E4, E3/E4) genotypes; with E3 being the most frequent among general population. Several groups have reported that the E4 allele frequency is higher in AD patients than in age matched controls. In the present study, we analysed the Apo E4 allele frequency of patients with late-onset AD that were listed in the Society of Alzheimer's Disease in Denizli, Turkey. 62 patients with AD (mean age 73.3 ± 6.8 years; 37 females, 25 males) and 56 healthy elderly people (mean age 70.8 ± 7.7 years; 26 females, 30 males) enrolled in the study. The Apo E gene polymorphism was detected by PCR-RFLP analysis. The Apo E genotype frequency in AD and healthy control are E2E3 13%, 14%; E3E3 48%, 68%; E3E4 33%, 18%; E2E4 3%, 0%; E4E4 3%, 0% respectively. Patients with at least one allele E4 were more frequent in patients with AD (21%) than controls (9%) ($P = 0.01$). We confirmed that the Apo E4 allele occurs more frequently in late onset AD.

PP-602
Pamam dendrimers' influence on the formation and disruption of amyloid fibrils

B. Klajnert[1], M. Cortijo-Arellano[2] and J. Cladera[2]
[1]*Department of General Biophysics, University of Lodz, Lodz, Poland,* [2]*Department of Biochemistry and Molecular Biology, Universitat Autonoma de Barcelona, Bellaterra, Spain. E-mail: aklajn@biol.uni.lodz.pl*

Inhibition of fibril assembly is a potential strategy for therapeutic intervention in neurodegenerative disorders such as prion and Alzheimer's diseases. It has recently been shown that polyamidoamine and polypropyleneimine dendrimers are promising candidates in the treatment of prion diseases. These relatively novel macromolecules are globular and are characterized by a densely packed surface. Dendrimers are built in a cyclic manner from a central core molecule that is surrounded by layers of branched monomers. The more layers are attached, the higher the so-called generation is. As generation increases, the amount of surface groups increases too, so the shape of a dendrimer changes from flat and ellipsoidal to globular. We have used the third, fourth and fifth generation of polyamidoamine dendrimers in order to study how dendrimers' structure and size determine their effect on amyloid aggregation. Amyloid fibrils were produced *in vitro* under aggregation conditions (pH 5.5 and the presence of heparin). The aggregation of the prion peptide PrP 185–208 and Alzheimer's peptide Aβ 1–28 were monitored using a dye thioflavin T (ThT) which fluorescence depends on the presence of amyloid structures. The fluorescence results were complemented with electron microscopy. The results show that the higher the dendrimer generation the larger the inhibition of the aggregate formation process and the more effective in the disruption of already existing fibrils.

PP-603
Mutational effects on brain spectrin tetramerization

A. Sevinc and L. W. -M. Fung
Department of Chemistry, University of Illinois at Chicago, Chicago, IL, USA. E-mail: lfung@uic.edu

Spectrin, a major constituent of the (cyto) skeleton in cells, forms a sub-membrane filamentous network through the tetramerization of heterodimers of α- and β-spectrin subunits (Spα and Spβ, respectively). It has been found that the N-terminal region of Spα associated with the C-terminal region of Spβ in spectrin tetramers. Recombinant model peptides of non-erythroid (brain) α-spectrin (SpαII-1-359) and β-spectrin (SpβII-1689-2137) have been used to characterize the interaction between SpαII and SpβII during tetramerization. Previous studies reveal that residue 22 of SpαII-1-359 is critical in SpαII-1-359 association with SpβII-1689-2137. It is interesting to note that a mutation at this position in erythroid spectrin has been shown to produce clinical symptoms in patients. We have replaced various amino acids at this position (V22A, V22D, V22M, V22F and V22W) using site directed mutagenesis techniques. Structural and functional features of these new peptides have been studied to provide insights on mutational effects on the association of SpαII with SpβII.

PP-604
A potential role of sulfite oxidase deficiency in xenobiotic metabolism

B. Tutuncu[1], A. Sen[1], V. Kucukatay[2] and **B. Sahin**[3]
[1]*Department of Biology, Faculty of Arts and Sciences, Experimental Research Unit, Pamukkale University, 20020 Denizli, Turkey,* [2]*Department of Physiology, Faculty of Medicine, Experimental Research Unit, Pamukkale University, 20020 Denizli, Turkey,* [3]*Faculty of Medicine, Experimental Research Unit, Pamukkale University, 20020 Denizli, Turkey. E-mail: begumtutuncu@mynet.com*

Sulfite oxidase (SOX) is an essential enzyme in the pathway of the oxidative degradation of sulfur containing amino acids. It protects cells from toxicity of sulfite which has both endogenous and exogenous provenances. Factors like species, sex, age, diet and genetic polymorphism alter xenobiotic metabolizing enzymes (XME) levels and causes considerable differences in biotransformation ability of individuals. The present project investigates the role of SOX deficiency on xenobiotic metabolism, which is the first report on changes of XME in SOX deficiency. In this study, male Wistar albino rats were used and SOX deficiency was produced by the administration of low molybdenum diets with concurrent addition of 200 ppm W to drinking water. First, hepatic SOX activity in deficient groups was measured to confirm SOX deficiency. Then, cytochrome b5 reductase (b5 RED), ethoxyresorufin O-deethylase (EROD), erythromycin N-demethylase (END), glutathione S-transferase (GST), N-nitrosodimethylamine N-demethylase (NDMA-ND) and penthoxyresorufin O-deethylase (PROD) activities were determined. Our results clearly demonstrated that SOX deficiency significantly elevated END and NDMA-ND activities while decreasing EROD and GST activities. No significant changes were observed with b5 RED and PROD activities. These alterations in XME can contribute to the varying susceptibility and response of these individuals to different drugs and/or therapeutics used for treatments.

PP-605
Beta-sheet structured oligomers of Alzheimer's beta-amyloid peptide perturb phosphatidylcholine model membranes

M. R. R. de Planque[1], S. A. Contera[1], V. Raussens[2], D. T. S. Rijkers[3], J. F. Ryan[1], F. Separovic[4] and A. Watts[5]
[1]*Bionanotechnology IRC, Physics Department, University of Oxford, Oxford, UK,* [2]*Structure and Function of Biological Membranes Laboratory, Free University of Brussels, Brussels, Belgium,* [3]*Department of Medicinal Chemistry, Utrecht University, Utrecht, The Netherlands,* [4]*School of Chemistry, University of Melbourne, Melbourne, Australia,* [5]*Department of Biochemistry, University of Oxford, Oxford, UK. E-mail: m.deplanque1@physics.ox.ac.uk*

Aggregation intermediates on the pathway from monomeric to fibrillar Aβ appear to be the most neurotoxic species in Alzheimer's disease. Unstructured monomeric Aβ binds to charged membrane surfaces, leading to membrane-disrupting structures with a high β-sheet content, but soluble beta-structured oligomers of Aβ are more potent membrane perturbers. We have studied Aβ (1–40) in uncharged bilayers of phosphatidylcholine, the most simple model membrane. Peptides and lipids were mixed in organic solvent and after solvent removal the mixed peptide-lipid film was hydrated, resulting in multilamellar proteoliposomes. No significant effect of Aβ on acyl chain order was observed with solid-state NMR. Although Aβ is primarily helical in the solvent mixture, CD and ATR-FTIR demonstrate that the peptide is present as β-sheets in unilamellar vesicles and aligned multilayers. When these vesicles are fused into an uncharged planar bilayer, electrical recordings show a significant but irregular increase in bilayer conductivity. The same vesicles were used to form a supported bilayer on mica and were imaged by AFM: initially interfacial packing defects were present but in time the entire bilayer disintegrated. We conclude that in PC bilayers Aβ (1–40) cannot form discrete ion channels, has a high propensity to assemble into beta-structured oligomers, and that these are potent perturbers of membrane integrity, irrespective of membrane surface charge.

PP-606
Molecular diagnosis of autosomal dominant spinocerebellar ataxias with CAG expansions in Turkey

N. Ersoy[1], E. Soydan[2] and A. N. Başak[2]
[1]*Department of Molecular Biology and Genetics, Halic University, Istanbul, Turkey,* [2]*Department of Molecular Biology and Genetics, Bogazici University, Istanbul, Turkey.*
E-mail: nagehanersoy@yahoo.com

Autosomal Dominant Spinocerebellar Ataxias (ADCAs) are a clinically and genetically heterogeneous group of neurodegenerative disorders. Among ADCAs, CAG/polyQ expansion mutations in the exons constitute the largest group, these include SCA 1, 2, 3, 6, 7 and 17. The prevalence of ADCAs with CAG expansions varies between populations, therefore establishment of molecular diagnostic criteria requires the investigation of normal and expanded CAG size ranges for each population. In this study, normal repeat ranges at the SCA1 and SCA2 loci were identified to be 26–34 and 21–24 CAGs, respectively. In addition, 146 individuals with cerebellar atrophy were subjected to molecular analysis at the above mentioned six ADCA loci, using capillary electrophoresis. Out of 38 patients with an apparent autosomal dominant inheritance, four were given the molecular diagnosis of SCA1, and 14 were identified to carry SCA2; others were shown to carry normal sized repeat alleles at all six polyQ loci. Negative correlation between the age at onset and repeat size were detected both in SCA1 and SCA2 patients; furthermore mutant alleles were shown to be expanded upon transmission in SCA2. In the remaining patient population, polyQ mutations were excluded. The results imply that the Turkish population is very much similar to the Italian population with respect to SCA prevalence, in whom SCA1 and SCA2 are the only ADCAs described, and the rest of the clinically diagnosed cases are genetically undefined.

PP-607
Microtubule severing protein, spastin is regulated by microtubule-associated proteins

S. Korulu and **A. Karabay**
Molecular Biology-Genetics and Biotechnology Program, Istanbul Technical University, Istanbul, Turkey. E-mail: korulu@itu.edu.tr

Spastin, microtubule (MT) severing protein leads to Hereditary Spastic Paraplegia disorder when mutated. It is especially important in nerve cells for breaking long MTs into short pieces so that they can move through the axon. It is essential that the activity of spastin has to be regulated so that the entire MT array is not severed continuously into subunits .Our hypothesis is that 'microtubule-associated proteins (MAPs) may regulate the severing of MTs by restricting the access of spastin to the MT lattice. The regulation is controlled locally in neurons by signaling cascades that affect the binding of relevant MAPs to the MT'. In this study, we investigated whether particular MAPs have the capacity to regulate severing activity. When spastin was overexpressed in fibroblasts, the microtubules broke into short pieces. However,

when the spastin was overexpressed together with MAP2c, the severing activity was markedly reduced; MTs were partially protected against the severing. In MAP1b overexpressed fibroblasts, no protection was observed. It is probably because of the differences in MT binding domains. We conclude that certain MAPs such as MAP2c protect MTs from being severed, while others like MAP1b do not. Especially MAP2c has a critical, regulative role in determination of MT length by spastin.

PP-608
Microtubule organization in neuronal regeneration by katanin

A. Yildiz and **A. Karabay**
Molecular Biology-Genetics and Biotechnology Program, Istanbul Technical University, Istanbul, Turkey. E-mail: yildizay@itu.edu.tr

Katanin is a heterodimeric protein that consists of 60 and 80 kDa subunits and severe microtubules (MT) by hydrolyzing ATP. P60 has the enzymatic activity to severe MTs whereas p80 has a role in localization of the protein complex. In mitotic cells, katanin reorganizes MTs, while it is responsible for elongation and elaboration of axons and possibly branching of dendrites in neurons. Neurons are terminally differentiated, hence their injuries cause irreversible paralysis of body parts. Since neurons cannot divide, if possible at all, nerve regeneration can only be achieved by reorganization of the neuronal cytoskeleton, specifically new branching of axons and dendrites this mechanism requires severing proteins, like katanin. For this purpose, chicken brain was dissected and total RNA was isolated. cDNA library was constructed by using oligo d(T) primers. Specific primers were used to perform PCR to clone p60 and p80. DNA sequences of clones were confirmed by sequencing. Cloned p60 and p80 were ligated into pEGFP-N1 expression vector. The achieved constructs will be transfected into chicken embryo primary neurons such as dorsal root ganglions, as the most suitable system to determine the function of katanin is the neurons that do not have the intrinsic branching feature. Activities of katanin dimer in non-branching DRGs will elucidate the possible functions of katanin in regeneration of neurons.

PP-609
Kif 15 interacting cytoskeletal proteins

A. Dilsizoglu[1], P. Baas[2] and **A. Karabay**[1]
[1]Molecular Biology-Genetics and Biotechnology Program, Istanbul Technical University, Istanbul, Turkey, [2]Neurobiology and Anatomy Department, College of Medicine, Drexel University, PA, USA. E-mail: dilsizoglu@itu.edu.tr

Kif15, member of kinesin motor protein family, opposes to the forces generated by other motors that move microtubules (MTs) to maintain the separation of spindle poles in mitotic cells. Kif15 motor domain interacts with MTs, while its tail domain interacts with other MTs or actin filaments, depending on the phase of mitosis. Kif15 is also found in neurons, but there its function is still a mystery. Many neurons are born far from their ultimate destinations and have to migrate to their target places. When neurons arrive to their target places, the forces generated by other motors that affect migration, have to be neutralized. It is thought that Kif15 crosslinks MTs and microfilaments (MFs), and as a result they oppose the capacity of other motors to generate independent MT movements. The aim of the project is to specifically identify the domains of Kif15 that interact with the above mentioned cytoskeletal elements, and ultimately to understand the molecular mechanisms of neuronal diseases, like Lissencephaly caused by abnormalities during migration. For this reason, adult rat cDNA was used as template. Motor and tail domains of Kif15 were cloned by using sequence-specific primers. Sequences of the clones were verified and then subcloned into fluorescent expression vectors. These vectors will be transfected into neuronal cells for identification of the interacting cytoskeletal elements.

PP-610
Relations of microtubules with LDH: clue for neurodegenerative diseases

M. Akkor, B. Binay, N. G. Karaguler and **A. Karabay**
Molecular Biology-Genetics and Biotechnology Program, Istanbul Technical University, Istanbul, Turkey. E-mail: akkor@itu.edu.tr

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by extracellular beta-amyloid-containing plaques, intracellular neurofibrillary tangles (NFT), reduced synaptic density and neuronal loss in selected brain areas. Abnormalities of cerebral metabolism contribute to the pathophysiology of AD. The expression level and the activity of Lactate dehydrogenase (LDH) increase significantly in several parts of AD brains, suggesting that LDH is a sensitive enzyme to neurodegeneration. Neuronal tau, a microtubule (MT)-associated protein, aggregates and forms the major components of NFTs. Neuronal tau functions a chaperone-like protein towards the enzymes of carbohydrate metabolism. Another study suggested that the simulated ischemia-induced MT disorganizations could rather be an early ultrastructural correlate of the cellular reaction to the metabolic challenge. LDH gene from *Bacillus stearothermophilus* was inserted into pEGFP-C2 (Green Fluorescent Protein) expression vector. In this study, the relation of LDH with microtubules will be analysed using the fluorescent protein as a marker in cell culture.

PP-611
Study on the neurotoxic mechanism of prion using the protease-resistant protein and the synthetic peptide 106-126

B. ELMoualij, I. Dupiereux, W. Zorzi, D. Zorzi, O. Pierard and E. Heinen
Institute of Pharmacy-CHU, Avenue de l'Hôpital, 1, Sart Tilman. E-mail: b.elmoualij@ulg.ac.be

Prion diseases are caused by the accumulation of an abnormal isoform of the cellular prion protein (PrPc), named PrPsc (PrP scrapie). The aim of the present work was to examine the implication of the lipid membranes and PrPc in the toxic mechanism induced by PrPsc. For this, we used a peptide corresponding to the 106-126 PrP sequence of PrP, bovine and human resistant PrP(PrPres). The effect of PrP 106-126, human (CJD) and bovine (BSE)PrPres was investigated on human neuroblastoma SH-SY5Y expressing basal and overexpressing murine PrPc(wtPrP). We show by MTS and ATP tests that PrPc expression does not modulate the toxicity of the peptide. Moreover, we investigated the effect of this peptide on a non-neuronal model, rabbit kidney epithelial A74 cells, which expresses a doxycycline-inducible murine PrPc gene. We show that PrP 106-126 does not exert any toxic effect on this cell line in the presence or absence of doxycycline. These results show that the PrP 106-126 induced cell death is independent of PrPc expression levels. It seems to rather act via an interaction with the lipid components of the plasma membrane as strengthened by our results showing the differential susceptibility of neuronal and non neuronal cell lines which differ significantly by their fatty acid membrane composition. In conclusion, we reinforce the hypothesis that the toxicity induced by PrPsc could be besides residual endogenous PrPc a direct interaction with lipid components of cell membranes.

PP-612
The effect of L-Carnitine on SOD, GSH and MDA following experimental spinal cord injury in rats

N. Canacankatan[1], L. Kayrin[1], **T. Erman**[2] and F. Karayandi[2]
[1]*Department of Biochemistry, Medicine Faculty, Cukurova University, Adana, Turkey,* [2]*Department of Neurosurgery, Medicine Faculty, Cukurova University, Adana, Turkey.*
E-mail: ncanacankatan@yahoo.com

Primary traumatic spinal cord injury (SCI) is defined as the injury occurring at the time of the trauma. The metabolic and biochemical processes lead to the secondary injury in the following hours after the primary trauma. Free radicals and ischemic reperfusion injury are two factors declared to contribute to the secondary injury. In this study we aimed to investigate the effect of L-Carnitine on superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA) and histopathological changes following experimental spinal cord injury in rats. Male Wistar albino rats, weighing 280 g were randomly allocated to four groups. Group I ($n = 10$) was control. Rats underwent six-segment laminectomy in Group II ($n = 10$). In Group III ($n = 10$) rats underwent six-segment laminectomy and SCI was produced by extradural compression of the exposed cord. The same procedures were applied to 10 rats in Group IV, but they also received one (100 mg/kg/day) i.p. injection of L-Carnitine hydrochloride. As a result, SOD enzyme activity and the level of MDA significantly decreased, while the GSH content significantly increased in the Group IV compared to the Group III. The histopathological findings revealed the preservation of spinal cord structure in the Group IV. In conclusion, L-Carnitine might reduce secondary structural changes in damaged rat spinal cord tissue, by a possible mechanism; impeding the production of free radical and ischemic reperfusion injury.

PP-613
Urinary 8-hydroxydeoxyguanosine levels and serum paraoxanase activity in Alzheimer's disease

O. Zengi[1], A. Karakaş[1], M. Şeneş[1], U. Ergün[2], L. İnan[2], G. Saydam[1] and D. Yücel[1]
[1]*Department of Clinical Biochemistry, Ankara Education and Research Hospital, Ministry of Health, Ankara, Turkey,* [2]*Department of Neurology, Ankara Education and Research Hospital, Ministry of Health, Ankara, Turkey.*
E-mail: oguzhanzengi@yahoo.com

In this study urinary 8-hydroxydeoxyguanosine/creatinine ratio (8-OhdG/creatinine), serum paraoxanase (PON1) and PON1/HDL cholesterol ratio were measured in patients with Alzheimer's disease (ages = 76 ± 8; 10 male, 11female). All the analytes were also measured in 21 age- and sex-matched healthy controls (ages = 81 ± 7; 11 male, 9 female). 8-OhdG/creatinine ratio was significantly increased in patients when compared with controls ($P = 0.045$). Serum PON1 activity was decreased in patient group as compared with controls ($P = 0.003$). PON1/HDL cholesterol ratio of patient group was also decreased when compared to control group, but the differences between the groups was not statistically significant ($P > 0.05$). There were no statistically significant differences between the groups for the concentrations of cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides ($P > 0.05$). Urinary 8-OhdG concentrations were measured by an HPLC-ECD method after solid phase extraction of urine samples. Performance characteristics of the method used were determined. A manual colorimetric method for PON-1 activity was applicated to a chemistry analyser. The results show that 8-OhdG/creatinine ratio can be used for the determination of oxidative DNA damage in Alzheimer's disease. Additionally, decreased serum PON1 activity may be an indicator of increased oxidative damage. Performance characteristics of the methods used are sufficient for clinical laboratories.

PP-614
Identification of three novel arylsulfatase A mutations as a cause of metachromatic leukodystrophy

E. Önder[1], İ. Sinici[1], F. M. Sönmez[2], M. Topçu[3] and H. A. Özkara[1]
[1]*Department of Biochemistry, Faculty of Medicine, Hacettepe University, Ankara, Turkey,* [2]*Department of Pediatrics, Faculty of Medicine, Karadeniz Technical University Trabzon, Turkey,* [3]*Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara, Turkey. E-mail: ozkara@hacettepe.edu.tr*

Metachromatic Leukodystrophy (MLD) is a lysosomal storage disorder caused by the deficiency of arylsulfatase A (ASA) or saposin B. This leads to the accumulation of sulfatide, which results in severe demyelination. In this study, clinically suspected patients were diagnosed as MLD by enzyme analysis using para-nitrocathecolsulfate as substrate. Eight exons and flanking regions of ASA gene of patients were amplified by PCR and then subjected to Single Stranded Conformational Polymorphism (SSCP) analysis. PCR products of suspicious exons in SSCP were purified and sequenced. DNA sequencing revealed three novel disease-causing mutations, two missense mutations (1567G to A, 307Glu to Lys in exon 5 which is together with a pseudodeficiency allele 2160C to T, 391Thr to Ser in exon 7; 1602G to T, 318Trp to Cys in exon 5) and one insertion mutation (G insertion at 1742 in exon 6). These 3 mutations are in highly conserved structural elements region of the ASA protein. Thus, missense mutations 307Glu to Lys in exon 5 and 318Trp to Cys in exon 5 probably change the active site conformation by disrupting the 6th alpha helix and the 12th beta-sheet structure of the ASA protein, respectively and cause deficiency in enzyme activity. Insertion mutation in exon 6 causes frameshift of the codons and premature termination after 22 aminoacids and probably leads to degradation of enzyme. This study provides the molecular basis for understanding the mechanism underlying MLD in Turkish patients.

PP-615
Specific inhibitors of the CAM/Ca^{2+}-regulated FKBP38 are potential drugs for the treatment of neurodegenerative diseases

F. Edlich[1], M. Weiwad[1], F. Jarczowski[1], D. Wildemann[1], F. Erdmann[1], S. Kilka[1], G. Jahreis[1], C. Lücke[1], F. Striggow[2] and G. Fischer[1]
[1]*Max-Planck Research Unit for Enzymology of Protein Folding, Halle, Germany,* [2]*KeyNeurotek AG, Zenit Technology Park, Leipziger Str. 44, D-39120 Magdeburg, Germany.*
E-mail: edlich@enzyme-halle.mpg.de

FKBP-type peptidyl prolyl cis/trans isomerases (PPIases) are folding helper enzymes involved in neurodegeneration, steroid receptor signalling, Ca^{2+} homeostasis, and immunosuppression. Our results demonstrate that the inactive human FKBP38 is

activated by formation of a FKBP38/Calmodulin/Ca^{2+} complex upon intracellular Ca^{2+} rise. The PPIase-active complex interacts with the apoptosis regulator Bcl-2 and thereby interferes with Bcl-2 function in neuronal survival. The Bcl-2/FKBP38 interaction is disrupted by application of FKBP38-specific inhibitors. Based on a comparative analysis of binding constants of various FKBP ligands towards the characterized FKBP activities, we identified a cycloheximide derivative to discriminate between FKBP38 and other human FKBP activities with up to 80-fold higher affinity to the CaM/Ca^{2+}-dependent PPIase. Thus, we synthesized N-(N', N'-dimethylcarboxamidomethyl)-cycloheximide (DM-CHX) with improved FKBP38 specificity and metabolic stability that was applied in a rat ischemia model. DM-CHX caused (a) neuronal protection, (b) neural stem cell proliferation, (c) neuronal differentiation, and (d) improved motor behaviour post insult. Through its interaction with Bcl-2, the CaM/Ca^{2+}-activated FKBP38 is therefore a regulator of neuronal cell death and proliferation, making FKBP38 inhibition by low molecular weight ligands or siRNA to a promising strategy for the treatment of acute and/or chronic neurodegenerative diseases.

PP-616
Effects of dihydrotestosterone on steroid 5α-reductase isozymes in the adult female rat brain.

J. M. Torres[1], P. Sánchez[2], P. Gavete[2], P. Vilchez[3] and **E. Ortega**[1]

[1]Department of Biochemistry and Molecular Biology, Faculty of Medicine. Institute of Neurosciences, University of Granada, Granada, Spain, [2]Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Granada, Granada, Spain, [3]Biochemistry Unit, San Cecilio University Hospital, Granada, Spain. E-mail: esortega@ugr.es

Neurosteroids are steroids produced within the central nervous system (CNS) of vertebrates which are involved in the regulation of stress responses, anxiety, and other important neurobiological processes and neuropsychiatric disorders. The enzyme 5a-reductase (5a-R) (EC 1.3.99.5) is the key enzyme in the biosynthesis of 3a, 5a-reduced neurosteroids. 5a-R exists as two isoforms, 5a-R type 1 (5a-R1) and 5a-R type 2 (5a-R2). It is known that neurological disorders are more frequent in the elderly, when the levels of sexual hormones are low. We demonstrated that both 5a-R isozymes are present in the CNS of the adult male rat and are regulated in an opposing way by androgens. In this work, we studied the effects of testosterone (T) and dihydrotestosterone (DHT) on mRNA levels of both 5a-R isoforms in the prefrontal cortex of the adult female rat by one-step quantitative RT-PCR coupled with laser-induced fluorescence capillary electrophoresis (LIF-CE). Our results demonstrate that 5a-R2 mRNA is slightly regulated by T and DHT. Surprisingly, 5a-R1 mRNA is not regulated by T in the intact female, whereas it is very positively regulated by DHT, a more potent androgen than T. These data indicate the great sexual dimorphism in the CNS with respect to both 5a-R isozymes, and suggest a crucial role of DHT in the sexual dimorphism of the CNS in the female. These results open up a new research line that may lead to a better understanding of the physiology of the CNS.

PP-617
Proteomic studies of pesticide-induced neurodegeneration in differentiating neuronal cell lines

W. Harris[1], J. Flaskos[2], M. Sachana[2] and A. Hargreaves[1]

[1]School of Biomedical and Natural Sciences, Nottingham Trent University, UK, [2]Laboratory of Biochemistry and Toxiciology, Aristotelean University of Thessaloniki, Greece. E-mail: wayne.harris@ntu.ac.uk

Increased pesticide use over the past few decades has led to increased incidence of occupational exposure, environmental pollution and accumulation in the food web. It has also heightened concern about the effects of low level exposure in man and livestock. We have developed a cellular system to study the neurodegenerative effects of pesticides. This model is being used in the present study to identify proteomic changes following exposure to the sheep dip pesticides diazinon and cypermethrin. Diazinon but not cypermethrin inhibited the growth of axon-like processes by differentiating N2a cells in a dose dependent manner. Probing of Western blots of cell extracts with anti-cytoskeletal antibodies indicated changes in the levels of neurofilament proteins but no overall change in the levels of tubulin in diazinon- treated cell extracts. Analysis of cell extracts by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) revealed that a number of polypeptides were selectively up-regulated or down-regulated in the presence of both pesticides either individually or in combination. Current work involves the identification of polypeptides of interest by MALDI-TOF mass spectrometry.

Reference:

1. Flaskos J, McLean WG, Fowler MJ, Hargreaves AJ. Tricresyl phosphate inhibits the formation of axon-like processes and disrupts neurofilaments in cultured mouse N2a and rat PC12 cells. Neuroscience Letters 1998; 242: 101–104.

PP-618
The effect of erythropoietin on caspase-8 activation induced by amyloid β in PC12 cells

S. Kizildag[1], S. Genc[2], S. Kizildag[3], M. Sakizli[3] and M. Fadiloglu[1]

[1]Department of Biochemistry, Medical Faculty, Dokuz Eylul University, Izmir, Turkey, [2]Research Laboratory, Dokuz Eylul University, Izmir, Turkey, [3]Department of Medical Biology and Genetics, Medical Faculty, Dokuz Eylul University, Izmir, Turkey. E-mail: servet.kizildag@deu.edu.tr

Objectives: Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by central nervous system degeneration and neuronal loss. It has been shown that in various models erythropoietin (EPO) has a neuroprotective effect. Evaluation of the impact of EPO administration on caspase cascade and the neuronal apoptosis in the neuronal death model induced by amyloid β peptide (Aβ) is aimed and planned.

Methods: Apoptotic neuronal death was induced in PC12 cells by the administration of $A\beta_{(25–35)}$ fragment. In evaluating levels of neuronal injury and the potential protective effect of EPO, dimethylthiazol diphenyltetrazolium bromide reduction cell viability assay and trypan blue exclusion were performed. Apostain immunofluorescein staining has been used for the evaluation of apoptotic cell death. Caspase-8 activation was evaluated by western blotting method.

Results: Erythropoietin administration was shown to protective effect on cell viability in Aβ induced neuronal cell death. Apostain revealed that EPO had an anti-apoptotic effect in this experimental model. A reduction in caspase-8 activation was observed after EPO administration.

Conclusion: Erythropoietin was demonstrated to have a cell protective effect in neurotoxicity induced by amyloid β peptide and to diminish the apoptosis by decreasing activation of caspase-8 which promotes apoptosis. This result suggest that EPO may have a role in management of amyloid β peptide neurotoxicity in AD.

PP-619
Inhibition of neurite outgrowth by phenyl saligenin phosphate: effects on neural transglutaminase activity.

R. Howden[1], P. L. R. Bonner[1], M. Griffin[2] and **A. J. Hargreaves**[1]
[1]*School of Biomedical and Natural Sciences, Nottingham Trent University, Nottingham, UK,* [2]*School of Life and Health Sciences, Aston University, Birmingham, UK. E-mail: alan.hargreaves@ntu.ac.uk*

Transglutaminases (TGases) are calcium-dependent enzymes that catalyse the transamidation of polypeptide bound glutamines. This can involve either the formation of protein crosslinks or the incorporation of polyamines into a variety of substrate proteins. Altered TGase activity is known to be involved in several neurodegenerative conditions that share common features with chemically-induced neuropathies, such as organophosphate toxicity. Furthermore, the organophosphate phenyl saligenin phosphate (PSP) inhibits neurite outgrowth, a developmental process in which TGase is known to be involved. In order to study the possible involvement of TGase in PSP-induced neurotoxicity, TGase assays were performed on a variety of extracts from mammalian brain and cell cultures incubated in the presence and absence of PSP. PSP treatment induced a significant increase in TGase activity in porcine brain cytosol extracts, and in lysates from differentiating neuronal cells, as determined by the incorporation of biotin-TVQQEL into casein [1]. Amine incorporation assays on cells differentiated in the presence of 5-(biotinamido) pentylamine also revealed higher levels of TGase activity in PSP-treated cells, with no overall change in the levels of TGase protein. Our findings suggest that altered TGase activity may be a key molecular event following exposure to PSP.

Reference:

1. Trigwell SM, Lynch PT, Griffin M, Hargreaves AJ, Bonner PLR. Analytical Biochemistry 2004; 330: 164–166.

PP-620
Neurotrophin 3, tumoral necrosis factor alpha, interleukin 6 and acute phase proteins in patients with ischemic stroke

D. Pasarica[1], M. Gheorghiu[1], C. Bara[1], M. Serban[1], T. Trandafir[1], F. Toparceanu[2], L. Ichim[2], C. Bleotu[2], C. Popa[3], M. Alaicescu[3] and **M. Stoian**[2]
[1]*Pathophysiology and Immunology Department,"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania,* [2]*Antiviral Therapy Department, "Ştefan S. Nicolau" Institute of Virology, Bucharest, Romania,* [3]*"Vlad Voiculescu" Institute of Cerebro-Vascular Diseases, Bucharest, Romania. E-mail: traiantrandafir@yahoo.com*

We investigated the possible correlation of NT3 with proinflammatory cytokines TNFalpha and IL6 in serum and CFS of patients with IS. NT3 and cytokines were determined using ELISA kits. We also investigated the serum and CSF level of AAT and TR in these patients. Twenty patients aged 72.65 ± 12.81 years. were investigated after computed tomography-confirmed IS. Outcoming patients were assessed using Glasgow outcome score (GOS). TNFalpha was detected in three patients with GOS 1 and in one patient with GOS 4 (7.75 ± 0.5 days after IS). Patients were divided into three groups, according to their serum and CSF levels of IL-6 and NT3: GROUP I with low serum concentration of IL6 (< 40 pg/ml) and high IL6 CFS level (> 76 pg/ml) or inversely [GOSs:1(n = 5), 2(n = 2), 3(n = 2)]; GROUP II with high concentration of IL6 (> 72 pg/ml) or NT3 (> 17 pg/ml) in serum and high level of IL6 (> 50 pg/ml) in CFS [GOSs:1(n = 2), 2(n = 1), 5(n = 2)]; GROUP III with low concentration of IL6 (< 45 pg/ml) and NT-3 (< 15 pg/ml) in serum and CFS [GOSs:3 (n = 1), 4 (n = 3), 5 (n = 2)]. High significant serum levels of ATT and TR were observed in group II non-survivors (ATT = 435 ± 113 mg% versus 7.5 ± 1.8 mg%, TR = 396 ± 91mg% versus 5.1 ± 1.4 mg%). The negative correlation between NT3 and IL6 is associated with improved outcome after IS. Absence of this correlation or presence of exacerbated serum levels of TNFalpha could be associated with imminence of death. Association of cerebral ischemia with inflammatory response is confirmed by increased level of acute phase proteins.

PP-621
Evaluation of the possible transmission of prions to sea bream

C. Panagiotidis[1], E. Eleftheriadis[2], F. Arapoglou[2], K. Teliousis[3], E. Kaldrymidou[3], G. Krey[2] and T. Sklaviadis[1]
[1]*Center of Research and Technology/Institute of Agrobiotechnology, Thermi, Greece,* [2]*National Agricultural Research Foundation/Fisheries Research Institute, Nea Peramos, Greece,* [3]*Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece. E-mail: cpanagi@pharm.auth.gr*

Transmissible spongiform encephalopathies (TSEs) are infectious neurodegenerative diseases in which the causative agent is thought to be PrPSc, an aberrant isoform of the normal cellular prion protein, PrPC. While TSEs have been studied extensively in mammals, very little is known about TSE pathogenesis in fish. With fish farming becoming an important industry that provides high protein nutrition for humans, both the prospect of a prion disease developing in fish and the possibility of farmed fish being contaminated with infectious mammalian PrPSc are of major concern. We have undertaken a study with sea bream to evaluate the possibility of transmission of TSEs to fish. Two groups of sea bream were force fed with infected sheep or cow brain homogenates, while similar control populations were fed with normal brain homogenates. Following this challenge the inoculated fish have been studied for clinical and behavioral signs of disease on a daily basis. At regular times during the post-inoculation period fish from each challenge group have been sacrificed and their tissues subjected to histopathological examination, immunohistochemical detection of residual mammalian PrP and western blot analysis for detection of both mammalian and fish PrPs. The results from the first 2 years of this study do indicate that prion disease has been transmitted to sea bream.

PP-622
Mitochondrial complex I and IV activities, ND2, ND4 gene mutations and expressions in idiopathic Parkinson's disease

A. Ercan[1], G. Kulaksiz[1], M. Muftuoglu[1], O. Dalmizrak[1], H. Ogus[1], L. Cavdar[2], L. Inan[2] and N. Ozer[1]
[1]*Department of Biochemistry, Faculty of Medicine, Hacettepe University, 06100 Sihhiye, Ankara, Turkey,* [2]*Department of Neurology, Ankara Research and Training Hospital, 06100 Sıhhiye, Ankara, Turkey. E-mail: naozer@hacettepe.edu.tr*

Parkinson's Disease (PD) is a common neurodegenerative disorder characterized by bradykinesia, rigidity, tremor and

pathologically by the death of dopaminergic neurons in substantia nigra. Oxidative stress, accelerated aging, and genetic predisposition have been implicated in the etiology of idiopathic PD. The discovery of selective inhibition of complex I in dopaminergic neurons and appearance of PD-like symptoms provide a model for investigating the importance of complex I enzyme in PD. In this study, muscle biopsies obtained from 19 idiopathic PD patients and 4 healthy subjects have been studied for mitochondrial complex I and IV activities. A significant decrease in the muscle complex I activity (19.73 ± 8.24 U/mg protein) was found in idiopathic PD patients compared to age matched control (31.49 ± 8.28 U/mg protein). Complex IV activities in idiopathic PD patients and control group were found to be 11.51 ± 6.45 U/mg protein and 30.02 ± 14.76 U/mg protein, respectively. The entire ND2 and ND4 genes were sequenced from muscle of 19 idiopathic PD patients. Although several different sequence variants are detected, there is no specific mutation associated to idiopathic PD. Finally, ND2 and ND4 expression levels by using relative quantitative RT-PCR were examined. We found a decrease in the expression of ND2 and in ND4 genes in two patients.

Acknowledgement: *A.E. and G.K. contributed equally to this work.

PP-623
A PI-TPα-dependent survival factor protects primary cultured neurons against serum deprivation-induced cell death

H. Bunte[1], M. Schenning[1], P. Sodaar[2], P. R. Bär[3], K. W. A. Wirtz[1], F. L. van Muiswinkel[2] and G. T. Snoek[1]
[1]*Department of Biochemistry of Lipids, Bijvoet Center for Biomolecular Research, Utrecht University, Utrecht, The Netherlands,* [2]*Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands,* [3]*Graduate School for Biomedical Sciences, University Medical Centre Utrecht, Utrecht, The Netherlands.*
E-mail: h.bunte@chem.uu.nl

Selective neuronal loss is a prominent feature in both acute and chronic neurological disorders. Recently, a link between neurodegeneration and a deficiency in the protein phosphatidylinositol transfer protein α (PI-TPα) has been demonstrated. In this context it may be of importance that fibroblasts overexpressing PI-TPα are known to produce and secrete bioactive survival factors that protect fibroblasts against UV-induced apoptosis. In the present study it was investigated whether the conditioned medium of cells overexpressing PI-TPα (CMα) has neuroprotective effects on primary neurons in culture. We show that CMα is capable of protecting spinal cord-derived motor neurons against serum deprivation-induced cell death. Since the CM of wild type cells was much less effective we infer that the neuroprotective effect of CMα is linked (in part) to the PI-TPα-dependent production of arachidonic acid metabolites. The neuroprotective activity of CMα is partly inhibited by suramin, a broad-spectrum antagonist of G-protein coupled receptors. Western blot analysis shows that brain cortex and spinal cord express relatively high levels of PI-TPα, suggesting that the survival factor may be produced in neuronal tissue. We propose that the bioactive survival factor is implicated in neuronal survival. If so, PI-TPα could be a promising target to be evaluated in studies on the prevention and treatment of neurological disorders.

Bioinformatics and Proteomics

PP-624
ITS DNA sequence utility for the relationship of *Heracleum L.* (umbelliferae) genus

M. Maras[1], **E. Aksöz**[2], Y. Menemen[1] and E. Hamzaoğlu[3]
[1]*Biology Department, Faculty of Science and Art, Kırıkkale University, 71450, Yahşihan-Kırıkkale, Turkey,* [2]*Biology Department, Faculty of Science, Hacettepe University, 06532, Beytepe-Ankara, Turkey,* [3]*Biology Department, Faculty of Science and Art, Erciyes University, Yozgat, Turkey*

The internal transcribed spacer (ITS) regions of 18–28S nuclear ribosomal DNA, separating the coding regions, evolve relatively rapidly and may be useful for reconstructing phylogenies at specific and generic levels. Phylogenetic relationships among seven species of *Heracleum* genus were inferred from nucleotide sequence variation in the internal transcribed spacer regions. Average pairwise sequence divergence values across both ITS1 and ITS2 regions among *Heracleum* species ranger from 0.11 to 0.92% of nucleotides. The combined analysis resulted in a well-resolved phylogeny with bootstrap supports. This study affirms that ITS sequences are useful for phylogenetic inference among closely related species of *Heracleum* genus.

PP-625
Five hundred proteins identified in human endothelial cells by Fourier transform mass spectrometry after 2-DE

B. Baudin[1], A. Bruneel[1], P. Marcelo[2], I. Haddad[2], A. Lor[1], J. Vinh[2], J. Rossier[2] and M. Vaubourdolle[1]
[1]*Biochimie A, Hôpital Saint-Antoine AP-HP, Paris, France,* [2]*ESPCI-CNRS/UMR-7637, Paris, France.*
E-mail: bruno.baudin@sat.aphp.fr

We applied Fourier Transform mass spectrometry (LC-FTQFT-MS) to complete the proteomic reference map of human umbilical vein endothelial cells (huvec.com). Proteins extracted from cultured HUVEC were separated by two-dimensional electrophoresis (2-DE) in 4–7 pH gradient and 8–18% SDS-PAGE. The 18 cm/0.5 mm gel was cut in 221 regular pieces, treated by trypsin and analysed by LC-FTQFT-MS. More than 400 proteins were identified and classified with the former into 10 categories:1–40 from sugar metabolism;2–31 from lipid metabolism and bioenergy;3–35 from amino-acid and vitamin metabolisms; 4–152 from cytoskeleton (contractile, intermediary filaments,

microtubules),proteins interacting with extracellular matrix, vacuoles, Golgi, endoplasmic reticulum and proteasome; 5–88 from nucleic acid metabolism, replication, transcription, ribosome, initiating and elongating factors for translation, tRNA-synthetases; 6–44 chaperoning and folding proteins; 7–36 membrane-receptors and proteins in transduction pathways (ionic channels, AMP-cyclases, GTP-proteins, phospho-inositol and phosphorylation mediated transduction); 8–30 for intercell relations; 9–32 involved in proliferation, apoptosis and cancerogenesis; 10–24 against oxidative stress or for detoxication. Thus, beside proteins involved in the general cellular machinery, HUVEC express a number of more specific proteins, for some of them not yet identified in this model or with an implication in pathological situations such as cancer and cardiovascular diseases.

PP-626
Dynamic protein domains: identification, interdependence and stability

S. O. Yesylevskyy[1], V. M. Kharkyanen[1] and A. P. Demchenko[2]
[1]*Department of Physics of Biological Systems, Institute of Physics, Natl. Acad. of Sci. of Ukraine. Prospect Nauki, 46, Kiev, Ukraine,* [2]*A. V. Palladin Institute of Biochemistry, Leontovicha st. 9, Kiev-01030, Ukraine; Research Institute for Genetic Engineering and Biotechnology, TUBITAK, Gebze-Kocaeli, 41470, Turkey.*
E-mail: yesint3@yahoo.com

Existing methods of domain identification in proteins usually provide no information about the degree of domain independence and of their stability. However, this information is vital for many areas of protein research. The recently developed Hierarchical Clustering of the Correlation Patterns (HCCP) technique provides machine-based domain identification in a computationally simple and physically consistent way. Here we present the modification of this technique, which allows to determine not only the most plausible number of dynamic domains but also to estimate the degree of their independence and stability. With this technique we provided domain assignments and calculated intra- and inter-domain correlations and inter-domain energies for more than 2500 test proteins. It is shown that mean intra-domain correlation of motions can serve as a quantitative criterion of domain independence, and the HCCP stability gap is a measure of their stability. Our data show that the motions of domains with high stability are usually independent. In contrast, the domains with moderate stability usually exhibit substantial degree of correlated motions. It is shown that in multi-domain protein the domains are the most stable if they are of similar sizes, and this correlates with the observed abundance of such proteins.

PP-627
Analysis of systematic RNAi screens

Z. Arziman, T. Horn and M. Boutros
Boveri-Group Signaling and Functional Genomics, German Cancer Research Center, 69120 Heidelberg, Germany.
E-mail: zeynep.arziman@dkfz.de

RNA interference (RNAi) screens have become a powerful genetic approach to systematically dissect pathways on a genome-wide scale. With an increasing number of screens performed, it has become more important to build comprehensive phenotype databases and develop systematic approaches to analyse RNAi datasets. RNAi libraries available for C. elegans and Drosophila target almost every predicted gene and allow genetic screens for a variety of phenotypes. One of the major concerns remains the potential off-target effects which may influence the information content obtained by RNAi screening. I will describe a database that collects all available RNAi phenotype datasets from Drosophila and allows the analysis of RNAi quality and phenotypes across multiple screens and species. RNAi phenotypes that are stored with a systematic way in a database can be compared and clustered for in-depth analysis of datasets. Clustering of genes with related phenotypes can allow predicting new functions. A combined RNAi database can also be integrated with different databases such as protein-protein interaction and expression databases. The database is publicly available at http://rnai.dkfz.de.

PP-628
About long-range correlation in proteins primary sequences

L. Unipan[1] and A. Isvoran[2]
[1]*University of Agricultural Science of Banat, Timisoara, Romania,* [2]*Department of Chemistry, West University of Timisoara, Str. Pestalozzi 16, 300115 Timisoara, Romania.*
E-mail: unipan_l@yahoo.com

The aim of this study is to analyse the existence of correlation in the sequences data of proteins. We have analysed 30 proteins: 10 from all alpha structural class, 10 from all beta structural class and 10 from alpha + beta structural class. We treated the primary structures of these proteins as time series. Too spatial series of data for each sequence of a protein are generated from numerical correspondences between each amino acid and a physical property associated with it: its electric charge and its polar character. For each series we have determined the spectral coefficient, the scaling exponent and the Hurst coefficient. The values obtained for these coefficients revealed non-randomness in the series of data.

PP-629
In silico prediction and analysis of IgE-binding epitopes in enolase

M. Piast, I. Kustrzeba-Wójcicka, M. Matusiewicz, M. Krzystek-Korpacka and T. Banaś
Department of Medical Biochemistry, Medical University, Wrocaw, Poland. E-mail: piast@bioch.am.wroc.pl

Enolase catalyzes the dehydration of 2-phospho-D-glycerate to phosphoenolopyruvate. It has been recognized as an allergen in *S. cerevisiae*. Further research confirmed its allergenic properties in *A. alternata, A. fumigatus, A. oryzae, C. albicans, C. herbarum* and *P. citrinum*. IgE antibodies specific to enolase have been found in patients allergic to these species. Although enolase has been acknowledged as an allergen in different moulds, structure of IgE-binding epitopes is still not known in most of these species. This work was focused on analysis of known enolase sequences from moulds in order to predict an accurate location of epitopes in the C-terminal domain of molecule. Analysis methods included multiple alignments and use of Peptide Similarity Search, tool supplied by SDAP Database. Enolase sequences were compared to *A. alternata* major allergen Alt a 1. The alignment of enolases pointed on two regions of potential IgE-binding – 124-AEKGVPL-130 and 136-DLAGTTKP-143, with minor differences in epitopes of *C. albicans* (125-AAQGIPL-131; 137-NISNAKKGK-145), *R. rubra* (124-AQKDVPL-130; 136-DISKAKEGK-144), and *S. cerevisiae* (123-AEKNVPL-129; 135-DLSKSKTSP-143). The Peptide Similarity Search resulted in one epitope – decapeptide 133-HISDLAGTKK-142, almost completely superimposing with second region observed in the alignment. Results shown may have important inference in synthesis of peptides used in mould's allergy diagnostics and clinical treatment.

PP-630
Complementation of an *Escherichia coli* DnaK defect by Hsc70-DnaK chimeric proteins

J. P. Suppini[1], **M. Amor-Mahjoub**[1], J. H. Alix[2] and M. M. Ladjimi[1]
[1]*Laboratory of Biochemistry FRE 2621, University Pierre and Marie CURIE, Paris, FRANCE,* [2]*Institut de Biologie Physico-chimique, Paris, France. E-mail: mounamor@yahoo.fr*

The heat shock proteins of 70 kDa (Hsp70s) are among the most conserved proteins and are found in most prokaryotic and in most compartments of all eukaryotic cells. They are known to protect cells against damage by high temperatures and to assist protein folding and assembly by ATP-dependent cycles of substrate binding and release. *Escherichia coli* DnaK and rat Hsc70 are members of Hsp70 family that show strong sequence and structure similarities and comparable functional properties in terms of interactions with peptides and unfolded proteins and cooperation with cochaperones. To gain insight into the structural origin of these differences, a series of chimeric proteins, made by swapping respective domains having similar structures but different functions, have been generated and analysed *in vivo* for the complementation of two *E. coli* phenotypes, growth at high temperatures and propagation of lambda phage. We show here that, while the DnaK protein is, as expected, able to complement an *E. coli* dnaK mutant strain for growth at high temperatures and lambda phage propagation, Hsc70 protein is not. However, an Hsc70 in which the peptide binding domain has been replaced by that of DnaK is able to complement this strain for both phenotypes, suggesting that the peptide-binding domain of DnaK is essential to fulfil the specific functions of this protein necessary for growth at high temperatures and for lambda phage replication.

PP-631
In vivo phosphorylation site analysis of liver glycogen synthase by MALDI-TOF mass spectrometry

O. Blanco-Presas[1], M. Garcia-Rocha[1], E. de Oliveira[2], J. C. Ferrer[3] and J. J. Guinovart[1]
[1]*Department of Biochemistry and Molecular Biology, IRB-Barcelona Science Park-University of Barcelona, Barcelona, Spain,* [2]*Proteomics Platform, Barcelona Science Park-University of Barcelona, Barcelona, Spain,* [3]*Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Barcelona, Barcelona, Spain. E-mail: oblanco@pcb.ub.es*

Liver glycogen synthase (LGS) is a key enzyme in the control of glycogen metabolism. Its activity is regulated by phosphorylation at multiple sites and its subcellular localization is dependent on the metabolic state of the hepatocyte. Our study aims to identify the residues of LGS that are phosphorylated *in vivo* in a range of metabolic situations in order to elucidate their role in the regulation of the activity and subcellular localization of LGS. To this end, LGS-enriched fractions were obtained after immunoprecipitation of lysates from isolated rat hepatocytes and were then subjected to polyacrylamide gel electrophoresis. The bands corresponding to LGS were excised and digested with trypsin. The phosphopeptide-enriched fraction obtained after ion metal affinity chromatography (IMAC) of the tryptic digestion mixture was analysed by MALDI-TOF mass spectrometry (MS). Tandem MS/MS analysis allowed us to identify two phosphorylated residues of rat LGS, Ser8 and Ser11. These are the first two phosphorylation sites of LGS determined using MS. The observation that Ser11 is phosphorylated only when Ser8 also carries a phosphate moiety suggests a hierarchy of phosphorylation/dephosphorylation events on these sites that could have functional consequences. We are currently studying the phosphorylation status of these and other residues of rat LGS in a range of experimental conditions which include the presence of glucose and/or hormones involved in glycogen metabolism.

PP-632
Multivariate analysis of expression and sequence characteristics of miRNAs

K. D. Kaya, O. Konu and **C. Yakicier**
Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey. E-mail: kkaya@bilkent.edu.tr

MicroRNAs are small ribonucleic acids, which bind to 3'UTR regions of mRNAs by base complementation. They play crucial roles in the regulation of embryonic development, and in many cellular events such as proliferation, apoptosis, and differentiation. Hence, understanding the mechanisms by which they are regulated is a key attractant for research intentions involving microRNAs. Accordingly we have investigated the significance of the bias in dinucleotide motif distribution of miRNAs using a randomization approach. The correlation of sequence characteristics with miRNA microarray expression values also was statistically assessed. Our results indicated that observed miRNA frequencies of multiple dinucleotide motifs significantly deviated from expected and their relative abundancies are close to genomic values. Moreover, variation in the level of miRNA expression could partly be explained by the presence or absence of a CpG motif. Our studies on the development of a database, which integrates the species-specific miRNA sequence information and the associated public microarray data, are in progress.

PP-633
The glycine specificity motif in cysteine proteases

A. Golubtsov and T. Ahola
Animal Virus laboratory, Institute of Biotechnology, Helsinki, Finland. E-mail: Andrey.Golubtsov@helsinki.fi

Knowledge on the protease cleavage specificity is the absolute requirement for understanding of their biological role and utilization in biotechnology. The group of cysteine proteases includes many enzymes of potential medical importance. We have described a new structural motif of these proteins associated with the substrate recognition. We have found that the majority of the cysteine proteases, which carry an aromatic residue (Trp, Tyr or Phe) immediately following the catalytic His, process polypeptides with Gly residue at the P2 position of a cleavage site. The motif is found in two clans of cysteine proteases, including several families of viral endopeptidases, ubiquitin and SUMO hydrolases, pseudomurein endoisopeptidases, bacteriocin processing enzymes. Despite the lack of sequence similarity, the proteins share a common architecture of the catalytic centers with the aromatic residue of the motif being in a direct contact with the penultimate Gly of the substrate. Based on the bioinformatics data, structural and biochemical information available, we suggest the glycine specificity motif (GSM) – the catalytic His followed by an aromatic residue – as a hallmark of this type of peptidases, which can be used for predicting of the specificity of newly discovered proteases.

PP-634
HP2SLs: a database for subcellular localizations of human proteome

B. Bilen[1], V. Atalay[2], M. Ozturk[1] and R. Cetin-Atalay[1]
[1]*Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey,* [2]*Department of Computer Engineering, METU, Ankara, Turkey. E-mail: rengul@bilkent.edu.tr*

The Human Proteome Subcellular Localizations Database (http://www.i-cancer.org/hP2SLs) contains the subcellular localization predictions of all human proteins. Protein localization classes over ER targeted, cytosolic, mitochondrial and nuclear are predicted using P2SL [1]. The sequence data and prediction class results are stored in a scalable, searchable and downloadable relational database. We extracted Homo sapiens protein sequences from PIR-NREF [2] and UniRef100 [3] databases which consisted of more than 128000, 86000 sequences respectively. The prediction class data in the hP2SLs database and the related information in the web site are automatically updated if any new releases exist in PIR-NREF or UniProt Databases. The hP2SLs database can be queried based on the PIR and UniRef100 database id, keyword, protein sequence fragment, and subcellular localization classes. Additional information on the predicted sequences can be expanded for further annotations through NCBI BLAST [4] analysis and source sequence web pages links. The hP2SLs database provides a reference source for Homo sapiens proteome scale subcellular localization information. In addition, it offers an environment for the experimentally designed peptide localization prediction through internal BLAST.

References:

1. Atalay and Cetin-Atalay. Bioinformatics, 2005
2. Cathy et al., Nucleic Acids Research, 2003
3. Apweiler et al., Nucleic Acids Research, 2004
4. Altschul et al., J. Mol. Biol, 1990

PP-635
CAPRIS: a database for cancer gene promoter related motif search

M. Iskar[1], A. Erdem[2], E. Erdem[2], A. Dickerman[3], M. Ozturk[1], V. Atalay[2] and R. Cetin-Atalay[1]
[1]*Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey,* [2]*Department of Computer Engineering, METU, Ankara, Turkey,* [3]*Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, USA. E-mail: rengul@bilkent.edu.tr*

CAPRIS (http://www.i-cancer.org/capris) database contains cancer gene classes that are grouped based on their 600 bp promoter region (−500 to +100) and DNA motifs extracted from these groups. CAPRIS database is constructed as a result of the analysis of 1150 cancer related gene promoter sequences using machine learning techniques. Sequences are labeled based on their relation to 21 different cancer types extracted from ENTREZ-Gene [1]. For each of the 1150 cancer related gene, a feature vector is formed based on the frequency statistics of nucleotides extracted from 600bp promoter region. These feature vectors are fed to Self Organizing Map (SOM) [2]. Sequences are clustered on the SOM with respect to these features. Next, we have selected SOM nodes that contain a group of sequences particular to a cancer type. Finally, a total of 130 clusters from neighboring SOM nodes are formed for 21 cancer types. Then we analysed these cancer related gene promoter groups with motif extraction tools in order to obtain common DNA sequence motifs. The extracted gene clusters and the motifs are stored in a searchable and downloadable relational CAPRIS database. CAPRIS can be queried based on gene name, ID, cancer type and nucleotide sequence fragment. In addition users can perform NCBI-BLAST analysis against predicted motifs.

References:

1. Maglott D et al. Nucleic Acids Res 2005
2. Kohonen T. Self-organizing Maps. Springer-Verlag, 1997

PP-635a
Sequence-structure-function relationships of apoptotic nuclease – DNase II

I. A. Cymerman[1], P. Schafer[2], G. Meiss[2] and **J. M. Bujnicki**[1]
[1]*Laboratory of Bioinformatics and Protein Engineering International Institute of Molecular and Cell Biology ul. Ks. Trojdena 4 02-109 Warsaw, Poland,* [2]*Institute of Biochemistry, Justus-Liebig-University, Heinrich-Buff-Ring 58, D-35392 Giessen, Germany. E-mail: iwona@genesilico.pl*

DNase II is an endodeoxyribonuclease involved in the apoptosis and essential for the mammalian development. Despite the understanding of biochemical properties of this enzyme, its structure and relationships to other protein families remained unknown. The lack of this information was a bottleneck for functional analyses. Using protein fold-recognition methods we found that DNase II shares a three dimensional fold and the active site with enzymes from the phospholipase D superfamily. The model explained the available experimental data and allowed for selection of amino acids potentially important for the function of the enzyme. The wild-type enzyme and its alanine mutants were overexpressed, purified and functionally characterized. We have also analysed the oligomeric state of DNase II. Our experimental results confirm the theoretical predictions and provide the platform for further functional studies and rational engineering of DNase II, which will lead to the better understanding of its regulation during apoptosis and its application as a potential mucolytic agent for improving pulmonary clearance in patients with cystic fibrosis.

PP-636
Calmyrin2 – a new member of Calmyrin family among neuronal calcium sensors

M. Blazejczyk[1], I. A. Cymerman[2], A. Sobczak[1], M. R. Kreutz[3], J. Kuznicki[1] and U. Wojda[1]
[1]*Laboratory of Neurodegeneration, International Institute of Molecular and Cell Biology, Warsaw, Poland,* [2]*Laboratory of Bioinformatics, International Institute of Molecular and Cell Biology, Warsaw, Poland,* [3]*Department of Neurochemistry/Molecular Biology, Leibniz Institute for Neurobiology, Magdeburg, Germany. E-mail: mblazejczyk@iimcb.gov.pl*

Precise Ca-signaling in neurons requires multitude of Ca-sensor proteins, many of which belong to Neuronal Calcium-Sensor (NCS) family containing 4 Ca-binding motives called EF-hands. Recently calmyrin1 (CaMy1, CIB1), a new NCS family member was described in details. In addition, 3 homologues of CaMy1 were found in human genome but little is known about their properties or expression. To gain insight in the CaMy protein subfamily, we analysed and compared CaMy2 and 1 expression profile, protein structure and biochemical features. We found CaMy2 was highly expressed in various regions of rat brain and its expression pattern differed from CaMy1. Homology modeling for CaMy2 showed high similarity with CaMy1 structure. However, CaMy2 seems to be less compact and contains an additional helix preceding EF1. EF1 in both proteins is non-functional, but in contrast to CaMy1 the structure of EF2 in CaMy2 suggests a proper amino acid configuration for coordination of Ca. To confirm our predictions we cloned CaMy2 and its N-terminal

fragment containing EF1 and EF2 and characterized their Ca-binding properties and Ca-induced conformational changes. We also showed that CaMy2, in contrast to CaMy1, did not interact with Alzheimer's disease associated presenilin2. Our studies indicate that despite CaMy2 and CaMy1 overall structural similarities, their biochemical features and expression in the brain differ suggesting various roles of these proteins in Ca-signaling.

PP-637
Molecular dynamics simulation of ubiquitin in water: on the interaction mechanism with some lysine rich proteins

F. M. Mazze and L. Degreve
Departamento de Química, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo (USP), Avenida dos Bandeirantes, 3900, Cidade Universitária 14040-901 Ribeirão Preto, SP, Brazil. E-mail: leo@ffclrp.usp.br

Ubiquitin (UB) is a well known small protein consisting of 76 highly conserved amino acids found in all eukaryotes that appears to function in several important cellular processes, such as: cell cycle control, antigen presentation, heat shock response, receptor signaling, transcriptional activation, and DNA repair. The UB/proteasome pathway is the principal mechanism for turnover of normal short-lived proteins in mammalian cells. In proteins targeted for degradation, UB couples to the α-amino group of LYS residues in the protein via its C-terminal GLY residue, forming an isopeptide bond. Recently, it has also been shown that UB is present inside different retroviruses. A putative causal link between budding and UB proposes that depletion of the intracellular pool of free UB inhibits budding. However nothing is known about the cellular mechanisms involved in the UB interactions with other proteins. Molecular dynamics simulations conducted at 298K and pH = 7.0 were used to get some insight on these mechanism. They have shown that the structure of 1–72 residues is well conserved while a complete lack of structure of the last four C-terminal residues (LRGG) was found. These residues are thus located in such a way that they are able to interact easily with a residue of another peptide as it is the case of LYS. These results can be interpreted as the role of the last four C-terminal residues as a search tool for finding LYS residues in other proteins.

Acknowledgment: Fapesp-CNPq.

PP-638
The peroxisomal proteome in *Saccharomyces cerevisiae*

C. Brocard, K. Grossschopf, S. Merich, I. Dohnal and A. Hartig
Max F. Perutz Laboratories, Department of Biochemistry, University of Vienna, Dr. Bohrgasse 9, 1030 Vienna, Austria. E-mail: andreas.hartig@univie.ac.at

Peroxisomes are highly dynamic organelles present in virtually all eukaryotic cells. These organelles play an important role in the cellular metabolism, which is illustrated by the occurrence of severe human diseases correlated with defects in peroxisome biogenesis and metabolism such as Zellweger syndrome and Adrenoleukodystrophy. The size, number and protein content of peroxisomes greatly fluctuate with the environmental and physiological state of the cells. Such flexibility has also been demonstrated in yeast upon growth on different carbon sources. Nevertheless, the knowledge about the enzymatic content of peroxisomes remains incomplete. To fully appreciate the vital role of peroxisomes for living organisms it is crucial to identify all proteins associated with this organelle. The yeast *Saccharomyces cerevisiae* was used as a model organism to apply an organellar proteomic approach. We established a novel isolation procedure for peroxisomes based on affinity purification. Proteins from the highly purified and intact organelles were fractionated and analysed by mass spectrometry. This procedure enabled us to identify a number of proteins previously not known to be associated with peroxisomes.

Acknowledgment: This research was supported by the EU project LSHG - CT-2004 - 512018.

PP-639
Bioinformatic-aided characteristic of coeliac-toxic peptides

M. Darewicz and M. Dziuba
University of Warmia and Mazury in Olsztyn, Chair of Food Biochemistry. E-mail: darewicz@uwm.edu.pl

It was found that small sequences of amino acids were common to the peptides linked with coeliac toxicity. However the motifs must be flanked by other amino acids. The aim of the research was to characterize proteins as a source of toxic tetrapeptides and extended motifs. The BIOPEP database (http://www.uwm.edu.pl/biochemia), SWISS-PROT database (http://www.expasy.org), BLAST (http://www.ncbi.nlm.nih.gov/blast) homology searching protocol and PREDICT 7 program were applied. Determination of potential possibilities of toxic peptides release from proteins induced by endopeptidases also was the objective of the research. Computer analysis of 345 sequences (wheat, rice, barley, oat, buckwheat, pea, taumatin and trypsin- and alpha-amylase-inhibitors) showed that coeliac tetrapeptides were present within 155 of them. Extended motifs were detected for 29 of analysed protein sequences i.e. for wheat, barley and oat protein sequences. The BLAST homology searching protocol proved the existence of high extent of evolutional homology. Random coil and beta-sheet were the only occurring secondary structures. They tend to be located on the hydrophilic surface of the protein molecule. In silico analysis of chosen wheat, oat and barley proteins showed that 28 of them had peptide bonds susceptible to hydrolysis by selected endopeptidases, which may consequently lead to the release of coeliac-toxic peptides. They were obtained as a result of proteinase K, thermolysin and prolyl endopeptidase.

PP-640
Genome organization of genes coding for carbohydrate active enzymes in *Bacteroides* species

A. Pallejà, P. Puigbò, E. Guzmán, J. M. Orellana, M. Marcet, M. A. Montero, S. Garcia-Vallvé and A. Romeu
Evolutionary Genomics Group, Department of Biochemistry and Biotechnology. Rovira i Virgili University, E-43005 Tarragona, Spain. E-mail: albert.palleja@estudiants.urv.net

The carbohydrate active enzymes (CAZY) are proteins that play a key role in the biology of the *Bacteroides* species: dominant members of our normal distal intestinal microbiota. The most abundant specie is *Bacteroides thetaiotaomicron* while *Bacteroides fragilis* is less abundant and it can be pathogenous. Putative functional clusters, and molecular evolution features of CAZY related genes are analysed. The *Escherichia coli* genome has been taken as reference. Normalized CAZY related genes (CAZY genes/total genes) in *B. thetaiotaomicron, B. fragilis* and *E. coli* are 0.08, 0.05 and 0.02, respectively. In all kind of CAZY group of genes about 60% is located in the leading DNA strand. Although the *B. thetaiotaomicron* genome shows a high level of

glycosidase and glycosyltransferase gene clusters. The number of overlapping CAZY related genes are described. The distribution of the genes in the genome is not random, so it is rather surprising that a significant number of genes overlap in the *Bacteroides* genomes. However, the origin and evolution of overlapping genes are still unknown. We identified about 50 pairs of overlapping genes in *B. thetaiotaomicron* and *B. fragilis* and studied their evolutionary patterns. Studies of the gene structures and overlapping patterns showed that only a small fraction of the analysed genes preserve exactly the same pattern in both organisms.

Acknowledgment: This study has been supported by BIO2003-07672 Spain Project and by BBVA Fundation Project.

PP-641
Study on the structure-function relationship of several *Helicobacter pyroli* proteins

B. J. Lee, S. J. Park, K. D. Han, S. J. Kang, M. D. Seo and J. H. Kim
College of Pharmacy, Seoul National University, Seoul, Korea.
E-mail: lbj7869@daum.net

The *H. pyroli* proteins of which molecular weight are below 15 kDa were selected for the investigation of their 3D structures. 60 target genes of *H. pyroli* were selected and cloned into *E. coli* expression vector. The expression system was chosen for pET/BL21 (DE3) series. Among them, we could determine the 3D structure of six targets. HP0559 is an acyl carrier protein that is predominantly associated with the biosynthesis of fatty acid. While the structures of acyl carrier proteins are highly conserved in various bacterial sources. HP0559 showed a distinct pH-dependent conformational characteristic. HP1073 is a putative copper binding regulatory peptide of 66 amino acid residues. It is supposed to be copper divalent ion binding protein while its homologous protein, CopZ shows a copper monovalent ion binding feature. HP 1073 adapts a babba fold, unlike babbab fold of CopZ. The other four proteins are unknown proteins, of which functions were evaluated by comparing their structures with the 3D models in the structural database (Dali server).

PP-642
HPV DNA testing in Cyprus

P. I. Neophytou, A. A. Albayrak and V. Tanos
Mendel Center for Biomedical Sciences, Egkomi, Cyprus.
E-mail: pav@mendelcenter.org

Cervical cancer is caused by human papilloma virus (HPV) infection. During the process of conducting an epidemiological study of the Cyprus population (including 521 Greek-Cypriots and 187 Turkish-Cypriots) we sequenced a number of putative novel HPV types. Cervical DNA was PCR amplified using My09 and My11 HPV-specific primers. Positive samples were typed by restriction digestions. Samples displaying a novel restriction enzyme pattern were DNA sequenced followed by FASTA searches and alignments. Our HPV DNA test included high-resolution typing and measurement of viral load that correlated with clinical results. We identified two novel HPV types, four novel subtypes and 10 variants. We also identified a number of human sequences amplified by HPV-specific primers My09 and My11. This allowed for an increased specificity of our test by elimination of false-positives. The frequency of HPV infection was 15% in 'normal' Greek-Cypriot women and 27% in Turkish-Cypriot women. The most important risk factors in Greek-Cypriot women were multiple sexual partners and smoking. In conclusion we conducted a prospective cross-sectional study of our populations using an excellent HPV DNA test and identified the most frequent types and the most important factors of risk. A number of novel HPV types, subtypes and variants have been identified. Furthermore a number of human loci amplified by HPV-specific primers have been identified, allowing for an increased specificity of the HPV DNA test.

PP-643
Analysis of Cdc25a and Mfn2 homologs isolated from the heart specific subtractive hybridization cDNA library

N. Erginel-Unaltuna
Department of Genetics, Institute for Experimental Medical Research, Istanbul University, Istanbul, Turkey.
E-mail: bilgeozsait@yahoo.com

A subtractive hybridization cDNA library specific to BALB/c mouse heart tissue was previously created in our laboratory. The purpose of this study is to analyse the transcripts isolated from the cDNA library in order to find novel genes that have important roles in cardiac formation and function. The analysis of the two transcripts will be summarized in this abstract in order to exemplify the study. The study design is composed of the analysis of the selected transcripts by bioinformatics studies and molecular genetics methods. The transcripts are selected depending on the results of comparative screening through the gene banks. One of the two transcripts had high homology (97%) to mouse Mfn2 gene. The second one had high homology (84%) to rat Cdc25a gene, although it was originally isolated from mouse cDNA library. At first, the transcripts are analysed by semi-quantitative PCR method using the cDNAs synthesized from total RNAs of adult mouse heart and skeletal tissue. Northern Blotting is done to confirm their heart expression. Subsequently, semi-quantitative PCR is done for embryonic, neonatal and adult stages. The expression values are calculated by gel densitometer. The beta-actin gene is used as control. This experiment provided us the knowledge of the expression patterns of the transcripts at different developmental stages. Describing the heart specific genes will make it possible to analyse their contributions in cardiac function and development.

PP-644
Gen expression analysis of carbonic anhydrase isoenzyme genes in mouse genome by *in situ* hybridization

D. Ekinci[1], S. Beydemir[1], O. I. Kufrevioglu[1], M. Ciftci[1], M. Cankaya[1], H. Ozdemir[1], G. Ilhami[1] and G. Eichele[2]
[1]*Chemistry Department, Faculty of Art and Science, Ataturk University, 25240-Erzurum, Turkey,* [2]*Max-Planck Institute of Experimental Endocrinology, 30625 Hannover, Germany.*
E-mail: dekinci@atauni.edu.tr; beydemir@atauni.edu.tr; okufrevi@atauni.edu.tr; ciftcim@atauni.edu.tr; mcanka

In the present study, the gene expression analysis of carbonic anhydrase isoenzymes that present in mouse genome was carried out using an automatic robotic system by *in situ* hybridization in 14.5 days old mouse embryo. Specific primers were arranged for these enzymes. Specific cDNA templates were prepared with these specific primers. Antisense mRNAs (*in vitro* transcription) were produced with specific cDNA templates. *In situ* hybridization in which was used these antisense mRNAs on mouse embryo (14.5 days) was made. Automated microscopic scanning of gene expression data for carbonic anhydrase isoenzyme genes was investigated and these results were transferred with software in internet (www.genepaint.org). The results of gene expression on mouse embryo (14.5 days) are given below:

Car1: lung, lens, cranial gland, cortex, retina, pons, spinal cord, thalamus; Car2: lung, liver, intestines, ear, blood vessels; Car3: muscles, liver, cartilage; Car4: kidney; Car5a: preform cortex; Car5b: not expression; Car6: not expression; Car7: ubiquitous low; Car8: cerebellum, lung, stomach, kidney, thalamus, pancreas, medulla, salivary gland, olfactory bulb; Car9: not resulted yet; Car10: ubiquitous low; Car11: thalamus, pancreas, medulla, salivary gland, olfactory bulb, striatum, spinal cord; Car12: tongue, skin, choroids plexus, salivary gland; Car13: low expression in skin, muscles and some other organs; Car14: choroids plexus; Car15: ubiquitous low.

PP-645
Improvement on serum analysis for comprising small peptides upon two dimensional gel electrophoresis

F. Shekari[1], M. Habibi-Rezaei[1] and Gh. Hoseini Salekdeh[2]
[1]*School of Biology, University College of Science, University of Tehran, Tehran, Iran,* [2]*Agricultural Biotechnology Research Institute of Iran, Karaj, Iran.*
E-mail: faezehshekari@khayam.ut.ac.ir

Although two dimensional electrophoresis can be very useful approach to diagnose biomarkers in patient's blood, existence of highly abundant proteins has limited utilizing this approach by masking the presence of low abundant proteins. Among these abundant proteins is albumin which constitutes at least about 60% of total serum proteins by itself. So, albumin depletion has attracted a lot of attentions and the effectiveness of chromatographical techniques or commercial albumin removal kits has been reviewed by many other scientists. Recently modified TCA/acetone precipitation procedure for albumin removal from serum has been reported. Based on some of the albumin physicochemical characteristics, such as surface hydrophobicity, here we are reporting new aspects on the case. From them, albumin resolubilization process at the interface between two dimensions is in the great importance and has been modified in this work. According to the fact that Laemelli SDS polyacrylamide gel electrophoresis can't be reliable in detecting low molecular weight proteins including biomarkers or communicational intercellular signaling proteins or peptides, we have examined other SDS-PAGE systems which have been shown to be more applicable.

PP-646
Preliminary identification of moderately halophilic microorganisms through metabolic footprinting

B. Sarıyar Akbulut[1], A. Salman Dilgimen[1], S. Ceylan[1], S. Perk[2], A. A. Denizci[3] and D. Kazan[1]
[1]*Chemical Engineering Department, Engineering Faculty, Marmara University, Goztepe 34722 İstanbul, Turkey,* [2]*Department of Chemical Engineering, Illinois Institute of Technology, Chicago, IL, USA,* [3]*TUBİTAK, Research Institute for Genetic Engineering and Biotechnology, PK:21, 41470 Gebze Kocaeli, Turkey. E-mail: bsariyar@eng.marmara.edu.tr*

There is a growing need for the development of methods for the rapid identification and characterization of microorganisms because of time consuming experimental labor. Metabolic footprinting offers the potential for preliminary characterization of microorganisms. Metabolic footprinting is based on the analysis of metabolites secreted by the microorganisms. Since such secretory activities reflect metabolic activity, global analysis of secreted products gives significant information on the families of microorganisms. The aim of this study was to use metabolic footprinting for the preliminary identification of newly isolated microorganisms from Çamaltý Saltern area in Turkey. Cell-free culture media of the microorganisms from different growth phases are initially analysed using an electrospray ionization time of flight mass spectrometer (Waters/Micromass). After reduction of dimension of the chromatographic raw data, principal component analysis was performed. Loading plots have enabled us to cluster the microorganisms in relation to Halomonas species and to each other. Further analysis of the score plots if combined MS-MS analysis may give us clues on the specific metabolites that are critical in making the distinctions between microorganisms.

Acknowledgment: This work has been supported by TÜBÝTAK-TBAG with the Project no TBAG2321 (103T069).

PP-647
Proteomics of boar reproductive organs

P. Manaskova[1], J. Peknicova[2], M. Ticha[3] and **V. Jonakova**[1]
[1]*Department of Biochemistry of Reproduction, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 37 Prague 6, Czech Republic,* [2]*Department of Biology and Biochemistry of Fertilization, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Videnska 1083, 142 20 Prague 4, Czech Republic,* [3]*Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, 128 40 Prague 2, Czech Republic. E-mail: vjon@img.cas.cz*

Boar seminal plasma, a mixture of secretory products of the male reproductive organs, contains proteins of spermadhesins (AQN, AWN, PSP), DQH protein, and beta-microseminoprotein (beta-MSP). Protein structures, biochemical and binding features of the proteins have been described. Their origin in the male reproductive organs has been not resolved. Fluids from boar reproductive organs were tested with antiAQN, antiAWN, antiPSP, antibeta-MSP, and antiDQH antibodies. Immunofluorescence (IMF) was employed for the distribution of proteins on tissue sections and spermatozoa. The isolation of mRNAs of the DQH protein and beta-MSP, and PCR were used for their expression in reproductive organs. Spermadhesins were immunodetected in epididymal, prostate and seminal vesicle (SV) fluids, beta-MSP in prostate and SV fluids, DQH only in SV fluid. IMF displayed the presence of PSP and beta-MSP on prostate and SV sections, and epididymal spermatozoa. DQH was immunodetected only on SV sections. The DQH mRNA was found only in SV, the beta-MSP mRNAs in testis, SV and prostate. Spermadhesins and the DQH protein in seminal plasma predominately originated from seminal vesicles. They bind on the sperm surface during ejaculation and they participate in consequential steps of the reproduction process in the oviduct. The occurrence of spermadhesins in epididymal fluid and their role in sperm epididymal maturation is not clear.

Acknowledgment: Supported by grants Nos. 303/04/P070 and 303/06/0895 GACR; and AVOZ50520514.

PP-648
Potential biomarkers for ischemic heart damage identified in mitochondrial proteins

N. Kim, H. Kim, J. B. Youm, W. S. Park, M. Warda, V. C. Dang and J. Han
Mitochondrial Signaling Laboratory, Department of Physiology and Biophysics, College of Medicine, Cardiovascular and Metabolic Diseases Research Center, Biohealth Products Research Center, Inje University, Busan, Korea. E-mail: phykimnr@inje.ac.kr

We used proteomics to detect regional differences in protein expression levels from mitochondrial fractions of control,

ischemia-reperfusion (IR), and ischemic preconditioned (IPC) rabbit hearts. Using 2-DE, we identified 25 mitochondrial proteins that were differentially expressed in the IR heart compared with the control and IPC hearts. For three of the spots, the expression patterns were confirmed by Western blotting analysis. These proteins included 3-hydroxybutyrate dehydrogenase, prohibitin, 2-oxoglutarate dehydrogenase, adenosine triphosphate synthases, the reduced form of nicotinamide adenine dinucleotide (NADH) oxidoreductase, translation elongation factor, actin alpha, malate dehydrogenase, NADH dehydrogenase, pyruvate dehydrogenase and the voltage-dependent anion channel. Interestingly, most of these proteins are associated with the mitochondrial respiratory chain and energy metabolism. The successful use of multiple techniques, including 2-DE, MALDI-TOF-MS and Western blotting analysis demonstrates that proteomic analysis provides appropriate means for identifying cardiac markers for detection of ischemia-induced cardiac injury.

PP-649
Comparative phylogenic analysis of small GTP-binding proteins of *Medicago truncatula* and *Lotus japonicus*

B. Yuksel and **A. R. Memon**
TUBITAK, Research Institute of Genetics Engineering and Biotechnology, P. O. Box.21, 41470 Gebze, Kocaeli, Turkey.
E-mail: bayramyuksel@gmbae.tubitak.gov.tr

Small GTP-binding proteins play key regulatory role in multitude of cellular processes such as vesicle-mediated intracellular trafficking in plants and animals. *Medicago truncatula* has emerged as a model leguminous plant for the molecular and genetic dissection of various plant processes such as rhizobial, mycorrhizal interactions. *Medicago* and *lotus* are legumes with large number of sequences on databases. We gleaned through the publicly available online resources for these plants from the websites such as http://www.medicago.org, http://www.tigr.org to collect GTP-binding protein homologs. The collected sequences and Arabidopsis orthologs were phyletically analysed to shed light on evolution and to putative functions. One of the main emphases of the study was on elucidating the possible involvement of GTP-binding protein homologs in establishment of symbiotic relationship in root nodules. High frequency of vesicle-mediated trafficking in nodules may support the idea of subfunctionalization of some paralogs of this family in legumes specifically for nodule formation and development. We are hoping to determine list of possible candidates of small GTPases denoted likely to be expressed in nodules. The sequences of these selected genes could be used in more detailed molecular genetic analyses for clarifying role of small GTPases in nodulation. This study is hitherto the most comprehensive comparative evolutionary analysis of small GTPases in any legume species.

PP-650
Determination of alterations in protein profiles of brain tissues of experimental epilepsy models by proteome analysis

O. Danis[1], S. Demir[1], R. Aker[2], A. Gunel[3], F. Onat[2] and A. Ogan[1]
[1]Faculty of Arts and Sciences, Marmara University, Marmara, Turkey, [2]Faculty of Medicine, Marmara University, Marmara, Turkey, [3]Faculty of Arts and Sciences, Ondokuz Mayis University, Turkey. E-mail: odanis@marmara.edu.tr

Epilepsy, a chronic disorder characterized by repeated seizures resulting from abnormal activation of neurons in the brain. Although studies defined mutations and polymorphisms in genes related to Na^+, K^+, Ca^{++} channels and neuronal signaling in some types of epilepsy, there are few studies showing protein changes. Proteomics technology which has been developed in recent years, can detect the structure, quantity and post-translational changes of proteins in cells and tissues. The difference of this technique from other protein analysis methods is that its ability of high throughput quantitative and qualitative detection of all the proteins in cells or tissues with their post-translational modifications at a given time. In this study we investigated the protein changes in a genetic rat model of absence epilepsy Genetic Absance Epilepsy Rats from Strasbourg (GAERS) with proteomics technology-two dimensional gel electrophoresis (2-DE). We isolated protein samples from the GAERS ($n = 5$) and control Wistar group's ($n = 5$) cortex, thalamus and hippocampus. 2-DE was carried out with an immobilized pH gradient strip in the first dimension and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the second dimension. Over 500 polypeptide spots were resolved with a silver staining protocol by computerized 2-D gel analysis. We observed that protein profile of the diseased group (GAERS) is changed, intensity of some of the spots decreased while some of them increased.

PP-651
MHC class 1 peptide motif determination using a novel association rule mining method

A. Kucukural, G. G. Yardimci and O. U. Sezerman
Biological Sciences and Bioengineering Program, Sabanci University, Istanbul, Turkey.
E-mail: kucukural@su.sabanciuniv.edu

Computational approaches for the prediction of peptides binding to major histocompatibility complex (MHC) problem are of crucial importance for vaccine design since these peptides can act as a T-cell epitope to trigger immune response. There are two main branches for peptide prediction methods; structural and data mining approaches. These methods can be successfully used for prediction of T-cell epitopes in cancer, allergy and infectious diseases. In this paper association rule mining methods are implemented to generate association rules of peptide selection by MHCs. To capture the binding characteristics, modified rule mining and data transformation methods are implemented in this paper. Peptides are known to bind to same MHC show sequence variability, to capture this characteristics, we used reduced amino acid alphabet by clustering amino acids according to their physico-chemical properties. Using the classification of amino acids and using the OR-operator to combine the rules to reflect the fact that different amino acids types and positions along the peptide may be responsible for binding are the innovations of the method presented. We can predict the MHC Class-I binding with more than 85% coverage and more than 82% accuracy. Moreover, we also predict the key peptide positions for binding and motifs for several MHCs which can be used for selection of candidate T-cell epitopes.

PP-652
Intrinsic disorder in the function of the mediator

A. Toth-Petroczy, I. Simon and M. Fuxreiter
Institute of Enzimology, Budapest, Hungary.
E-mail: tothagi@enzim.hu

The Mediator is a large macromolecular complex comprising more than 20 different protein components that bridges RNA polymerase II and myriad DNA binding regulatory proteins. It

transduces both positive and negative signals that turn on and off messenger RNA synthesis in response to the ever changing micro-environment of the cell. Upon interacting with activator and repressor proteins the Mediator often undergoes a conformational change that is proposed to propel the assembly and disassembly of the pre-initiation complex. Thus for proper functioning, excessive and malleable binding surfaces of the Medator proteins are required that can accommodate versatile substrates and adopt alternative structures accordingly. For such purpose intrinsically unstructured segments can offer various functional benefits: flexibility, extremely large interacting surface and weak transient contacts with the partners that enable binding to multiple targets with separate or overlapping binding surfaces. We predict the intrinsically unstructured segments in sequences of mediator subunits and analyse their conservation among proteins from different sources. We propose that disordered regions can play pivotal role in regulatory machinery by maintaining the Mediator's enormous binding capacity.

PP-653
Structure-function relationship of restriction endonucleases

P. Doruker[1], O. Kurkcuoglu[1], **A. Uyar**[1] and L. Nilsson[2]
[1]*Chemical Engineering Department and Polymer Research Center, Bogazici University, Istanbul, Turkey,* [2]*Center for Structural Biochemistry, Karolinska Institute, Department of Biosciences, Huddinge, Sweden. E-mail: arzu.uyar@boun.edu.tr*

The collective dynamics of *Eco*RI-DNA complex is analysed with the computationally efficient elastic network model. For comparison, multiple molecular dynamics simulations are also performed on the complex, each of 1.5 ns duration. Our results indicate that the residues making specific and nonspecific interactions with DNA are relatively immobile even in the absence of DNA and preserve the shape of the DNA binding pocket. The interdigitated monomers of *Eco*RI form a stable inner core domain beneath the DNA binding region due to the clustering of hot spot residues, which also include the 'crosstalk ring' residues responsible for communication between distant active sites. Moreover, high positive cross-correlations are observed between the active sites in slow modes. The collective motions observed mainly result from the inner and outer loops, for which the stable inner core domain forms the base. Other domains that indicate mobility in certain slow modes about the inner core domain are the outer helices α1, α2, and αii. It is known that DNA distortion during binding, which is indispensable for bringing DNA and protein's functional groups together for recognition and catalysis, is energetically unfavorable. Our coarse-grained model indicates a favorable vibrational entropy change of the protein, $T\Delta S_{vib}$, which can partially compensate for the energetically unfavorable kink formation.

PP-654
Towards compartmentalised selections of protein-protein interactions using a split-protein sensor

P. Mathonet[1], H. Leemhuis[1], A. D. Griffiths[2] and F. Hollfelder[1]
[1]*Department of Biochemistry, University of Cambridge, Cambridge, UK,* [2]*Institut de Science et d'Ingenierie Supramoleculaires, Universite Louis Pasteur, Strasbourg, France. E-mail: pm354@cam.ac.uk*

Protein-protein interactions are key mediators of functions of life in the cell. They play crucial roles in regulation of enzyme action, in signal transduction and inter-cellular communication. If interactions between proteins are the central transmitters of function, then identification and prediction of protein interactions will be the basis for understanding the complexity of cellular networks. The overall goal of the project is to set-up a system to select protein-protein interactions by *in vitro* compartmentalisation in microdroplets. If two proteins A and B are interacting, an optical signal is generated in the droplet. Fluorescent microdroplets are sorted (by fluorescence activated cell sorting) and the pairs of genes contained therein recovered, amplified, and either characterised or re-selected. Model selections are carried out with bona fide interacting pairs, but we hope to be able to use this method for large-scale proteomic studies.

PP-655
Does information technology really help us?

M. Cihan[1], O. Cihan[2] and K. Erbil[1]
[1]*Biochemistry and Clinical Biochemistry Department, Gulhane Military Medical Faculty, Ankara, Turkey,* [2]*Information Management Department, Hacettepe University. E-mail: mcihan@gata.edu.tr*

Data management comprises all the disciplines related to managing data as a valuable resource. Data mining, also known as knowledge-discovery in databases (KDD), is the practice of automatically searching large stores of data for patterns. To do this, data mining uses computational techniques from statistics, machine learning and pattern recognition. Today internet is everywhere and also everyday all data about science is growing. Are we really able to manage this data? Data managers are not the persons who is interested in all the sciences about living organisms. There is a dilemma here. We use the data that is prepared by others. To solve this problem, we have some suggestions and hypothesis about more beneficial and efficient data management.

PP-656
The protein substrates of calpain-3: a proteomic approach.

I. Bertipaglia[1], D. Branca[1], I. Richard[2], A. Pahlman[3], L. Andersson[3], J. James[3] and E. Carafoli[1]
[1]*Department of Biochemistry, Venetian Institute of Molecular Medicine, University of Padova, Padova, Italy,* [2]*Généthon, Centre National de la Recherche Scientifique, Unitè de la Recherche Associèe, Evry, France,* [3]*Protein Technology, Lund Institute of Technology, Lund University, Sweden. E-mail: ilenia@gmail.com*

Limb-girdle muscular dystrophy type 2A is an autosomal recessive disorder, generated by inactivating mutations in the gene for the muscle specific protease calpain-3. Calpain-3 is a monomeric multidomain protein which differs from conventional calpains by three exclusive insertion sequences (NS, IS1, IS2). Domain I has a regulatory role, domain II is the proteolytic module, domain III links the catalytic domain to the Ca^{2+}-binding domain IV. Calpain-3 is unstable, and undergoes rapid Ca^{2+}-dependent autolysis in solution. Therefore, its heterologous expression and purification have been difficult. One open problem is the nature of the substrates of the protease. Their identification will help understanding why the absence of calpain-3 leads to muscle dystrophy. A comprehensive study aimed at identifying the substrates of calpain-3 was thus performed by carrying out MS analysis on digested 2D electrophoretic spots from myotube samples differently in the gel. Among them: Desmin, Nestin, Spectrin and PDLIM1 are of particular interest of calpain-3 knockout mice. The investigation has so far identified 21 protein spot that

migrate. We therefore assayed calpain-3 proteolytic activity against these proteins *in vitro*. Calpain-3 did not cleave the three cytoskeletal proteins, PDLIM1, instead, appears to be an *in vivo* substrate of the protease.

PP-657
Investigation of *Mycobacterium phlei* cell extracts by MALDI-MS

E. Buber[1], B. Orhan[1], O. Celikbicak[2], B. Salih[2], M. S. Keskin[1], Z. Saribas[3], A. Alpaslan[3], H. Ozen[4] and **N. L. Acan**[1]
[1]*Department of Biochemistry, Faculty of Medicine, Hacettepe University, Ankara, Turkey,* [2]*Department of Chemistry, Faculty of Science, Hacettepe University, Ankara, Turkey,* [3]*Department of Microbiology, Faculty of Medicine, Hacettepe University, Ankara, Turkey,* [4]*Department of Urology, Faculty of Medicine, Hacettepe University, Ankara, Turkey. E-mail: bagnuorhan@yahoo.com*

Researches with *Mycobacterium phlei* are carried away to find an efficient therapeutic agent for the treatment of some type of superficial bladder carcinoma. It is proposed that the components of the mycobacterial cell wall has both immunostimulating and direct apoptotic activities. Several different types of *Mycobacterium phlei* cell extracts were prepared by biophysical procedures and the immunostimulating activity of these fractions were searched. It was found out that some of the fractions showed TNF-alpha and IL-12 response. All the samples were analysed by MALDI-MS using alpha-cyano-4-hydroxycinnamic acid matrix and observed that detergent soluble proteins having molecular weight around 7800 and 12700 Da seem to be responsible for cytokine activation.

Acknowledgment: This work is a part of the project supported by Hacettepe University, Research Unit, Project No. 03G31.

PP-658
Alkaline proteome analysis of *Acinetobacter radioresistens* S13

P. Fattori[1], R. Mazzoli[1], M. G. Giuffrida[2], C. Lamberti[1], C. Barello[2], E. Pessione[1] and C. Giunta[1]
[1]*Human and Animal Biology Department, University of Turin, Turin, Italy,* [2]*ISPA, CNR, Colleretto Giacosa (TO), Italy. E-mail: paolo.fattori@unito.it*

Acinetobacter radioresistens S13, a bacterial strain selected for its ability to biodegrade phenol, biosynthesizes, during growth on aromatic carbon sources, a number of proteins which are absent in control conditions. The aim of this research was to identify proteins, specifically induced by aromatic substrates (benzoate and/or phenol), having alkaline isoelectric points (between 6 and 11), thus integrating results obtained in previous works, concerning the analysis of auxiliary, citosolic and membrane, proteins with acidic pI (pH 4–7). The comparative proteome analysis showed 25 spots differentially expressed: at present six of them have been identified by ESI-MS/MS spectrometry. The biological role of the six identified proteins can be led back to four classes: (a) Proteins involved in uptake of nutrients inside the cell or in extrusion of toxic molecules (tsp Protease); (b) Proteins involved in attenuation of direct and indirect toxic effects of aromatic molecules on bacterial cells (tsp protease, S2 protein of the 30S ribosomal subunit, pseudouridine synthase β subunit);
(c) Proteins connected with energy metabolism (soluble transhydrogenase for pyridine cofactors, ATP synthase γ subunit) (d) Proteins connected with pH homeostatis (glutaminase/asparaginase). These results improved our knowledge on the physiology of this Acinetobacter radioresistens strain by giving new insights on regulation and adaptation mechanisms of bacterial cells to aromatic exposure.

Clinical Proteomics

PP-659
Analysis of SLAM (CD150) expression on subacute sclerosing panencephalitis patients.

P. Akpinar[1], B. Anlar[2] and A. K. Piskin[3]
[1]*Department of Medical Biology, Faculty of Medicine, Hacettepe University, Ankara, Turkey,* [2]*Department of Pediatric Neurology, Faculty of Medicine, Hacettepe University, Ankara, Turkey,* [3]*Department of Biochemistry, Faculty of Medicine, Hacettepe University, Ankara, Turkey. E-mail: akpinarpinar@hotmail.com*

Measles virus (MV) causes an acute childhood disease which still claims roughly 1 million lives a year. Major reasons of mortality are the secondary infections due to the immunosuppressive effect of measles virus. The most common late complication of measles is subacute sclerosing panencephalitis (SSPE). Signaling lymphocytic activating molecule (SLAM) is the membrane receptor for all species of measles virus. It is a member of immunoglobulin superfamily and an integral membrane glycoprotein having a molecular weight of approximately 70 kDa. SLAM is expressed especially by activated T and B lymphocytes and memory cells. We examined the expression of SLAM's sub-types on the mononuclear cell surface under different conditions and we have analysed the receptor by western blot technique and immunoblotting after analysing the plasma membrane proteins of activated peripheral blood lymphocytes and leukocytes of subacute sclerosing panencephalitis (SSPE) patients. Furthermore we examined the expression of SLAM's subtypes on brain tissue of SSPE patients. As a result we found that different SLAM-subtypes are expressed especially on the surface of monocytes and lymphocytes of SSPE patients and we examined more SLAM Subtype bands on the brain tissue of SSPE patients. As addition to that, when we activated periferal blood leukocytes of SSPE patients by con-A or LPS, we saw an increase in the size of the SLAM's band on immune blot.

PP-660
α1-acid glycoprotein is marker of cancer progression

A. Mikhailov
Engineering of Cell, Institute of Biophysics and Cell Engineering, NAS, Belarus. E-mail: alexbunder@yahoo.com

α1-acid glycoprotein (AGP) is acute phase protein which concentration is rapidly increased in different diseases including wide spectra from tuberculosis to cancer. For diagnostics of these cases the clinicists need fast and not time-consumed test of determination of inflammatory markers in human blood. AGP consist of two parts: protein and carbohydrate chains, which form a glycan core in glycoprotein. We developed a fluorescent method of AGP determination in human blood using fluorescent probe Quinaldine Red which specifically binds with AGP on cationic binding site of protein part. Fluorescent measurements strongly correlate with IFA methods but IFA is more expansive and need additional setups in clinical laboratories. With close collaboration of Byelorusian Cancer Center we measured more then 50 samples

of patient's plasma having breast and lung cancer and concluded that our method is high sensitive and adequate for all requirements. In case of breast cancer the concentration of AGP has increased ten-folds. In several patients we did not observe increasing of AGP level but we detected increasing of tryptophan fluorescence we explained as the conformational changes of AGP is following to the movement of Trp residues to the inner pocket of protein part. Thus we should take in consideration as concentration of AGP as binding properties, which are depended on conformational state of glycoprotein.

PP-661
Expression of hypothetical *B. garinii* protein from *Erp* gene family in *E. coli*.

R. Ranka and **V. Baumanis**
Laboratory of molecular microbiology, Biomedical Research and Study Centre, Riga, Latvia. E-mail: renate_r@biomed.lu.lv

Borrelia burgdorferi, the Lyme disease spirochete, undergoes changes in antigenic composition as it cycles between its arthropod and mammalian hosts. *Erp* gene family is cp32 plasmid-encoded lipoprotein genes with highly polymorphic nature. It was shown, that outer membrane surface proteins from *Erp* family bind host complement regulatory factor H. This mechanism helps the bacteria to avoid killing by the alternative complement pathway during vertebrate infection. These proteins were shown to be immunogenic, so that they may be used as potential targets for the serodiagnosis of Lyme disease. In Europe, *B. garinii* and *B. afzelii* are main species caused Lyme disease in humans. Our research indicated the presence of potential gene in *B. garinii* genome similar to those of *Erp* gene family. Sequence analyses revealed that it predicted to be antigenic and surface exposed. The gene was amplified by PCR and cloned in pBAD/TOPO expression system. Different levels of expression were observed in *E. coli* depending on growing conditions. Because the target protein was obtained both in soluble and insoluble fractions, here is the possibility to purify both the native and denatured protein. Analysis of this potential protein will give additional information about the pathogenesis of *B. garinii* infection and may be useful in serodiagnosis of Lyme disease.

Acknowledgement: This work has been supported by European Social Fund.

PP-662
Resolution of bovine plasma Gla proteins by two dimensional gel electrophoresis

F. Uras, U. Gurbuz, H. Aksoy and T. Yardimci
Biochemistry Department, Faculty of Pharmacy, Marmara University, Istanbul, Turkey. E-mail: furas@marmara.edu.tr

Prothrombin, factor VII, factor IX, factor X, protein C, protein S and protein Z are well-known vitamin K dependent (Gla proteins) proteins which have been purified systematically by time consuming chromatographic methods. It may be useful in clinical practice/research to perform a less time consuming procedure for simultaneous isolation/quantification of Gla proteins from plasma. The purpose of this study is to resolve the Gla proteins by two-dimensional (2-D) gel electrophoresis. Gla proteins isolated from normal bovine plasma by barium citrate adsorption and ammonium sulfate elution was subjected to 2-D gel electrophoresis. It was carried out with a pH gradient in the first dimension and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the second dimension. More than eight protein spots were visualized by staining with Coomassie Blue R-250. The number of protein spots was more than the number of well-known plasma Gla proteins. Some of them might be contamination of plasma proteins or degradation products. 2-D method can be used for resolution of human plasma Gla proteins. Further mass spectrophotometric evaluation of the 2-D pattern will be providing useful data for research/clinical studies. Computer assisted densitometry of the human protein pattern of 2-D may be helpful in clinical studies for simultaneous quantification of plasma Gla proteins.

Acknowledgment: This study was supported by a grant from Marmara University Scientific Research Project Commission (SAG-039/131102).

PP-663
Necrotic injury of a myocardium at the experimental crush syndrome and an opportunity of its treatment

G. Kevorkian[1], A. Kanayan[2], H. Hayrapetyan[2], A. Guevorkian[1], L. Melkonyan[1] and A. Galoyan[3]
[1]Laboratory of Pathological Biochemistry, H. Buniatian Institute of Biochemistry, Yerevan, Armenia, [2]Department of Clinic Pathological Anatomy, Medical University, Yerevan, Armenia, [3]Department of Neurohormones, H. Buniatian Institute of Biochemistry, Yerevan, Armenia. E-mail: kevork_neurochem@mail.ru

Trauma of skeletal muscle with long-time compression, widely known as crush syndrome (CS). There are numerous data indicating that the main intoxication of the organism occurs during decompression, in which toxic metabolic products of the peptides nature are released into the blood from the damaged tissue and kidney and accumulated at the myocardium. CS was induced by compression of femoral soft tissues for 2 and 5 h. The animals were divided into the following groups: intact, control (2-and 5 h of compression), and experimental (2, 4, 24, and 48 h of decompression). As a result of hyperkalemia during decompression period there are cardiac arrests in connection with infringement of potential of atrioventricular node. The nature development of necrotic injury of a myocardium remains unknown. We found out five peptides containing 5–9 amino acids residues which are absent in myocardium during compression and start to appear from 2–4 h of decompression period. These peptides are localized in atrioventricular node of myocardium. Most likely they are a 'myocardium depressing factors' causing necrosis of myocardium and cardiac arrest. Allocated in the damaged by CS myocardium, peptides, after intravenous administration at the intact animals, get collected in a myocardium and cause necrotic injury of myocardium. Introduction of hypothalamic natural cytokine 'proline rich peptide' (PRP) interfere with formation of toxic peptides and their moves into a myocardium.

PP-664
Clinical utility of serum clusterin isoforms in colorectal cancer diagnosis and prognosis

A. M. Rodríguez-Piñeiro[1], M. Páez de la Cadena[1], L. De Chiara[1], A. López-Saco[2] and F. J. Rodríguez-Berrocal[1]
[1]Departamento de Bioquímica, Genética e Inmunología, Universidad de Vigo, Vigo, Spain, [2]Servicio de Cirugía, Complejo Hospitalario Universitario de Vigo, Vigo, Spain.
E-mail: anarp@uvigo.es

Clusterin (CLU) is an enigmatic protein expressed in a wide range of tissues and altered in a variety of diseases, including cancer. Regarding human colorectal cancer (CRC), tissue CLU has been proposed as a potential indicator for diagnosis. Furthermore, its differential expression in highly aggressive colon tumours and metastatic nodes suggests a possible prognostic and

predictive role. However, to date there are not many studies regarding CLU in serum. Therefore, we studied the serum levels of CLU in healthy donors and CRC patients, aiming to analyse its clinical utility for diagnosis and prognosis of CRC. Sera were processed through a Concanavalin A chromatography, obtaining a fraction (FI) enriched in O- and non-glycosylated proteins, and another one (FII) enriched in N-glycoproteins. Total CLU serum levels were analysed by ELISA, whereas FI and FII were inspected by immunodetection after slot blot. Regarding total serum, we found a significant increase of CLU levels in CRC patients. The construction of a ROC curve showed a cut-off point that allowed 48% sensitivity with 91% specificity, yielding 70% diagnostic efficiency. On the other hand, immunoquantification showed a 4.2-fold increase in patients FI. Furthermore, the amount of this CLU fraction was significantly related to the existence ($P = 0.004$) and distance ($P = 0.011$) of metastasis in patients. Therefore, serum CLU behaves as a potential clinical marker both for CRC diagnosis and prognosis.

PP-664a
The new enzyme methods for determination of glutamate in clinic and in food stuffs

Zh. Kudiyarova, B. Tyimebaeva and Zh. Rakhmetova
M. A. Aytkhozhin's Institute of Molecular Biology and Biochemistry. E-mail: 13575@mail.ru

The problem of determination of glutamate has great value for public health care. It is known, that glutamate is neurotoxic substance. However till now there are no satisfactory and high-sensitivity methods for determination of glutamate. Used for this purpose Gdh from the bovine liver is very unstable enzyme and has low affinity to glutamate. In M. A. Aytkhozhin's Institute of Molecular Biology and Biochemistry was discovered the new enzymes complex malate dehydrogenase - glutamate oxaloacetateaminotransferase (EC). EC catalyses the next consequence of reactions: MDh oxidizes malate to oxaloacetate and reduces NAD to NADH; then oxaloacetate transaminate with glutamate and formes aspartate and 2-oxoglutarate. As EC catalyses irreversible sequence of reaction this makes very convenient for determination of glutamate while on activity of EC did not influence the products of reactions. We develop the effective methods for purification of EC from cheaper plant materials by ion-exchange and gel chromatographies. Test of purified EC for determination of glutamate in biological liquids and in foods stuffs shown high sensitivity, reliability and reproducibility. For one analysis is necessary 2–3 min.
Application of this method will allow to diagnose quickly and precisely various pathological conditions in clinic and determination of glutamate in food stuffs for juvenile children will allow to prevent the damaging of their brains.

PP-664b
Interaction between bovine serum albumin loaded with fatty acids and polyamidoamine dendrimer

D. Shcharbin and M. Bryszewska
Department of General Biophysics, University of Lodz, Lodz, Poland. E-mail: shcharbi@biol.uni.lodz.pl

Dendrimers are newly synthesized globular, large monodisperse polymers suitable for a wide range of biomedical applications. Their application for drug targeting requires studying their interactions with serum albumins – the main binding and transport proteins of blood. In blood serum albumins are usually loaded with various fatty acids (up to 30%). We examined the interaction between bovine serum albumin (fatty acids free), bovine serum albumin loaded with oleic acid, linoleic acid, oleic + linoleic acids, oleic + linoleic + arachidonic acids and polyamidoamine dendrimer of 5th generation, using fluorescence technique. The data show that dendrimer quenches intrinsic fluorescence of protein, increases its polarization, affects the position of the spectrum maximum, changes the ability of protein to bind fluorescent probe ANS. These changes depend on loading of protein with fatty acids. It can be concluded that dendrimers can affect protein conformation and its binding properties and the strength of that interaction depends on type of fatty acid attached.
Acknowledgement: Dr. Dzmitry Shcharbin is a beneficiary of a Marie Curie International Incoming Fellowship within the 6th EU Framework Programme (grant 510018).

PP-665
HPLC and HPCE determination of homocysteine as a criterion of vascular diseases risk factor

I. O. Melnikov[1], I. V. Nazimov[2], E. A. Stukacheva[2]and Yu. M. Glubokov[1]
[1]Department of Analytical Chemistry, M. V. Lomonosov Moscow State Academy of Fine Chemical Technology, Moscow, Russia, [2]Laboratory of Protein Analytical Chemistry, M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia. E-mail: myabstracts@yandex.ru, nazimov@ibch.ru

Nowadays increased level of homocysteine (Hcy) in blood is generally excepted vascular diseases risk factor. The most sensitive analysis of Hcy requires its derivatization by fluorogenic reagents. The derivatization of Hcy by monobromobiman (mBrB) as well as by 5-iodoacetamidofluorescein (5-IAF) was investigated. Fluorescent derivatives of Hcy were isolated and characterized by high performance liquid chromatography (HPLC), high performance capillary electrophoresis (HPCE) and mass-spectrometry (MS). Methods of Hcy quantitative determination by reversed phase (RP) HPLC with fluorometric (FL) and direct UV detection were developed. Sensitivity of FL detection (1 nmol/ml) noticeably exceeds the sensitivity of direct UV detection. mBrB and 5-IAF Hcy derivatives were separated by HPCE and identified with direct UV and FL detection. The same derivatives were characterized by MS (MALDI-TOF, ESI-MS). Micellar electrokinetic chromatography was more preferred for Hcy determination using mBrB as a marker. Detection limits: direct UV detection – 250 nmol/ml, FL detection – 5 nmol/ml. RP HPLC and HPCE Hcy quantitative determination in blood (physiological concentration range 5–50 nmol/ml) was compared. HPCE provided better peak resolution, but sensitivity of direct UV detection was essentially worse using standard procedure of analysis. The lowest level of Hcy quantitative determination in blood (RP HPLC with FL detection) was 1–5 nmol/ml.

DNA and Protein Microarrays

PP-666
Noninvasive breast cancer diagnostics based on extracellular DNA analysis in blood and urine.

E. Rykova, T. Skvortsova, S. Tamkovich, O. Bryzgunova, V. Vlassov and P. Laktionov
1Institute of Chemical Biology and Fundamental Medicine, Siberian Division of the Russian Academy of Sciences, Novosibirsk, Russia. E-mail: rykova@niboch.nsc.ru

Extracellular RNA and DNA concentrations were detected using fluorescense-based assay. In healthy women cirDNA were found in the bloodstream predominantly (98%) attached to the surface of blood cells. In the blood of 80% of breast cancer patients of cirDNA were found in plasma in increased amounts and were undetectable in the cell-surface-bound fractions. Patients with fibroadenoma demonstrate increase of cirDNA in plasma and detectable amounts of cell-surface-bound cirDNA. Thus increasing amount of free cirDNA along with disappearance of cell-surface-bound cirDNA correlate with breast cancer development. Presence of methylated RARβ2, RASSF1A and Cyclin D2 gene promoters in different fractions of cirDNA in blood and urine in healthy donors and patients with breast tumors was investigated. Analysis of methylated markers in the total extracellular DNA (cell-free and cell-bound) in blood and urine provides 95% diagnostic coverage in breast cancer patients, 60% in patients with benign lesions without false positive results in healthy women. Results of the study indicate that the total cirDNA provides more valuable and informative material for methylation – specific PCR analysis, compared with free plasma and urine extracellular DNA only. Analysis of RARβ2, RASSF1A and Cyclin D2 promoter methylation in the total extracellular DNA from blood and urine can be used as the reliable marker of breast tumor development.

PP-667
SNP microarray analysis in human hepatocellular carcinoma cell lines reveal new candidate miRNAs as cancer causing genes

K. Demir and C. Yakicier
Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey. E-mail: kdemir@bilkent.edu.tr

Changes in DNA copy number is one of the mechanisms that may result in abnormal expression and function of genes, including non-coding micro RNA (miRNA) genes. Lately, miRNAs have been shown to be involved in gene regulation and carcinogenesis. Amplifications (AMP), homozygous deletions (HD), loss of heterozygosity (LOH) and mutations are common mechanisms of carcinogenesis in human epithelial cancers and hepatocellular carcinomas (HCCs). Recently developed high throughput genotyping technique with single nucleotide polymorphisms (SNPs) is an efficient method to detect genome-wide copy number changes (CNC). In the framework of this study, we analysed CNCs in a panel of 14 human HCC cell lines (SkHep1, Huh7, HepG2, PLC, MV, Focus, SNU182, SNU387, SNU398, SNU423, SNU449, SNU475) using 10K Affymetrix SNP array. Our results confirmed CNCs in previously described regions; 4q and 8q (HD), 16q and 17p (LOH) and 1q and 20q (AMP) in HCC cell lines. Additionally, our data revealed novel HDs at 9p21 region, where hsa-mir-31 miRNA maps and miRNA rich 14q32.2-14q33.1 border, harboring hsa-mir-342 and 345 miRNAs. These miRNAs are candidates to be involved in HCC development. Additionally, we observed frequent chromosomal aberrations in telomeric and non-genic regions. These results may provide new insights in our understanding of HCC biology. Our ongoing research on HCC genesis, as well as recent results on new and previously identified regions and candidate miRNAs will be discussed.

PP-668
Ets-homologous factor as a candidate cirrhosis marker among upregulated genes in senescent clones

G. S. Bagislar[1], N. Ozturk[1], H. Ozdag[2], T. Yagci[1] and M. Ozturk[1]
[1]Molecular Biology and Genetics, Bilkent University, Turkey, [2]Department of Biotechnology, Ankara University, Turkey. E-mail: sevgib@fen.bilkent.edu.tr

Cirrhosis represents the final common histologic pathway for a wide variety of chronic liver diseases. In attempts to find the explanation for the pathophsiology of cirrhosis, it has been showed that telomeres were significantly shorter in cirrhosis compared to noncirrhotic samples. We think that fibrotic scarring at the cirrhosis stage may be a consequence of hepatocyte telomere shortening and senescence. Our group has recently identified that some clones in HCC cell lines enter senescence as a result of telomere shortening. Expression microarray analysis of these senescent clones revealed approximately 2700 significantly altered genes when compared to immortal clones. Approximately 1600 of these were significantly overexpressed in senescent clones. Ets-homologous factor (EHF) is upregulated 23.3 fold in senescent clones compared to non-senescent immortal clones. After confirmation of the microarray result of EHF by RT-PCR, we investigated the expression level of it in HCC cell lines, and also in cirrhotic, HCC, normal liver biopsy specimens. None of the tested HCC cell lines, except Snu423 had EHF expression. EHF revealed very strong expression in all (4) cirrhosis biopsy samples. Whereas, only one of six HCC samples and none of two normal liver samples had EHF expression. As a conclusion, EHF may be a good candidate to be used as cirhossis marker among upregulated senescent associated genes emphasizing that senescence may be hallmark of the liver cirrhosis.

PP-669
RHEB expression in fibroadenoma of the breast

A. Han[1], C. Ying[2] and **K. Park**[2]
[1]Department of Surgery, Wonju Christian hospital, Yonsei University, Korea, [2]Department of Pathology, Wonju Christian hospital, Yonsei University, Korea. E-mail: abba@yonsei.ac.kr

Although fibroadenoma is the most common benign tumor of the breast, specific genes of the tumor have not been acknowledged. Fibroadenoma is the most common tumor that can be mistakenly diagnosed as a breast cancer in fine needle aspiration cytology. In this study, we experimented to find a tumor marker for diagnosing fibroadenomas by discovering specific genes for the tumor. Using Platinum Human Cancer 3.0k chip (Genocheck, Korea), microarray analysis was performed. In five fibroadenoma samples, HDAC, ROS, TNFRSF10A, WASP2, TYRP1, WEE1, and RHEB were genes that expressed more than twofold increase. In 20 breast cancer tissue extracts from infiltrating ductal cell carcinoma patients and 20 fibroadenoma tissue

samples, the result of RT-PCR for RHEB showed that the expression of RHEB in fibroadenoma tissue samples increased, when compared to the level of RHEB expressed in breast cancer samples. When tested for real time PCR, the average RHEB/b-actin ratio was 2.46 times increase, when compared to infiltrating ductal cell carcinoma samples. It showed a significant change statistically. ($P < 0.01$) In fibroadenoma samples, immunohistochemical staining showed increase in the expression of RHEB. In conclusion, RHEB has a potential to be used as the specific marker to diagnose fibroadenomas.

PP-670
Detection of gastrointestinal pathogens using DNA microarrays

S. Y. Lee, D. H. Kim, J. E. Min and **Y. C. Kim**
School of Life Sciences, Chungbuk National University, Cheongju, Korea. E-mail: youngkim@chungbuk.ac.kr

Microarrays were used for accurate and rapid detection of gastrointestinal infectious pathogens such as *Vibrio* spp. We developed a microarray platform for detecting genus-specific, species-specific and virulence genes from gastrointestinal pathogens. Oligonucleotide probes (about 20-mers) for these targets at the 5' end of each probe was tailed with 15 dTTP molecules (T-spacer) to increase the on-array accessibility of spotted probes to target DNA. In addition, the 5'-terminal nucleotide of each probe was aminated to allow covalent coupling of the oligonucleotide to aldehyde group-coated slide by using a MicroGrid II arrayer. DNA for hybridization were amplified from complex genomic DNA by using ϕ29 polymerase-plus-Klenow tandem random amplification strategies and labeled with Cy3-dCTP simultaneously. Slides were imaged by using an aQuire scanner and the image was analysed by using a GenePix Pro 6.0 software. Furthermore, we performed multiplex PCR assays in order to confirm the efficiency of tandem random amplification. Random amplification approach was more effective than PCR-based amplification such as a multiplex PCR, because it was able to provide a more uniform genetic locus representation and perform reactions of numerous probes simply.

PP-671
Preamplification of sample limited specimens for real-time gene expression analysis

S. Stanley[1], J. Wilde[1], C. Lee[1], Y. Tesfaigzi[2] and **J. Stevens**[1]
[1]Molecular Biology, Applied Biosystems.Foster City, USA, [2]Asthma and Immunology Program, Lovelace Respiratory Reserach Institute, Albuquerque, USA.
E-mail: stevenjn@appliedbiosystems.com

Translational clinical researchers often face the difficulties of analysing multiple gene expression profiles using limited sample quantities. We have developed a robust solution for uniform amplification of cDNA prior to quantitative, real-time PCR. TaqMan(Preamp Master Mix allows preamplification of up to 100 gene targets simultaneously using the TaqMan (Gene Expression Assays as the source of pooled gene-specific primers. TaqMan assay-based preamplificaion preserves equilibrium of targets and retains the relative copy numbers of starting targets in a reproducible and precise manner. Uniformity of preamplification was demonstrated using clinical samples such as bronchial brushings. Bronchial brushings were obtained by bronchoscopy from subjects without lung disease, from mild asthmatics, and from chronic bronchitics. RNA extracted from clinical samples was reverse transcribed to cDNA using High-Capacity cDNA archive kit (P/N 4322171). A range of 1–250 ng of cDNA was used for preamplification. Various quality of RNA samples were examined for preamplification. TaqMan assay based preamplification is amenable to partially degraded RNA. RIN (RNA integrity number) can be as low as seven without losing the uniformity of 100-plex preamplification. TaqMan assay based preamplification has no 3' bias. The simple workflow enables researchers to enrich the amount of limited RNA samples uniformly within 1.5 h.

PP-672
Identification of binding proteins to transglutaminase 2 using protein microarray

S.-S. Park and S.-Y. Kim
Molecular Oncology Branch, Research Institute, National Cancer Center, Goyang, Gyeonggi-do, Korea.
E-mail: sspark01@gmail.com

Transglutaminase 2 (TGase 2) is a multifunctional enzyme that plays an important role in various physiological and pathological processes. To understand roles of TGase 2, we analysed proteins interacting with TGase 2 under various conditions using protein microarray and monoclonal anti-TGase 2 antibody. The microarray data showed that the binding proteins to TGase 2 are also dependent on Ca^{2+} ion or GTP. In the absence of Ca^{2+} ion, TGase 2 prefers to interact with polo-like kinase 3 (PLK3). Interestingly, in the presence of Ca^{2+} ion, TGase 2 interacted with multiple proteins including zinc finger FYVE domain containing 28 (ZFYVE28), v-ros UR2 sarcoma virus oncogene homolog 1 (ROS1), reticulon 1, Bcl2 modifying factor, and 3-phosphoinositide dependent protein kinase-1 except PLK3. In the presence of GTP, TGase 2 interacted with multiple binding proteins including ZFYVE28 and ROS1, ortholog of mouse D11lgp2, and TBP-like 1. Physiological roles of those binding proteins are under investigation. The substrate specificity of transamidation will be analysed using biotinylpentylamine and Alexa Fluor 647-tagged streptavidin in near future. This study will guide us to the important clues to the roles of TGase 2 in various pathogenic processes.

PP-673
The prevalence of MEFV gene mutations in west Mediterranean region of Turkey

R. Sutcu[1], T. Ercan[2], S. Mehmet[2], A. Onur[1], A. Osman[2] and D. Namik[1]
[1]Biochemistry, Suleyman Demirel University Medical Faculty, Isparta, Turkey, [2]Internal Medicine, Suleyman Demirel University Medical Faculty, Isparta, Turkey.
E-mail: recepsutcu@med.sdu.edu.tr

Familial Mediterranean Fever (FMF) is an autosomal recessive disease characterized clinically by recurrent short, self-limited attacks of fever accompanied by severe abdominal pain due to inflammation of the abdominal cavity. FMF is mostly prevalent four ethnic groups: Armenians, Arabs, Turks and Jews. MEFV gene, mapped to chromosome 16p13.3, codes the protein pyrin, which has an important role in the etiology of disease. Assay that we use for the identification of MEFV gene mutations based on polymerase chain reaction (PCR) and reverse-hybridization. 274 cases that have been suspected with FMF disease were analysed 12 mutations in the MEFV gene: M694V, M694I, M680I (G/C), M680I (G/A), E148Q, V726A, K695R, R761H, P369S, A744S, F479L, I692del. In 149 cases out of 274 MEFV gene mutations was observed (54.3%). And there were no MEFV gene mutations in 125 cases. In 42 individuals (39.25%) compound heterozygosity was determined that include mostly M694V, M680I (G/C), E148Q, V726A gene mutations. Other 107 mutation cases were

comprised of 26 (24.3%) homozygote and 39 (36.44%) heterozygote mutations. Most of the mutations observed in our cases were in codon 694 and 680 in our series. The diagnosis of FMF is not easy all the time. Today, finding the molecular alterations in MEFV gene helps the correct diagnosis.

PP-674
Detection of gastrointestinal pathogens using DNA microarrays

S. Y. Lee, D. H. Kim and **Y. C. Kim**
School of Life Sciences, Chungbuk National University, Cheongju, Korea. E-mail: youngkim@chungbuk.ac.kr

Microarrays were used for accurate and rapid detection of gastrointestinal infectious pathogens such as *Vibrio* spp. We developed a microarray platform for detecting genus-specific, species-specific and virulence genes from gastrointestinal pathogens. Oligonucleotide probes (about 20-mers) for these targets at the 5' end of each probe was tailed with 15 dTTP molecules (T-spacer) to increase the on-array accessibility of spotted probes to target DNA. In addition, the 5'-terminal nucleotide of each probe was aminated to allow covalent coupling of the oligonucleotide to aldehyde group-coated slide by using a MicroGrid II arrayer. DNA for hybridization were amplified from complex genomic DNA by using ϕ29 polymerase-plus-Klenow tandem random amplification strategies and labeled with Cy3-dCTP simultaneously. Slides were imaged by using an aQuire scanner and the image was analysed by using a GenePix Pro 6.0 software. Furthermore, we performed multiplex PCR assays in order to confirm the efficiency of tandem random amplification. Random amplification approach was more effective than PCR-based amplification such as a multiplex PCR, because it was able to provide a more uniform genetic locus representation and perform reactions of numerous probes simply.

PP-675
Mutation detection by SPR approach in a model of hemoglobin S

E. Ustel, A. Atalay and E. O. Atalay
Department of Biophysics, Medical Faculty, Pamukkale University, Denizli, Turkey. E-mail: eustel@pau.edu.tr

Surface plasmon resonance (SPR) is a novel approach to be used for the characterization of molecular interactions. Basically this approach is an optical technique using evanescanet wave phenomenon to measure changes in refractive index as a function of the interactions occurred in between two molecules under interest. The molecular interactions can be in between protein-protein, protein-nucleic acid, protein-ligand, nucleic acid-nucleic etc. In our study; the Hb S (GAG- > GTG) mutation at codon 6 of human beta globin gene was used as model mutation detection system. We examined the use of SPR system in premarital screening of the mutations for the abnormal hemoglobins like Hb S (beta6, GAG- > GTG), Hb D-Los Angeles (beta121, GAA > -CAA), Hb C (beta6, GAG > AAG), especially in the regions that these abnormal hemoglobins observed frequently like Denizli, an Aegean geographical region of Turkey. According to our preliminary results, SPR system can be used as a cheap and powerful approach for the quick molecular detection of abnormal hemoglobins in premarital screening programs meriting for further research and development studies.

Pharmacogenomics and Toxicogenomics

PP-676
Carcinogen-activating cytochrome P4502E1 variants in Turkish population

G. Ulusoy, E. Arinç and O. Adali
Biochemistry Graduate Programme and Department of Biological Sciences, Middle East Technical University, 06531, Ankara, Turkey. E-mail: gulusoy@metu.edu.tr

CYP2E1, a carcinogen-activating isoform of cytochrome P450, possess several polymorphisms throughout its gene that are thought to affect the risk of susceptibility to related diseases, including cancers. As the allele frequencies show inter-ethnic variability, it is of great importance to determine the allele frequencies of *CYP2E1* in Turkish population. In this study, three polymorphisms, namely *CYP2E1*5B, *6,* and **7B* allele frequencies were determined in a sample of 206 unrelated healthy individuals representing Turkish population. For this reason, DNA was isolated from whole blood and genotyping was performed by PCR amplification followed by digestion with *Pst*I/*Rsa*I, *Dra*I and *Dde*I restriction enzymes for **5B, *6,* and **7B* polymorphisms, respectively. The allele frequency for **5B* allele (G-1293C/C-1053T; *Pst*I/*Rsa*I RFLP) was found to be 1.9%. *CYP2E1*6* (T7632A; *Dra*I RFLP) allele frequency was determined as 8.3%, and **7B* (G-71T; *Dde*I RFLP) allele frequency as 6.8% for Turkish population. *CYP2E1*5B* and **6* allele frequencies for Turkish population were found to be similar to Caucasian populations –like German, French, Italian, while they were significantly different from Chileans, Mexican-Americans and Asian populations like Chinese, Taiwanese and Japanese populations ($P < 0.005$). There is limited data on **7B* allele frequency in literature, but there is no significant difference between Turkish and German population. Population studies like this could be useful in assessing the susceptibility of different populations to diseases related to CYP2E1 polymorphisms.

PP-677
Alcohol toxicity, Arg47His alcohol dehydrogenase and Glu487Lys aldehyde dehydrogenase polymorphisms in a Mexican population

B. E. Bastidas-Ramírez[1], C. Montes-Gomez[1], D. Gordillo[1], E. Gordillo[1], M. González-Hita[2], M. A Jimenez[3], J. J. Garcia-Bañuelos[1] and A. Panduro[2]
[1]Molecular Biology and Genomics Department, University of Guadalajara, Mexico, [2]Service of Molecular Biology in Medicine of the Civil Hospital, Mexico, [3]Hospital Valentin Gomez Farias, ISSSTE, Mexico. E-mail: bastidas@cencar.udg.mx

Introduction: Alcohol dehydrogenase (ADH2) and aldehyde dehydrogenase (ALDH2) are the main alcohol metabolizing enzymes in its conversion to acetaldehyde and acetate. Substituting Arg47 to His in ADH2 gene, renders a 100 times more active enzyme than the wild type. Substituting Glu487 to Lys in ALDH2 gene, results in an inactive enzyme. These polymorphisms could lead to acetaldehyde accumulation, and exert its toxic liver effects in persons who drink alcohol.

Objective: To determine the frequency and association of these polymorphisms to alcohol induced liver damage in a Mexican alcoholic population.
Methods and Patients: We studied 30 patients with alcoholic cirrhosis (AC) from the civil hospital of Guadalajara and 70 healthy individuals as control (C). DNA was isolated from 3 ml peripheral blood and studied the polymorphisms using RFLPs.
Results: Wild ADH2 allele was observed in 84% of C and 97% of AC. Polymorphic ADH2 allele frequency (16%) was higher ($P < 0.05$) than that observed in AC (3%). Genotype Arg47His/Arg47His was not observed. Regarding ALDH2, we could not observe the polymorphic Glu487Lys allele in either C or AC group.
Discussion and Conclusions: A very low frequency of the polymorphic Arg47His ADH2 allele was found. Protection against drinking habit has been attributed to Arg47His ADH2 allele and Glu487Lys ALDH2. Therefore, the high frequency of alcoholic cirrhosis in our country could be influenced by the lack of these protective alleles.

PP-678
The association of polymorphisms in biotransformation enzymes with colorectal cancer

I. Hlavata[1], M. Hubackova[1], S. Susova[1], J. Novotny[2], P. Vodicka[3], P. Soucek[1], M. Bortlik[4] and M. Kunovska[5]
[1]Group for Biotransformations, Center of Occupational Medicine, National Institute of Public Health, Srobarova 48, Prague 10, 100 42, Czech Republic. E-mail: hlavata@szu.cz, [2]1st Medical Faculty of Charles University and Department of Oncology, General Teaching Hospital, U nemocnice 2, Prague 2, 128 08, Czech Republic. E-mail: onkologie@seznam.cz, [3]Department of Genetic and Molecular Toxicology, Institute of Experimental Medicine, Czech Academy of Sciences, Videnska 1083, Prague 4, 142 20, Czech Republic. E-mail: pvodicka@biomed.cas.cz, [4]4th Department of Internal Medicine, U Nemocnice 2, Prague, 128 08, Czech Republic. E-mail: m.bortlik@hotmail.com, [5]Clinic of Internal Medicine – Gastroenterology, Jihlavska 20, Brno, 624 00, Czech Republic, kunovska@fnbrno.cz. E-mail: hlavata@szu.cz

Carcinogenesis is a complex process associated with genetic and lifestyle factors. One of the most common forms of cancer is colorectal cancer (CRC). CRC affects about 5% of worldwide population. The majority of CRC cases are sporadic forms in which susceptibility is influenced by polymorphisms in low-penetrance genes. Genetic polymorphisms in biotransformation enzymes may result in variations in detoxification capacity and thus modify the risk of CRC. We followed frequencies of functional polymorphisms in cytochrome P450 1B1, epoxide hydrolase 1, glutathione S-transferases M1, T1, P1, NADP(H)-quinone oxidoreductase (NQO1), superoxide dismutase 2 and myeloperoxidase in 610 CRC cases and 550 healthy controls. We found: 1/ the lack of association between particular polymorphisms and CRC risk; 2/ that female carriers of variant genotype in NQO1 were at more than three-fold risk of CRC in comparison with those carrying wild-type genotype ($P = 0.034$). There was no association of this polymorphism with CRC risk in males, but previously we reported its role in breast cancer on Czechs and Austrians. 3/ Age played no role as confounding factor. First such study on Czech population showed that polymorphisms in biotransformation enzymes may present risk factors in CRC. Further study should be focused on searching for differences in exposure between genders and assessment of importance of polymorphism combinations.

Acknowledgment: This project was supported by grants GACR 310/05/2626 and IGA 8563-5.

PP-679
Inter-individual variation in *CYP2A6* genotypes in relation to lung cancer risk among habitual tobacco smokers in turkey

S. Zencir[1], N. S. Gümüş[2], F. Lermioglu[2], F. Kılıç[3], T. Göksel[4], M. Fujieda[5], T. Kamataki[5] and Z. Topçu[6]
[1]Biochemistry, Ege University Faculty of Science, Izmir, Turkey, [2]Department of Pharmaceutical Toxicology, Ege University Faculty of Pharmacy, Izmir, Turkey, [3]Ege University Faculty of Agriculture, Izmir, Turkey, [4]Department of Chest Diseases, Faculty of Medicine Ege University, Izmir, Turkey, [5]Laboratory of Drug Metabolism, Department of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan, [6]Department of Biotechnology, Ege University Faculty of Pharmacy, Izmir, Turkey. E-mail: s_zencir@hotmail.com

Cytochrome P450 (CYP) is a heme-containing enzyme responsible for the metabolism of numerous endogenous and exogenous compounds. Approximately 20 individual CYPs have been identified in human. Among these enzymes, the *CYP2A6* family is characteristic of its catalytic properties to metabolize nitrosamines. Our laboratory previously reported a lower risk for *CYP2A6* gene deletion (*CYP2A6*4C/*4C*) in oral carcinoma among betel quid chewers in SriLanka. In this study we investigated the relationship between inter-individual difference in *CYP2A6* (**1A/*1A*, **1A/*1B*, **1B/*1B*, **1A/*4C*, **1B/*4C*, **4C/*4C*) genotypes in relation to lung cancer among habitual tobacco smokers in Turkey. Blood samples from a total of 81 tobacco smoker subjects with or without lung carcinoma (small cell, non-small cell; 45.7% and 54.3%, respectively) and a total of 14 control subjects were obtained and genotyping was performed with restrictional analyses of PCR-amplified samples of isolated DNA. Our current results suggest that *CYP2A6* genotype do not differ significantly among the two groups while the **1A* and **1B* allelic distribution was significantly different, which suggests the involvement of these enzymes in developing lung carcinoma.
Acknowledgment: *This study was supported by Turkish State Planning Organization (Grant 04 DPT 005).

PP-680
Inter-individual variation of *GSTM1* and *GSTT1* genotypes in relation to lung cancer risk among habitual tobacco smokers in turkey

S. N. Gumus[1], F. Lermioglu[1], T. Goksel[2], T. Kamataki[3], M. Fujieda[3] and **Z. Topcu**[4]
[1]Department of Pharmaceutical Toxicology, Ege University Faculty of Pharmacy, Izmir, Turkey, [2]Department of Chest Diseases, Ege University Faculty of Medicine, Izmir, Turkey, [3]Laboratory of Drug Metabolism, Department of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan, [4]Laboratory of Biotechnology, Department of Pharmaceutical Biotechnology, Ege University Faculty of Pharmacy, Izmir, Turkey. E-mail: serragumus@hotmail.com

Glutathione-S-transferases (GST) are a multifunctional family of enzymes that catalyse the conjugation of glutathione (GSH) to a variety of electrophiles. Among GST enzymes, *GSTM1* and *GSTT1* detoxify the metabolites of polycyclic aromatic hydrocarbons (PAH) found in tobacco smoke and epidemiological studies

suggest that individuals lacking *GSTM1*and *GSTT1* genes could potentially be at higher risk for lung cancer. We investigated distribution of *GSTM1* and *GSTT1* genotypes in 95 patients with lung cancer (ages between 42 and 76) and 14 control subjects (ages between 40 and 52) with habitual tobacco smoking by polymerase chain reaction amplification of isolated DNA from blood samples. Cases were investigated in two groups, based on histopathological findings; small cell carcinoma (SCC) (19.6%) and non-small cell carcinoma (non-SCC) (80.4%). We found that 38.1% and 25.1% of the patients exhibited *GSTM1* null genotype for SCC and non-SCC, respectively, while the distribution of *GSTT1* null genotype was 33.3% and 9.3% for SCC nd non-SCC, respectively. The null genotypes for *GSTM1* and *GSTT1* were 7.1% and 14.3%, respectively, for control subjects. Our results show that SCC and non-SCC groups do not differ significantly in *GSTM1* and *GSTT1* genotype profiles.

Acknowledgment: *This study was supported by Turkish State Planning Organization (Grant 04 DPT 005).

PP-681
The effects of the OPRM1 gene polymorphisms on pain control in Turkish patients.

M. Ozcan Caliskan[1], E. Manguoglu[1], I. Keser[1], M. Akbas[2], B. Karslı[3], H. Akbas[4] and G. Luleci[1]
[1]*Department of Medical Biology and Genetics, Faculty of Medicine, Akdeniz University, Antalya, Turkey,* [2]*Department of Algology, Faculty of Medicine, Akdeniz University, Antalya, Turkey,* [3]*Department of Anesthesiology, Faculty of Medicine, Akdeniz University, Antalya, Turkey,* [4]*Central Laboratory, Faculty of Medicine, Akdeniz University, Antalya, Turkey. E-mail: muallaozcan@akdeniz.edu.tr*

The human mu-opioid receptor (OPRM1) is major site for the analgesic action of most opioid drugs such as morphine, methadone and heroin. Single nucleotide polymorphisms (SNPs) in the OPRM1 gene might alter the affinity between mu-opioid receptor and morphine interaction. The purpose of the study was to investigate whether the genetic polymorphisms in the OPRM1 gene influence the human pain tolerance and morphine addictions. We studied 50 patients who were given morphine after operation as preliminary work. Sequencing analysis were performed to detect the SNPs in the OPRM1 gene. Morphine levels in the blood serum were measured by Cobas Integra 400. Four different SNPs were found, among these, three were in exon 1 (A118G, C17T, A208C), one was in intron 2 (IVS2 + 691G > C). In addition, another four different rare SNPs were detected, two in exon 1, one in exon2, and one in exon3. The frequencies of the A an G alleles were found as 85%and 15% respectively for A118G change. Our results show that the patients who were carrier for variant G allele of A118G polymorphism needed more morphine to achieve pain control compared to wild type individuals. Also, the novel variant in exon 1, A208C, may alter morphine requirement in pain control. In conclusion, SNPs in the mu-opioid receptor, especially A118G and A208C polymorphisms should be screened to achieve pain control and to regulate of the morphine pharmacokinetic.

PP-682
A simple and fast method for genotyping drug metabolizing enzymes CYP2D6, CYP2C19, and TPMT

M. Kampa[1], O. Rasouli[1], **C. Mitropoulos**[2], E. Tsagaraki[1], C. Tsatsanis[1], K. Glinou[2], V. Tsaousis[2], A. Margioris[1], E. Castanas[1] and A. Gravanis[1]
[1]*Department of Pharmacology, Exp. Endocrinology and Clinical Chemistry, School of Medicine University of Crete, Heraklion, Greece,* [2]*Medicon Hellas SA, Athens, Greece. E-mail: gravanis@med.uoc.gr*

We have developed a simple method for the detection of known SNPs in genes encoding two cytochrome P450 enzymes (CYP2D6, CYP2C19) and S-Thiopurine methyltransferase (TPMT) that metabolize a large menu of therapeutic agents and where a genotype-phenotype relationship is well established. The method utilizes two established technologies PCR and ELISA. A gene specific PCR is performed followed by allele specific hybridization-amplification and ELISA. In brief, genomic DNA was extracted from EDTA anticoagulated peripheral blood. Specific primers were designed to amplify the specific region of the relevant mutations with PCR. Amplicons were confirmed with sequence analysis, then they were labeled with biotinylated primers complementary to the mutation site. Two parallel hybridization-polymerization reactions were set for each mutation, one with the wild type biotinylated primer and the other one with the mutant primer. The product of the second PCR was transferred to a streptavidin coated microplate well, incubated with horseradish peroxidase conjugated antibody raised against digoxigenin, and developed with 3, 3', 5, 5'- tetramethylbenzidine solution. The optical density of each well was measured at 450 nm in a microtiter plate reader. Our method is fast, highly specific and sensitive, can be automated for screening of large numbers of specimens and implemented in small health units, not requiring sophisticated equipment.

Glycobiology and Glycomics

PP-683
Effect of lysozyme on carbohydrate composition of plasma membranes. Lectin binding assay

V. Mikaelyan, **G. Poghosyan**, E. Stepanyan and K. Gasparyan
Institute of Biochemistry, Yerevan, Armenia. E-mail: VGasparyan@excite.com

Lysozyme has defensive function in protection of host organism against bacterial infections. Saliva, tears, milk are rich sources of the enzyme which confirms this view. However it is secreted by some tumor lines and during some pathologies that are not associated with bacterial infections. Therefore its role has to be reconsidered. In particular it is unclear if the enzyme is secreted by tumors as an invasive agent or a compensatory agent. We have studied the effects of lysozyme on cells of some organs to elucidate its role. So the changes in lectin binding properties of the cells as a result of lysozyme action were investigated. It was shown that the enzyme action brings to changes of wheat germ agglutinin and concanavalin A binding properties of liver and heart cells. The procedure of enzyme immune like analysis on polyacrylamide slices permits to detect small changes on surface of the cells during lysozyme action. Physiological and medical aspects of these changes are discussed.

PP-684
Myeloma diagnosis by determination of antibodies to carbohydrates

Z. Melkonyan[1], S. Dagbashyan[2], A. Melkikyan[2] and K. Gasparyan[1]
[1]*Institute of Biochemistry, Yerevan, Armenia,* [2]*Center of Haematology, Yerevan, Armenia.*
E-mail: VGasparyan@excite.com

Myeloma is diagnosed by various biochemical and cytochemical methods. The biochemical methods (total proteins, lysozyme immunoglobulins A, M, G, and E etc) have some interference and these indexes are changed during other pathologies also. Cytochemical methods are time consuming. Here is presented ELISA method for detection of antibodies to carbohydrates (N-acetyl glucosamine) as diagnostic tool for detection of myeloma. Sepharose covalently bound with N-acetyl glucosamine by epichlorohydrine was applied as matrix for ELISA. Assay involved NAG + serum + washing + anti human IgG-peroxidase + staining. Specificity of reaction was demonstrated by addition of anti human IgG to reaction. In this case the inhibition of binding takes place. Specificity of the test to myeloma was evaluated by analysis of other oncological patients. Possibility for the assay in diagnosis of myeloma is considered.

PP-685
Peculiarities of metabolism of glycoconjugates in monitoring of ovarian cancer treatment

M. Knyazyeva[1], A. Prokopyuk[2] and O. Babaeva[3]
[1]*Department of Biochemistry, Kharkov National University named after V.N Karazin, Kharkov, Ukraine,* [2]*Department of Oncogynaecology, Civil Organization "New thinking in medicine", Kharkov, Ukraine,* [3]*Department of Biochemistry, Civil Organization "New thinking in medicine", Kharkov, Ukraine.*
E-mail: marina_k@tnss.kharkov.ua

In order to define optimal number of neoadjuvant chemotherapy (NChT)-courses and to improve the treatment efficiency of patients having ovarian cancer (OC) we determined summary content of glycosaminoglycans (GAG) and their fractional composition as well as summary content of chondroitinnsulfates (Ch-S) in the blood serum. 47 women having OC of III-IV stages before and after NChT in dynamics and 12 women having benign tumor (BT) have been examined. Platinum preparations in the combination with cyclophosphan and doxorubicine were used for NChT (1–6 courses). The obtained results show that before NChT summary content of GAG in the blood serum of patients having OC was higher than summary content of GAG in the patients having BT as well as GAG fraction I contained mainly Ch-6-S and fraction II contained Ch-4-S and dermatansulfate. The first course of NChT led to the reduction of summary content of GAG to the GAG level in patients having BT. It also led to the reduction of GAG fractions II and III (contained heparansulfates, keratansulfates and heparin), but at the same time the content of Ch-6-S increased as compared with the GAG level in patients having BT and OC without NChT. Structural degradation of matrix effects the GAG spectrum and precedes invasion and tumor metastasis. Great number of NChT courses (6) modules neoplastic activity best of all and prevents invasion and metastasis that affects the GAG spectrum in the blood serum of patients.

PP-686
Glycan analysis of serum ribonuclease 1 indicates its endothelial origin

S. Barrabés[1], L. Pagès-Pons[1], G. Tabarés[1], E. Fort[2], P. M. Rudd[3], R. de Llorens[1] and R. Peracaula[1]
[1]*Biology Department, University of Girona, Girona, Spain,* [2]*Digestive Unit, Hospital Universitari J. Trueta, Girona, Spain,* [3]*Glycobiolgy Institute, Oxford University, Oxford, UK.*
E-mail: silvia.barrabes@udg.es

Human pancreatic ribonuclease 1 (RNase 1) is a glycoprotein expressed mainly by the pancreas, also present in body fluids and other tissues, like endothelial cells. Pancreatic Cancer (PaC) simptomatology is not clear and its diagnosis remains difficult, therefore the search for a sensitive and specific tumour marker is required. Glycosylation pattern modifications in all sort of glycoconjugates is distinctive of tumour cells. Previous studies showed that RNase 1 glycans from human healthy pancreas were all neutral while RNase 1 glycans from PaC cell lines contained sialylated structures. Our goal was to determine whether these glycan differences could be present in serum RNase 1 from PaC and also, to establish the main origin of the serum RNase 1. In order to determine possible glycan modifications on serum RNase 1 in tumour situation, and its origin, serum RNase 1 glycans from PaC patients' sera and control patients' sera and RNase 1 glycans from the endothelial cell line EA.hy926 were purified and characterized. Mono and disialylated biantennary core fucosylated structures were the main glycans detected in both normal and tumour serum RNase 1 samples. RNase 1 glycans from EA.hy926 contained the same main structures detected in the serum samples, except for the lack of some minor tetraanntennary glycans and complex polylactosamines, what indicates that endothelial cells are probably the main source of serum RNase 1.

PP-687
Primary structure and homology modeling of the novel alpha-galactosidase from marine bacterium

L. A. Balabanova, G. N. Likhatskaya, I. Ju. Bakunina, S. V. Petruk, V. B. Kozhemyako and V. A. Rasskazov
Pasific Institute of Bioorganic Chemistry, Vladivostok, Russia.
E-mail: balaban@piboc.dvo.ru

The chromosomal gene encoding the novel cytoplasmic 80 kDa alpha-galactosidase from marine bacterium *Pseudoalteromonas* sp. whose enzymatically active form occurs as homodimer was cloned by PCR-based methods. It has an open reading frame encoded 710 amino acids protein and isoelectric point of 5.5. Inspection of the sequence revealed high content of amino acid residues such as Leu (10%), Ser (8%), Val, Ala (7%), Glu (7%). The predicted amino acid sequence of alpha-galactosidase from *Pseudoalteromonas* sp. includes a classic domain of the glycosyl hydrolyses family 36 and revealed closest homology with alpha-galactosidases RAFA from *E. coli* (39%) and Thermotoga maritima (29%). Therefore, the fold of the novel alpha-galactosidase is more closely related to galactosidases from family 27. Homology model of alpha-galactosidase from *Pseudoalteromonas* sp. was obtained with MOE on the base of the crystal structures of rice and Thermotoga maritima alpha-galactosidases as templates. The structure demonstrates the catalytic mechanisms of enzyme with the participation of Asp residues that approximates to the position of Asp 51, 130, 185 of catalytic domain in rice alpha-galactosidase. Both alpha-galactosidase from *Pseudoalteromonas* sp. and glycosidases from family 27 attracted attention due to their ability to convert blood group A or B antigens into blood group O antigen to produce the universal donor blood type.

Acknowledgment: Work was supported by RFBR N 05-08-50285-a, FEBRAS N06-III-A-05-123 grants.

PP-688
Structural studies of ligand binding of natural killer cell receptor, protein CD69

O. Vanek[1], P. Novak[2], V. Kopecky Jr.[3], V. Kren[2], J. Brynda[4] and **K. Bezouska**[2]
[1]*Department of Biochemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 12840, Prague, Czech Republic,* [2]*Institute of Microbiology, Academy of Sciences of the Czech Republic, Videnska 1083, 14220, Prague, Czech Republic,* [3]*Institute of Physics, Faculty of Mathematics and Physics, Charles University in Prague, Ke Karlovu 5, 12116, Prague, Czech Republic,* [4]*Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Flemingovo namesti 2, 16636, Prague, Czech Republic. E-mail: kenav3@seznam.cz*

Protein CD69, an early activation antigen of human lymphocytes, is one of the most studied surface receptor molecules involved in tumour recognition by natural killer cells. It belongs to a group of C-type lectin-like lymphocyte receptors and till now several types of possible ligands have been identified or proposed: calcium cation, various carbohydrate structures and even peptides. We present our structural approach to describe CD69 binding properties together with list of novel therapeutically interesting ligands identified by inhibition tests or equilibrium dialysis. After recombinant production and optimalization of *in-vitro* refolding of soluble CRD domain of CD69 protein, we analysed its homogeneity by FT-ICR mass spectrometry and its secondary structure was determined by Raman spectroscopy. Further, ligand binding was examined both by Raman spectroscopy and by protein crystallization. We tested several types of proposed CD69 ligands: single monosaccharide ligand N-acetyl-D-glucosamine, antenary oligosaccharides isolated from hen egg white protein ovomucoid, synthetic peptidomimetic ligands based on calixarene core, heptapeptide ligand from mycobacterial heat shock protein hsp60. Although we found crystallization conditions at neutral pH necessary for ligand binding and we achieved high resolution crystal structures (the best at 1.8 Å), none of the ligand molecules were found in, except for calcium cations, which were proven by anomalous scattering on synchrotron.

PP-689
Carbohydrate specificity of an insecticidal lectin isolated from the leaves of *Glechoma hederacea* towards mammalian glycoconjugates

A. M. Wu[1], T. Singh[1], W. J. Peumans[2], P. Rougé[3], E. J. M. Van Damme[2], R. A. Alvarez[4] and O. Blixt[5]
[1]*Glyco-Immunochemistry Research Laboratory, Institute of Molecular and Cellular Biology, Chang-Gung University, Kwei-san, Tao-yuan, 333, Taiwan,* [2]*Department of Molecular Biotechnology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Gent, Belgium,* [3]*Surfaces Cellulaires et Signalisation chez les Végétaux, UMR-CNRS 5546, Pôle de Biotechnologie végétale, Chemin de Borde-Rouge, 31326 Castanet Tolosan, France,* [4]*Department of Biochemistry and Molecular Biology, University of Oklahoma, Health Sciences Center, Oklahoma City, Oklahoma 73104, USA,* [5]*Department of Molecular Biology, The Scripps Research Institute, La Jolla, California 92037, USA. E-mail: amwu@mail.cgu.edu.tw*

Preliminary studies indicated that the potent insecticidal lectin from the leaves of *Glechoma hederacea* (Gleheda) preferentially agglutinates human erythrocytes carrying the Tn (GalNAcα1-Ser/Thr) antigen. To corroborate the molecular and physiological function of Gleheda, it is necessary to identify the recognition factors involved in the Gleheda-glycotope interaction. In this study, fine affinity profile of Gleheda binding were evaluated using enzyme-linked lectinosorbent inhibition assay (ELLSA), a glycan array and molecular modeling. From the results, it is concluded that a high-density polyvalent Tn-containing glycoproteins (gps) were the most potent factors for Gleheda binding. They were on a nanogram basis 6.5×10^5, 1.5×10^4 and 3.1×10^3times more active than Gal, GalNAc and Tn, respectively. Among mono- and oligosaccharides examined, simple clustered Tn was the best, being 37.5 and 1.7×10^3times better than GalNAc and Gal, respectively. GalNAc glycosides were significantly more active than Gal glycosides, indicating N-acetyl group at carbon-2 plays an important role in Gleheda binding. The results of glycan array and molecular modeling support the conclusions drawn with respect to the specificity of Gleheda based on the ELLSA assays. The extraordinary binding feature of Gleheda for gps demonstrates the importance of affinity enhancement by high-density polyvalent glycotopes in the ligand-lectin interactions in biological processes.

PP-690
Studies of capsular carbohydrates in *Klebsiella pneumoniae* isolated from a diabetic patient

J. H. Wu[1], C. G. Tsai[1], A. M. Wu[2] and T. S. Wu[3]
[1]*Department of Microbiology and Immunology, Chang Gung University, Kwei San, Tao Yuan, Taiwan,* [2]*Institute of Biochemistry and Molecular Biology, Chang Gung University, Kwei San, Tao Yuan, Taiwan,* [3]*Division of Infectious Disease, Department of Internal Medicine, Chang Gung Memorial Hospital, Kwei San, Tao Yuan, Taiwan. E-mail: jhwu@mail.cgu.edu.tw*

Emerging evidences indicate that nosocomial infections by *Klebsiella pneumoniae* (KP) frequently cause severe bacteremia and liver abscess. KP contains thick capsule that is reported to associate with virulence. We propose that KP surface may contain molecules that contribute to the bacterial persistence in host and their migration to liver. The purified KP capsular sugars were assayed for binding to lectins and anti-Lewis antibodies and the compositions were investigated by capillary high performance chromatography. Survival rates of diabetic mice infected either with hepatic-KP or urinary tract infection (UTI)-KP strains and the liver tissue sections for the presence of bacteria were examined. The hepatic KP from diabetic patients contains high amount of fucose. Lectin binding assay revealed surface galactose and sialyl Lewis a, Lewis a and Lewis x in hepatic KP and surface mannose in UTI-KP. Diabetic mice infected with hepatic KP were rapidly fatal while those infected with UTI-KP showed 75% survival rate during 30-day observation. Liver sections of the infected diabetic mice contained many bacteria while liver sections obtained from diabetic mice infected with UTI-KP did not. Literature search indicates that cancer cells that express large amount of Lewis antigens show increased hepatic metastasis and adhesion to endothelial cells. Our results support our hypothesis that the KP contains surface molecular moiety that contributes to bacteremia and hepatic tropism.

PP-691

Exposure to hyperbaric pressure alters ganglioside expression in rat liver following partial hepatectomy

V. Cikes Culic[1], T. Ticinovic Kurir[2], S. Radic[3], T. Zemunik[4], M. Mesaric[5] and A. Markotic[1]
[1]Department of Biochemistry, Medical School, Split University, Split, Croatia, [2]Department of Pathophysiology, Clinical Hospital Split, Split, Croatia, [3]Undersea and Hyperbaric Medicine, Naval Medicine Institute of the Croatian Navy, Split, Croatia, [4]Department of Biology, Medical School, Split University, Split, Croatia, [5]Department of Chemistry and Biochemistry, Zagreb University Medical School, Zagreb, Croatia. E-mail: vcikes@bsb.mefst.hr

Introduction: Remnant liver after partial hepatectomy (PH) demands an increased amount of oxygen to restore hepatic energy. Modulation of the ganglioside content of hepatocyte plasma membrane could provide a mean for upregulating cellular responsiveness to a variety of factors.
Materials and Methods: Rat liver gangliosides were analysed by high performance thin layer chromatography (HPTLC) followed with immunostaining 54 h after PH. Non-operated and three different groups of operated rats were studied according to the hausing conditions before or after operation: recovering under normal ambient conditions after operation (1), treating with hyperbaric pressure before operation and recovering under normal ambient conditions (2 or preHB), and recovering under hyperbaric pressure after operation (3 or postHB).
Results: Liver of preHB group showed the highest expression of ganglioside GD1a, a marker of a peculiar transition phase of liver regeneration. Same animals showed enhanced expression of the several gangliosides: GM2, GM1a, GD1b, GT1b, GalNAcGM1b, nLc4, nLc6 and nLc8.
Conclusion: Since galactosyl- and N-acetylgalactosaminyltransferase catalyse final step of synthesis of GM2, GM1a, GD1b, GalNAcGM1b, nLc4, nLc6, and nLc8, our results point at possible role of galactosyl- and N-acetylgalactosaminyltransferase in regulation of the liver growth after PH.

PP-692

Serum leptin levels and insulin resistance in patients with superficial fungal disease

O. Goruroglu Ozturk[1], U. Guvenc[2], B. Cimen[1], U. Tursen[2], G. İkizoglu[2] and U. Atik[1]
[1]Department of Biochemistry, Faculty of Medicine, Mersin University, Mersin, Turkey, [2]Department of Dermatology, Faculty of Medicine, Mersin University, Mersin, Turkey.
E-mail: ozlem_goruroglu@yahoo.com

Various factors may be involved in dermatophytosis such as increased hydration of the skin with maceration, skin surface lipids, trauma, and atopy. However, no epidemiological studies have been done to show a relationship between leptin levels, insulin resistance and increased susceptibility to dermatophytosis. 70 patients with dermatophytosis and 25 healthy control subjects were enrolled in the study. The levels of insulin, glucose, leptin, triglyceride, cholesterol and lipoproteins were analysed to determinate the insulin resistance. The levels of leptin were analysed by a competitive enzyme immunoassay (active human leptin ELISA kit, lot no: 08314-B) and the insulin resistance of the patients were calculated by HOMA (The Homeostasis Model Assessment). For statistical analyses independent simple *t*-tests were performed. The mean insulin level of the patient group was found 9.8 ± 6.1 μU/ml and the control group was found 12.4 ± 5.4 μU/ml. The mean leptin level of the patient group was found 61.9 ± 60.6 ng/ml and the control group was found 64.9 ± 60.7 ng/ml. The mean glucose level of the patient group was found 5.04 ± 6.2 mmol/l and the control group was found 5.16 ± 4.75 mmol/l. The mean insulin resistance of the patient group was found 2.27 ± 1.54 and the control group was found 2.9 ± 1.3. Consequently, when leptin levels and insulin resistance were compared in patients with dermatophytosis there have been no significant results detected in this study.

PP-693

Estrogen induced cholestasis leads to increase of b-series gangliosides and a redistribution of GM1 in the rat liver

F. Majer[1], M. Jirkovska[2], L. Vitek[1], Z. Marecek[3] and F. Smid[1]
[1]Institute of Clinical Biochemistry and Laboratory Diagnostics, U Nemocnice 2, Prague, [2]Institute of Histology and Embryology, U Nemocnice 2, Prague, [3]General Military Hospital, U Nemocnice 2, Prague. E-mail: majerf@email.cz

Introduction: Composition of liver gangliosides and changes in GM1 ganglioside localisation were investigated in ethinylestradiol (EE)-induced cholestasis in Wistar rats.
Methods: Cholestasis was induced by sc. injection of EE for 18 days. Isolated hepatic gangliosides were separated by TLC detected with resorcinol-HCl reagent and evaluated by densitometry. GM1 localization was detected by an immunohistochemistry with the cholera toxin B-subunit binding specifically to GM1.
Results: Ethinylestradiol administration resulted in severe cholestasis. Total lipid hepatic sialic acid was two-fold increased in cholestatic rats ($P < 0.01$). While control rats had predominance of a-series gangliosides, in EE-treated rats a significant increase of b-series gangliosides GD3 (25-fold, $P = 0.002$), GD1b (54-fold, $P = 0.002$) and GT1b (107-fold, $P = 0.002$) was detected. In the control rat liver, the GM1 immunoreaction was detected in peripheral and middle zone of liver acini, while in EE-treated animals, GM1 immunoreactivity was markedly shifted to the sinusoidal membrane in all acinar zones. As confirmed by linear regression analysis, the degree of cholestasis correlated with redistribution of GM1-immunopositivity in the liver acinus.
Conclusion: Dramatic increase of b-series gangliosides and the shift of GM1 into sinusoidal membrane of hepatocytes in the central lobular zone might contribute to resistance of hepatocytes against harmful levels of bile acids during EE-cholestasis.

PP-694

Effect of cross clamping on sialic acid and CKMB in coronary sinus blood in patients undergoing cardiopulmonary bypass

C. Kazezoğlu[1], **S. Süer Gökmen**[1], B. Sunar[1] and H. Sunar[2]
[1]Departments of Biochemistry, Trakya University School of Medicine, Edirne, Turkey, [2]Departments of Cardiovascular Surgery, Trakya University School of Medicine, Edirne, Turkey.
E-mail: selmasuer@hotmail.com

Ischemia is a decrease in arterial blood flow below the minimum level necessary to meet the metabolic demands of a tissue or an organ. Damage to the cell membrane results in the release of intracellular contents and some membrane components. Sialic acids are structural constituents of both insoluble and soluble components of tissues and cells. The role of sialic acid in the pathogenesis of atherosclerosis and as a predictor of cardiovascular events has attracted much attention in recent years. The aim of this study is to investigate the effect of peroperative myocardial ischemia on serum total sialic acid (TSA), lipid-bound sialic acid (LSA) and CKMB mass levels in patients ($n = 20$) undergo-

ing cardiopulmonary bypass. Coronary sinus blood samples were obtained before aortic cross clamping (preischemic sample) and just before clamp removal (ischemic sample) in the end of ischemic period. The mean total cross clamping time was 55.28 ± 18.04 min (range 27–77). Serum TSA, LSA and CKMB mass levels were measured with the methods of Warren and Katopodis, and commercial kits, respectively. Repeated Measures ANOVA test was used to analyse the results. Only CKMB mass level in ischemic blood sample was significantly higher than those in preischemic blood sample ($P < 0.001$).As a result, we can report that peroperative myocardial ischemia may play an important role for the increase in CKMB mass levels, whereas, total and lipid bound sialic acid levels are not affected. This study has been supported by Trakya University Scientific Research Appropriation.

PP-695
TGFβ2 upregulates biglycan and lumican gene transcription in normal fibroblasts and fibrosarcoma cells.

A. Berdiaki, A. Zafiropoulos, E. Fthenou and G. N. Tzanakakis
Department of Histology, School of Medicine, University of Crete, Heraklion, Greece. E-mail: berdiaki@edu.med.uoc.gr

Transforming growth factor β2(TGFβ2) and basic fibroblast growth factor (bFGF) regulate SLRPs expression during matrix remodelling, migration and tumour growth. High SLRP levels have been identified in malignant tumors as well as in fibrotic lesions of lung, hepatic and renal tissues. In this study, we investigated the mRNA levels of decorin, biglycan and lumican in normal fibroblasts (DLF) and fibrosarcoma (B6FS) cell lines and their regulation by TGFβ2 and bFGF. DLF and B6FS cells were treated for 24 h with TGFβ2 or bFGF after a 24 h serum starvation period. Proteoglycan mRNA levels were analysed using Real time PCR. We observed higher expression of biglycan in B6FS cells while lumican and decorin mRNA levels were higher in DLF cells. Biglycan was significantly increased by TGFβ2 in both cell lines (DLF: $P < 0.001$; B6FS: $P < 0.01$) and by bFGF in DLF cells ($P < 0.05$). TGFβ2 significantly decreased lumican ($P < 0.001$) in DLF cells and significantly increased ($P < 0.001$) the latter in B6FS cells. Lumican was significantly decreased by bFGF in both cell lines (DLF: $P < 0.001$; B6FS: $P < 0.05$). Decorin mRNA levels, in B6FS cells, were significantly decreased by both growth factors (TGFβ2: $P < 0.01$; bFGF: $P < 0.05$). In conclusion, our study identified high basal level transcription of biglycan specifically in fibrosarcoma cells while TGFβ2 enhanced even further its expression. Differential SLRPs expression and regulation in normal and cancerous cells, by TGFβ2 and/or bFGF, supports the concept of a specific role of SLRPs in fibrosarcoma pathophysiology.

PP-696
TGF-β2 differentially regulates lumican and biglycan expression in two human osteosarcoma cell lines

D. Nikitovic, M. Assouti, A. Zafiropoulos and G. N. Tzanakakis
Laboratory of Histology, School of Medicine, University of Crete, Heraklion, Greece. E-mail: tzanakak@med.uoc.gr

Pathogenesis of the primary bone tumor, osteosarcoma, is closely related to extracellular matrix (ECM) remodeling. Small-leucine –rich-proteoglycans (SLRPs), which are major ECM components, have an important role in cancer growth partly through an autocrine mechanism involving transforming growth factor-β (TGF-β) signalling. The aim of this study was to examine the expression of the SLRPs lumican and biglycan among two human osteosarcoma cell lines (MG-63 and Saos 2) of different metastatic capability, and to determine the effect of TGF-β2 on their expression. Real-time PCR and western blotting were the techniques utilized. Lumican mRNA was detected, for the first time to our knowledge, in both osteosarcoma cell lines in varying amounts and lumican protein was found to be secreted to their culture media. The glycosaminoglycan chains bound in to the lumican core protein in both cell lines were identified to be keratan sulfate. MG-63 cells were found to express biglycan modified with chondroitin sulphate chains. TGF-β2 strongly decreased lumican mRNA expression in Saos 2 but had no effect on MG-63 cells. In contrast TGF-β2 caused a significant upregulation of biglycan mRNA in MG-63 cells. In conclusion, TGF-β2 differentially regulates the expression of the SLRP proteoglycans lumican and biglycan, among the high and low metastatic potential osteosarcoma cell lines.

PP-697
Chondroitin sulfate a specifically modulates the mitogenic effects of PDGF-BB on normal fibroblasts

E. Fthenou, A. Zafiropoulos, A. Berdiaki and G. N. Tzanakakis
Department of Histology, School of Medicine, University of Crete, Heraklion, Greece. E-mail: fthenou@edu.med.uoc.gr

Platelet derived growth factor (PDGF-BB) is a major mitogen for mesenchymal cells and physically interacts with glycosaminoglycans (GAGs). Binding of PDGF-BB to matrix and cell associated GAGs can regulate its mitogenic activity. The aim of our study was to evaluate the effects of various GAGs to the mitogenic function of PDGF-BB on normal fibroblast proliferation. PDGF-BB significantly stimulates normal fibroblast cell growth. In order to examine the possible participation of various cell associated GAGs to PDGF-BB function, cells were treated with chondroitinase ABC and heparitinase. Chondroitinase ABC stimulated both basal and PDGF-induced proliferation, while treatment of heparitinase was inhibiting. Exogenous addition of chondroitin sulphate A (CSA) and heparin inhibited PDGF-BB-induced proliferation, while dermatan sulphate (DS) had no effect. We performed quantisation of the PDGF receptor α and β mRNA by real-time PCR, to investigate if the inhibitory effect of CSA on the PDGF-BB-induced cell growth was caused by modification of the PDGF receptor levels. PDGF-BB decreased PDGFRα and PDGFRβ mRNA levels, while heparin upregulated both receptors. DS upregulated PDGF Ra transcripts, while CSA had no effect. In conclusion, our results indicate that CSA either free or attached on membrane proteoglycans can modulate the fibroblast mitotic response to PDGF-BB, with a mechanism which does not utilize transcriptional downregulation of the PDGF receptors.

PP-698
A novel finding of lumican expression in human malignant melanoma cell lines

M. Assouti, M. Sifaki, A. Berdiaki, D. Nikitovic and G. N. Tzanakakis
Laboratory of Histology, School of Medicine, University of Crete, Heraklion, Greece. E-mail: tzanakak@med.uoc.gr

Melanoma is a frequent and therapy-resistant human malignancy. Its progress is closely linked to the changes in extracellular matrix (ECM), which may affect proliferation, adhesion and migration of the tumor cells. Lumican is a member of the small leucine-rich proteoglycans (SLRPs), important to ECM assembly

and function, with either tumor promoting or anti-oncogenic properties. Our aim was to investigate the expression of lumican in two human melanoma cell lines (WM9 and M5), as well as in normal neonatal human melanocytes (HEMN). Real-time PCR and western blotting were utilized. Lumican mRNA was detected in both melanoma cell lines but not in the normal melanocytes. Using anti-lumican antibody, lumican protein was found to be secreted by both melanoma cell lines. In addition, we found that lumican is substituted with keratan sulphate chains, susceptible to enzymic degradation with keratanase II. This is the first report, to our knowledge, which demonstrates the expression of lumican in human melanoma cell lines, while the lack of its expression in normal melanocytes suggests that lumican may have a role in malignant melanoma progression.

New Proteomic Assays for Cancer Biomarkers

PP-699
Carcinoembryonic antigen and carbohydrate antigen 19-9 in serum from patients with cholangiocarcinoma

N. Serdarevic, F. Bilal and J. Foco-Solak
Department of Clinical Chemistry and Biochemistry, School of Medicine, University of Sarajevo, Bosnia and Herzegovina. E-mail: serdarevicnafija@yahoo.com

The aim of study was to define usefulness of determination serum concentration of carbohydrate antigen 19-9 (CA 19-9) and carcinoembryonic antigen (CEA) in patients with different disease. We make determination of serum concentration CA 19-9 and CEA in patients with cholangiocarcinoma ($n = 20$), benign biliary diseases ($n = 60$) and healthy individuals ($n = 20$). Using AxSYM Abbott immunoassay we measured serum CA 19-9 and CEA concentrations at 100 patient. The AxSYM CA 19-9 and CEA is Microparticle Enzyme Immunoassay (MEIA) for the quantitative measurement of 1116-NS-19-9 and CEA in human serum. The obtained concentration at healthy individuals for CA 19-9 have range 0–37 U/ml and for CEA 0–2.5 ng/ml. The mean difference between CA 19-9 (306.5 ± 18.9 U/ml) and CEA (3.8 ± 1.0 ng/ml) in patients with cholangiocarcinoma and CA 19-9 (18.38 ± 11.72 U/ml) and CEA (1.7 ± 0.9 ng/ml) in patients with benign biliary diseases was statistically significant for $P < 0.05$ using Student t-test. Serum concentration of CA 19-9 (306.5 ± 18.9 U/ml) and CEA (3.8 ± 1.0 ng/ml) in patients with cholangiocarcinoma compared with CA 19-9 (11.0 ± 1.6 U/ml) and CEA (1.7 ± 0.5 ng/ml) in healthy individual was statistically significant for $P < 0.05$ using Student t-test. A comparison of results between CA 19-9 and CEA in patients with cholangiocarcinoma showed correlation ($r = 0.402$). Serum CA 19-9 level may add useful information in patients with cholangiocarcinoma.

PP-700
Ultrafiltration for biomarker sample preparation

S. Urbain
Bioscience Division, Millipore SAS, Molsheim, France. E-mail: Samuel_Urbain@millipore.com

Significant research activities are dedicated to discover biological markers ('biomarkers') associated with all varieties of disease conditions. Biomarkers can provide powerful clues to genetic susceptibility, disease progression, and predisposition, as well as offer information on physiological and metabolic profiling of diseases and drug response. One of the major impediments to the discovery of new biomarkers is the fact that plasma or serum contains a significant number of proteins, salts, and lipids which make it difficult to detect and analyse peptides by mass spectrometry. Therefore, the complexity of these biological samples needs to be reduced by extensive sample preparation in order to use sensitive analytical techniques. This poster describes a combination of ultrafiltration and solid-phase extraction (SPE) techniques that allow researchers to purify peptides from biological samples in a bench-top, high-throughput format, ready for MS and MS/MS analysis, using any available mass spectrometer and related laboratory equipment.

PP-701
6D5 monoclonal antibody: a new promising marker for hepatocellular carcinoma

E. Öztaş[1], A. Özcan[2], E. S. Sayraç[1], M. Öztürk[1] and T. Yağcı[1]
[1]*Molecular Biology and Genetics, Bilkent University, Ankara, Turkey,* [2]*Pathology, Gulhane Military Medical Academy, Ankara, Turkey. E-mail: oemin@bilkent.edu.tr*

Hepatocellular carcinoma (HCC) is one of the most common cancers with high morbidity and mortality rates and, its incidence is increasing worldwide. Liver cirrhosis is the most important risk factor for HCC development. Therefore, the development of novel markers for HCC with stronger sensitivity and specificity is of great importance for the surveillance of patients with chronic hepatitis and liver cirrhosis, which are at high risk to develop liver tumor. We recently developed a monoclonal antibody, namely clone 6D5, and assessed its reactivity with HCC cell lines and 130 paraffin-embedded liver tissue specimen consisting of 14 cases of HCC, 18 normal liver and 98 non-cancerous liver pathologies. In western blot experiments, the antibody reacted with cell lysates of 13 HCC cell lines. In immunohistochemical (IHC) studies, 6D5 differentially recognized HCC and failed to react with normal liver and other non-cancerous tissues. More interestingly, high reactivity was also observed in non-tumoral area adjacent to HCC, suggesting a role for 6D5 protein in tumor and tumor microenvironment crosstalk. Cytoplasmic location of the protein was confirmed with both immunofluorescence and IHC staining assays. We also demonstrated the secretion of the protein in well-differentiated HCC cell lines, implicating early expression of 6D5 ligand in liver carcinogenesis. This latter issue encourages us to investigate the antibody as a potential serum biomarker.

PP-702
Production of monoclonal antibody to N-myc downstream regulated gene 2 and determination of NDRG2 by protein chip

D. Y. Yoon[1], M. Y. Park[2], K. E. Baek[2], J. S. Kim[2], J. T. Kim[2], M. Y. Cho[2], S. C. Choi[2], J. S. Lim[3], H. G. Lee[2], J. W. Kim[2], J. W. Kim[4] and E. Y. Song[2]
[1]*Department of Molecular Biotechnology, Konkuk University, Seoul, Korea,* [2]*Cellomics Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejon, Korea,* [3]*Department of Biological Science Sookmyung Women's University, Seoul, Korea,* [4]*Department of Laboratory Medicine, Dankook Medical School, CheonAn, Korea. E-mail: eysong@kribb.re.kr*

Recently, N-myc downstream-regulated gene 2 (NDRG2) has been reported as a new candidate for cancer suppressor gene. We

developed a protein microarray assay to assess NDRG2 level in human tissue and cell lines. We synthesized recombinant NDRG2 protein, and produced monoclonal antibodies (mAb) to the NDRG2 protein. To determine NDRG2 concentration, the samples of NDRG2 protein, cell lysate or tissue lysate were spotted onto a slide and allowed to react with the mAb to the NDRG2 protein. The reaction was followed by incubation with horseradish peroxidase (HRP) conjugated anti-mouse IgG antibodies. The addition of dimethylaminobenzidine (DAB) developed color which was measured and analysed by GenePix program. NDRG2 protein concentration in tissue or cell line was calculated based on the standard curve with the known amounts of NDRG2. The dose-response relationship between NDRG2 and developed color intensity showed a linearity in the range 0∼10 ng/ml and the sensitivity of 50 pg/ml. We have determined the NDRG2 concentration in various tissue specimens and cell lines using the new protein microarray technique. The results of the new microarray assay, compared with those of Western blot analysis, showed a good agreement. Thus, we conclude that the new protein microarray method may be adopted to assess specific proteins in human tissues or cell lines, particularly in the field of cancer and pathological research.

PP-703
Matrix metalloproteinase-7, 13, 2 and 9 in colorectal carcinoma: relationship to clinicopathological variables

G. Oktay[1], C. Terzi[2], Z. Cavdar[1], H. Islekel[1], A. E. Canda[2], M. Fuzun[2], A. Kupelioglu[3] and G. Guner[1]
[1]*Department of Biochemistry, School of Medicine, Dokuz Eylul University, Izmir, Turkey,* [2]*Department of General Surgery, School of Medicine, Dokuz Eylul University, Izmir, Turkey,* [3]*Department of Pathology, School of Medicine, Dokuz Eylul University, Izmir, Turkey. E-mail: gulgun.oktay@deu.edu.tr*

Matrix metalloproteinases (MMPs), extracellular matrix-degrading enzymes, are considered to play important roles in cancer invasion and metastasis. In this study, we examined the tissue levels of four different MMPs (MMP-2, 7, 9, 13) and two tissue inhibitors of MMPs (TIMP-1 and 2) in 43 colorectal tumour and paired normal mucosa. Concentrations of MMP-7, 13 and TIMP-1 and 2 were measured in the supernatants using the corresponding enzyme-linked immunosorbent assay (ELISA). Gelatinolytic activities of proform and active form of MMP-2 and MMP-9 in the tissue extracts were examined by gelatin zymography. The levels of MMP-13 and the activities of proform and active form of MMP-2 and MMP-9 were significantly higher in the tumour tissues than paired normal tissues ($P < 0.005$). The increase of MMP-7 and TIMP-1 levels were not significant. TIMP-2 levels of tumour tissues were significantly lower than normal tissues ($P < 0.05$). Among the clinicopathological variables, significant correlations were shown between pathological stage of disease versus MMP-7, proMMP-2 and active MMP-2; distant metastasis versus MMP-7 and pro MMP-2; tumour differentiation versus MMP-13 and TIMP-1; lymphatic invasion versus MMP-13 and tumour location versus MMP-7 ($P < 0.05$). The increased MMP activity and concentrations and the observed clinicopathological correlations may encourage tumour invasion and metastasis. This study points to MMP-7 as being of potential major importance in the development of colorectal cancer.

PP-704
Proteomic identification of heat shock proteins 90/70 as potential targets for Ewing tumor treatment

A. S. Martins, A. García-Sanchez, C. Mackintosh, D. Herrero Martín, M. Campos, J. L. Ordoñez and E. de Alava
Laboratory of Molecular Pathology, Centro de Investigación del Cáncer, Salamanca, Spain. E-mail: anasofia@usal.es

The Ewing Tumor (ET) cell survival and proliferation depend on several major autocrine loops and therefore their blockade is a promising therapeutical approach. We previously reported the *in vitro* impact of IGF1R/c-Kit pathway blockade on ET cell line proliferation, apoptosis, cell cycle and pathway phosphorylation. We now extended our observations to the level of proteomic changes induced by treatment. ET cell lines A4573 and A673 were treated with ADW742 (a specific IGF1R inhibitor) and/or Imatinib (a selective inhibitor of SCF-KIT loop) for 24 and 72 h. Proteins were resolved on 2D SDS gels, spot patterns with and without treatment compared and different spots selected and analysed by mass spectrometry (MALDI-ToF). The proteins showing the highest fold change were involved in the regulation of proliferation, apoptosis and stress induced responses, such as PA2G4 (Proliferation Associated protein 2G4), HSP90/70 (Heat Shock Protein 90/70), EF1G (Elongation Factor 1-gamma) and RhoA. Protein and mRNA levels were analysed by Western Blotting and qRT-PCR, confirming the results obtained. Heat Shock Proteins 90/70 were dramatically increased in treated cells, at both RNA and proteomic levels, suggesting that these molecules (against whom several specific inhibitors are available) could be interesting candidate targets for ET treatment in combination with IGF1R inhibitors.

Acknowledgment: *Partially funded by Fundación Ramón Areces and Spanish Ministry of Health (PI020828).

Imaging and Noninvasive Techniques: Fluorescence Techniques

PP-705
Investigation of serum albumin conformation by fluorescent probes under conditions of hypokinesia

I. Loryan
Department of Pharmacology, Yerevan State Medical University, Yerevan, Armenia. E-mail: loryani@freenet.am

The goal of the study was to investigate the postsynthetic conformational changes in the molecule of albumin by measuring the maximum and intensity of fluorescence of the probes 1-anilino-8-naphthalenesulfonate (ANS) and carboxiphenylimide of dimethylaminonaphthalenedicarbonic acid (K-35) in serum under conditions of hypokinesia-physical inactivity. Hypokinesia was achieved by placing rats in the cages during 15, 30 and 45 days. Concentration of albumin in the serum was determined at 605 nm using bromcresol purple. The binding property of albumin was investigated by fluorescent probes: ANS and K-35. Statistical data were analysed by SPSS11.0. There was no changes in albumin concentration. The maximum of fluorescence of ANS was unchanged

in whole periods of hypokinesia. The intensity of fluorescence of ANS was statistically increased in 30th and 45th days of hypokinesia by 12.2% and 11% ($P < 0.01$), respectively. Investigation of fluorescence of K-35 has shown statistically valid displacement in longwave region on 30th and 45th days of hypokinesia (505 ± 0.7 nm-control; 520 ± 8.8 nm–30th days; 515 ± 4.08 nm–45th days). The intensity of fluorescence of K-35 was statistically decreased on 45th days of hypokinesia by 32.64% ($P < 0.01$) in comparison with control. We suppose, that postsynthetic conformational changes in the molecule of albumin more impressed in the II-a domain. We recommend using fluorescent probes ANS and K-35 in clinical practice for estimation albumin binding ability.

PP-706
Chemical permeabilized cells, as a model for monitoring of [Ca^{2+}]ER and studying of exocytosis

O. Kopach[1], N. Voitenko[2] and N. Fedirko[1]
[1]*Ivan Franko National University of Lviv, Lviv, Ukraine,* [2]*Bogomoletz Institute of Physiology, Kiev, Ukraine.*
E-mail: kopach_o@yahoo.com

We have elaborated methods for direct measurements of luminal Ca^{2+} inside the Ca^{2+} stores in acinar cells of rat submandibular salivary gland. Digitonin and β-escin were used for the permeabilization of the acinar cells, Arsenazo III dye – to measure the total calcium content, Fura 2/AM and Mag-fura 2/AM – to detect free cytosolic calcium concentration ([Ca^{2+}]i) and free Ca^{2+} concentration inside the endoplasmic reticulum (ER) ([Ca^{2+}]ER), respectively. Total protein content was measured by Lowry method. It was found that the digitonin-permeabilized cells perform $^{2+}$-dependent protein secretion, which level depends on the digitonin concentration and duration of detergent action in a bell-shape mode. The ability of permeabilized cells to perform thapsigargin-sensitive ATP-dependent $^{2+}$ transport was also shown. Thus, digitonin-permeabilized cells are useful model for studying the $^{2+}$-dependent exocytosis and Ca^{2+} stores functioning. Moreover, we have shown that achetylcholine induces Ca^{2+} release from the ER in β-escin permeabilized cells, which was completely blocked by heparin. These data suggest that permeabilization with β-escin leads to penetration of the plasma membrane yet retains coupled receptors. Thus, β-escin permeabilized cells could be used for the studying the role of ER for the intracellular $^{2+}$ signaling mediated by membrane receptors activation.
Acknowledgment: Supported by JDRF#1-2004-30.

PP-707
Color variants of *Galaxeidae* monomeric green fluorescent protein for multicolor imaging

Y. Niino, K. Hotta and K. Oka
School of Fundamental Science and Technology, Keio University, Yokohama, Japan. E-mail: niino@bpni.bio.keio.ac.jp

Aequorea victoria green fluorescent protein (GFP) and Anthozoan GFP-like proteins have been popularly utilized and extremely contributed to extensive biological researches. With their expanded color variations, multicolor imaging has recently come into fashion. In comparison with recent enrichment of red, orange and yellow fluorescent proteins, applicable blue-cyan-emitting variants are limited to *Aequorea victoria* cyan fluorescent proteins (CFPs) such as Cerulean and CyPet. Thus, the additional color variants of bright blue-cyan-emitters must be required. Here we report new spectral variants of a monomerized *Galaxeidae* GFP-like protein, monomeric Azami-Green (mAG). The blue-shifting mutations of CFPs, that have been previously reported, were incorporated into mAG and some of the resulting mutants show blue-cyan fluorescence. Although the cyan-emitter has nearly same fluorescent properties as a dimeric cyan fluorescent protein Midoriishi-Cyan (MiCy), the color of the blue-emitter is especially unique, whose emission peak is between EBFP and ECFP. In addition, a red-shifting mutation generated a lime-emitting mAG mutant, which fluoresces with an intermediate color of EGFP and EYFP. It could be adequate to a partner of the blue-emitter in fluorescence resonance energy transfer (FRET). These mutants would be useful for the development of multicolor imaging applications aided by linear unmixing of fluorescent signals.

PP-708
Recombinant human M3 muscarinic receptors combining several modifications: design and functional characterization

D. O. Borroto-Escuela[1], M. Perez-Alea[1], D. Y. Borroto[2], W. Romero-Fernandez[1] and P. Garriga[1]
[1]*Department of Chemical Engineering, Technical University of Catalonia, Barcelona, Spain,* [2]*Department of Biochemistry, Havana University, Havana, Cuba.*
E-mail: dasiel.borroto@upc.edu

The Human M3 muscarinic receptor is a cholinergic receptor in the central and peripheral nervous systems that is implicated in several neurodegenerative diseases. In order to better characterize and study of this receptor, different recombinant hM3 receptors combining several modifications were designed and overexpressed in COS[-]7 and CHO cells. The different fluorescent chimera were obtained through fusion of the receptor N and C terminus with enhanced green fluorescent protein, potential glycosylation sites and a large part of the third intracellular loop were deleted, a HA tag sequence was introduced at the receptor N terminus, and, a FLAG epitope was either fused to the receptor N or C terminus. The high expression levels and ligand binding properties were almost identical to those of the wildtype hM3 receptor. These results together with confocal microscopy imaging demonstrated that the recombinant proteins were correctly folded and targeted to the plasma membrane. Despite the numerous modifications introduced within the hM3 sequence, all receptors retained nearly normal values in the ligand binding assays. The presence of the different epitopes was confirmed by immunocytochemistry and immunoprecipitation. Our work demonstrates that these fluorescently [-] labelled hM3 receptors are valuable tools for further functional, biochemical, and structural studies of muscarinic receptors.

PP-709
Antinuclear antibody in diagnosis of immune disorders

M. Demir, N. Cevahir, I. Kaleli, M. Gurbuz, E. Mete, C. Ergin and M. Sengul
Microbiology, Pamukkale University, Denizli, Turkey.
E-mail: mdemir@pamukkale.edu.tr

Antinuclear antibody (ANA) tests are very important in diagnosis of immune disorders. ANA results vary widely depending on the substrate and immunohistochemical methods used for detection. In this retrospective study, we analysed ANA and anti-Extractable nuclear antigen (anti-ENA) results of our laboratory. From January 2005 to December 2005, 1375 samples were tested for ANA by indirect immunofluorescence at our laboratory and 204 (14.8%) of 1375 samples were positive (titer ≥ 1 : 100). Immunofluorescent patterns of ANA positive serum samples are as follow; homogeneous (38.7%), speckled (22.5%), nucleolar (13.7%),

cytoplasmic (6.4%), centromere (3.4%) and mixed (11.3%). Anti-ENA (n = 76) and anti- ds DNA (n = 145) tests were performed in ANA positive serum samples. Anti-ENA detected by line immunoassay and Crithidia luciliae coated slides were used for detection of anti-ds DNA. Anti-ENA results are as follow; anti-SSA 4.1%, anti-Ro-52 10.8%, anti-RNP 2.7%, anti-Sm 1.4%, anti-Scl70 4.1%, anti-Cenp-B 1.4% anti-M2 1.4% and two or more pattern 37.8% . Anti-dsDNA found in 6.9% positive. In Conclusion, the data from this analysis are useful in estimating the probabilities of detecting specific ANA in our province.

Biocompatibility of Materials for Advanced Therapies

PP-710
In vitro cytotoxicity and genotoxicity of metallic and polymeric materials

S. Gülçe[1], S. İ. Gürhan[1], N. Hasırcı[2] and T. Endoğan[2]
[1]*Department of Bioengineering, Ege University, Izmir, Turkey*
[2]*Department of Chemistry, Middle East Technical University, Ankara, Turkey E-mail: sultangulce@yahoo.com*

Biomaterials are the materials that can replace whole or a part of the body tissues or organs. The most important property of the biomaterials is its biocompatibility. Tests to ensure the biological compatibility of materials have been specified by regulatory organizations. In this study; different metallic and polymeric materials were tested due to the test protocols of ISO and OECD. Metallic materials tested were; Ti grade 4, alloy 625, alloy 304 L, and alloy 321; and also three different polymeric materials, PMMA, PMMA + MMA and PMMA + MMA + HA were tested. Those consists of polymethylmetacrylate polymer, polymethylmetacrylate and methylmetacrylate monomer and, polymethylmetacrylate plus methylmetacrylate monomer and hydroxyapatite polymer respectively. To determine *in vitro* cytotoxicity of materials, cytotoxicity test with biomaterial extract and cell attachment tests were done. To determine genotoxicity of biomaterials, *in vitro* micronucleus assay with human peripheral blood lymphocytes were done. As a general result, metallic materials are more biocompatible than polymeric ones. If the metallic materials are compared among each other, Ti grade 4 is the least cytotoxic and genotoxic one; alloy 625, alloy 304 L, and alloy 321 (most toxic) follows it. And if we compare the polymeric materials among each other, PMMA is the most toxic one, PMMA + MMA + HA polymer found to be the least toxic. HA addition as a compound reduces cytotoxic and mutagenic effects of MMA monomers.

PP-711
Inhibitory effect of *Morus alba* L. on 3-hydroxy-3-methylglutaryl-coa(HMG-CoA) reductase

S.H. Kim[1], S.K. Oh[1], D.Y. Kwon[1], J.H. Seo[2], S.Y. Ryu[2], Y.S. Kim[2], **M.S. Koo**[1]
[1]*Food Functional Research Division, Korea Food Research Institute, Songnam-Si, Korea(South)* [2]*Korea Research Institute of Chemical Technology, Daejeon, Korea(South)*
E-mail: kiimmy@hanmail.net

HMG-CoA reductase catalyzes the first committed step in lipid (sterol, isoprenoide) biosynthesis. Therefore, inhibition of this enzyme is widely applied to the therapeutic method on the prevention of cardiovascular diseases (hypertension, coronary artery disease, stroke, bone and joint disease, cancer etc.) Syrian Hamster HMG-CoA reductase was overexpressed in *E. coli* and purified. The HMG-CoA reductase inhibitory activity was assayed spectrophotometrically which measured the rate of decrease in absorbance at 340 nm due to the oxidation of NADPH. The methanol extract of *Morus alba* L. was evaluated on the inhibitory effect of HMG-CoA reductase and partitioned to methylene chloride, ethyl acetate (EtOAc) and water soluble fraction. The EtOAc fraction of *Morus alba* L. exhibited a remarkable inhibitory effect of HMG-CoA reductase with 85% inhibition at the concentration of 40 μg/ml. The chromatographic separation of the EtOAc fraction of *Morus alba* L. led to the isolation of three compounds, sanggenon C, mordacin P, and daucosterol. Their structures were established by chemical and spectroscopic methods. Sanggenon C and mordacin P showed significant inhibitory effect on HMG-CoA reductase with 73%, 90% inhibition at the concentration of 40 μg/ml. These results indicate that sanggenon C and mordacin P are to be active compounds of *Morus alba* L. with HMG-CoA reductase inhibitory action.

PP-712
Improved biological properties of non-woven PGA/PVA scaffolds for artificial cartilage

M. Rampichova[1], E. Filova[2], L. Kolacna[2], E. Kostakova[3], L. Ocheretna[3], D. Lukas[3], A. Lytvinets[4] and E. Amler[5]
[1]*Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague, Czech Republic Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic,* [2]*Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague, Czech Republic,* [3]*Faculty of Textile Engineering, Technical University of Liberec, Liberec, Czech Republic,* [4]*Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic,* [5]*Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague, Czech Republic Institute of Biophysics, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic.*
E-mail: m.rampichova@centrum.cz

Polyglycolic acid (PGA) and polyvinylalcohol (PVA) are biodegradable polymers commonly used in medical practice. Our study was focused on PGA and PVA scaffolds for artificial cartilages. The scaffolds were prepared by the wet-laid method. PGA/PVA scaffolds were subsequently treated with PVA solution (PVA/PGA/PVA scaffolds), PGA scaffolds with hyaluronic acid solution (PGA/HA scaffolds) and/or subsequently processed by needle punching (PGA/PVA and PGA/HA scaffolds). Supplementation with nanofibres was also employed. Chondrocytes cultured on polystyrene (PS) were used as a control. Rabbit chondrocytes were cultured for 28 days and seeded onto the scaffolds at a density of 8×104 cells/cm^2. Proliferation and viability of chondrocytes were tested using the MTT test, fluorescence and confocal microscopy. The absorbance of PVA/PGA/PVA, PGA and PS cultured cells 24 h after seeding was significantly higher compared with the other scaffolds. After 7, 14, and 21 days, scaffolds containing PVA (PVA/PGA, PVA/PGA/PVA) showed the highest proliferation rate, comparable with polystyrene cultures. A good pH stability of culture medium was observed. On the other hand, scaffolds prepared with HA showed the lowest proliferation of chondrocytes, accompanied by acidification of the culture medium. In this study, the best proliferation of chondrocytes on three-dimensional non-woven PVA/PGA, PVA/PGA/

PVA scaffolds was shown. These results prove their potential for cartilage repair utilization.

PP-713
Investigation of the model protein interaction with novel di-block copolymers

A. Asady and M. N. Sarbolouky
Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran. E-mail: asady@ibb.ut.ac.ir

Amphiphilic di-block copolymers are typical nonionic polymeric surfactants with tunable hydrophilic and hydrophobic segments (synthesized here) was used to study of their effects on bovine serum albumin and lysozyme as a model proteins. Effects of different biodegradable di-block copolymers on the thermal stability and conformation of proteins in aqueous solution was studied by UV-Vis, flouresence and (near and far) UV-CD spectroscopic techniques. Results showed that these polymers have negligible effects on structure and a little or no increase thermal stability of these proteins. It can be concluded that these polymers are compatible for proteins and hence may be used as additives for several proteins and good candidates for drug delivery purposes and protein pharmaceuticals

Parasitic Diseases

PP-714
New approach aimed at investigation of genomic polymorphism of the Russian *M. tuberculosis* population

N. A. Gvozdevskiy[1], T. L. Azhikina[1], A. O. Botvinnik[1], I. Y. Evsyukova[1], I. G. Shemyakin[2] and E. D. Sverdlov[1]
[1]Russian Academy of Science, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia, [2]State Research Center for Applied Microbiology, Obolensk, Moscow reg., Russia. E-mail: zasulich80@yahoo.com

Mycobacterial population are characterized by considerable genetic heterogeneity. The purpose of our work was identification of genomic differences among various strains of *M. tuberculosis* that would help to better understand mechanisms of their functional differences. We compared genomes of four clinical strains widespread in Russia with the laboratory strain H37Rv by subtractive hybridization. The majority of the found insertions were shared by all the strains, with remarkable exception for 1540 that possess a peculiar genes, absent from other clinical strains. It is significant that among the strains under study 1540 reveals the highest virulence. Two of the differential genes encode putative membrane proteins and may be supposed to change the way of *Mycobacterium* interaction with a host cell, enhancing virulent properties of the strain. With the differences obtained using genomic PCR method we developed diagnostic tool called ID-typing aimed at identification of mycobacterial strains. 172 Russian clinical strain were analysed with the technique to characterize the population structure. We demonstrated good general correlation of the grouping results obtained by ID-typing with other methods in use such as spoligotyping and IS RFLP-typing. Since identification of unknown strains can be achieved quickly and at low cost in terms of consumables and equipment, ID typing may serve as a powerful complement to the existing epidemiological tools for the *M. tuberculosis* complex.

PP-715
L-proline and glucose transport in intracellular forms of *Trypanosoma cruzi*.

A. M. Silber[1], R. R. Tonelli[2], W. Colli[2] and M. J. Manso Alves[2]
[1]Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brasil, [2]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brasil. E-mail: asilber@iq.usp.br

Trypanosoma cruzi, the etiological agent of Chagas' disease affecting 16–18 million people in the Americas and with 80–100 million people at risk, has an obligatory intracellular cycle in the mammalian host. This intracellular cycle initiates with the invasion of mammalian cells by trypomastigotes (TRY) followed by a sequence of differentiation steps into amastigotes (AMA), intracellular epimastigote (IE), and again into TRY. The uptake of glucose and proline, the main energy and carbon sources of the parasite, and the intracellular free proline concentration were determined along the intracellular cycle of *T. cruzi*. Employing the host cell line CHO-K_1 (auxotrophic for this amino acid) it was established that proline is important for the differentiation of IE to TRY and, consequently, for the burst of TRY. IE has the highest proline transport activity (30 and 13 times higher than TRY and AMA, respectively) and the lowest free proline concentration (0.7 mM, in comparison to 6.6 mM for AMA and 2.7 mM for TRY). Conversely, the transport of glucose is 3.5 higher in TRY than in IE. No glucose transport was detected in AMA. Thus: (a) L-proline is essential for the differentiation of IE to TRY; (b) along the mammalian infection a metabolic switch occurs in the parasite from a glucose to a proline-based metabolism. These facts point to the proline transport and metabolism as targets for the development of therapeutic drugs.
Acknowledgment: Supported by: FAPESP and CNPq.

PP-716
Odorant-binding protein (OBP) genes in mosquitoes

M. S. Sengul and Z. Tu
Department of Biochemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA. E-mail: msengul@vt.edu

Olfaction plays a crucial role in host-seeking behaviour of mosquitoes. Odorant-binding proteins (OBPs) are one of the key components of the olfactory pathways in insects and are believed to bind and transport odorants to odorant receptors in olfactory sensory neurons. Little is known of the structural and functional properties as well as the regulation of OBP genes in mosquitoes. Here we report the identification of the selected OBP genes among different mosquito species by bacterial artificial chromosome (BAC) library screening. Multi-species comparison of the upstream regions of the orthologous OBP genes identified conserved non-coding regions, which might be potential regulatory elements of these genes. We characterized the complete gene structure of two OBP genes in Anopheles stephensi, named Ast-OBP1 and Ast-OBP7 because of its high amino acid similarity with Anopheles gambiae AgOBP1 and AgOBP7 respectively. The genome segments containing these two genes share conservation of gene order among mosquitoes as well. We also determined

temporal and spatial expression of these genes of An. stephensi using RT-PCR. A possible decrease in the expression of Ast-OBP1 and Ast-OBP7 after blood feeding may indicate their involvement in host-seeking. The identification and characterization of OBPs in different mosquito species is important for a better understanding of olfactory processes and also develop tools to interfere with the pathways to control mosquito-borne diseases.

PP-717
The regulation of proteolytic activity by endogenous inhibitors produced by dermatophyte fungi

M. Drobnic Kosorok, B. Premrov Bajuk, D. Mihelcic and N. Kozuh Erzen
Institute of Physiology, Pharmacology and Toxicology, Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia.
E-mail: marinka.drobnic-kosorok@vf.uni-lj.si

Microsporum canis is the most common fungal pathogen isolated from cats while Trichophyton mentagrophytes prevails in rabbits. Both species are considered to cause the most important zoonoses. During the invasion they secrete numerous enzymes and other metabolic products that are factors of pathogenesis and can interfere with the host defence system. Among the most important proteins produced by the fungi we can find several proteolytic enzymes. The proteases of all four classes were found to be produced by dermatophyte fungi. Among them, the role of serine protease seems to be the most clarified. It is well known that the regulation of proteolytic activity by specific inhibitors seems to be of great importance since any misbalance in enzyme action can lead to unwanted proteolysis and therefore to pathologic conditions in the cell or tissue. Protease inhibitors have been shown to be important also as the regulators of plant and microbial proteolytic activity. They can protect the host cell against invaders proteases or block the host defensive proteolytic activity during the contagion. In the present work the regulation of proteolytic activity with protein and small molecular mass fungal inhibitors will be demonstrated.

PP-718
Serum macrophage migration inhibitory factor and leptin levels in patients with acute trichinellosis

I. Sari[1], L. D. Kozaci[2], S. Akar[1], M. Birlik[1], **T. Kavak**[2], N. Akkoc[1] and F. Onen[1]
[1]*Division of Immunology and Rheumatology, Department of Internal Medicine, School of Medicine, Dokuz Eylul University, PK 35340, Balcova, Izmir, Turkey,* [2]*Department of Biochemistry, Faculty of Medicine, Adnan Menderes University, 09100, Aydin, Turkey. E-mail: kavaktulay@yahoo.com*

Trichinellosis is a zoonotic infection caused by parasites of the genus *Trichinella*. Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine which has an important role in the regulation of inflammatory-immune responses. Leptin is an adipokine which is also present within lymphoid organs and has an important metabolic and immunomodulatory role. In this study, we investigated the serum levels of MIF and leptin during acute infection and after the treatment of *Trichinella britovi* which occurred in an outbreak in Izmir, Turkey. Serum samples were collected from 36 confirmed acute trichinellosis patients during acute infection and shortly after the treatment MIF and leptin levels in patients sera were measured by a specific sandwich cytokine ELISA. Serum hs-CRP levels were measured by a sensitive turbidimetric method. Both serum MIF and leptin levels increased after therapy whereas hs-CRP decreased. Neither serum MIF nor leptin levels correlated with hs-CRP in acute period. Acute period after treatment: (a) hs-CRP (mg/l) – 20.6 ± 19.4, 1.35 ± 2.94; (b) MIF (pg/ml) – 5415 ± 8965, 7503 ± 10082 and (c) Leptin (pg/ml) – 7888 ± 5682 9590 ± 3893. There was no difference in levels of MIF between acute and after treatment periods. Leptin production was significantly decreased during acute trichinellosis and elevated after treatment. This was in accordance with the decreasing levels of acute phase reactants after the treatment which was pointing out a role of inflammatory cytokines during acute infection.

PP-719
Identification of genotypes of *C. parvum* in children with diarrhea in central Anatolian region by using various techniques

H. Şimşir[1], **S. Sümer**[1] and A. Metin[2]
[1]*Biology Department, Hacettepe University, Ankara, Turkey,* [2]*Department of Pediatric Immunology, Social Security Children's Hospital, Ankara Turkey. E-mail: handesimsir@gmail.com*

Protozoan parasites of the genus *Cryptosporidium*, infect the gastrointestinal track of humans, domestic animals and other vertabrates. Epidemiological evidence indicates that *C. parvum* is one of the most common enteropathogens causing diarheal illness in livestock and humans. While the infection is self-limiting in otherwise healthy subjects, in malnourished children and immunocompromised patients *C. parvum* may cause a chronic diarrhea that can be life threatening. In this study, we collected samples of whole feces from 257 children with diarrhea at Social Security Children's Hospital, Ankara, Turkey. The aim of this study was to determine the reliability of molecular techniques and to show that the molecular techniques are more sensitive than conventional microscopic staining techniques in the diagnosis of cryptosporidiosis. After detecting the oocysts by modified acid-fast staining method, we have also used direct immunofluorescent assay to screen the samples. For molecular studies, a fragment of the *Cryptosporidium* COWP and SSUrRNA genes encompassing the hypervariable region was amplified by PCR and then RFLP analysis was performed for distinguishing the *Cryptosporidium* species in the samples. In RFLP analysis Rsa I, Ssp I and Vsp I enzymes were used to identify the species/genotypes of *Cryptosporidium*. In the samples studied, we have determined only *C. parvum* and its two genotypes. The prevalence of *Cryptosporidium* positivity was about 4.0–5.0%.

PP-720
Leishmaniasis in Bolivia: new therapeutic strategies

S. Revollo, **V. Peralta**, R. Lopez, I. Callisaya, S. Gomez, M. L. Soto, E. Chuquimia and W. Strauss
Facultad de Ciencias Farmacéuticas y Bioquímicas, Universidad Mayor de San Andrés, Av. Saavedra, 2224 La Paz, Bolivia.
Phone: (591) 2.2.222436. Fax: (591) 2.2.224895.
Email: susanarevollo@hotmail.com

In this study, we proposed to develop different schemes of treatment on Bolivian patients with Leishmaniasis. The first based in conventional treatment; then, we will use the Evanta extract, used by the Tsimane ethnic group. The treatment with Glucantime was evaluated using Universal PCR in order to amplify products of three complexes of leishmania found in the new world. However, serological and Giemsa stain assays were also used. Ninety venous blood samples were collected. All patients

were positive to the universal PCR for Leishmaniasis while only 54.4% patients were positive with microscopical examination. A total of 52.2% patients had IgG anti- mixed of three complexes of leishmania. Twenty seven patients completed the treatment with glucantime. The control of treatment was performed at the end of treatment (T1) and 1 month after (T2). The patients with mucous and mucocutaneus leishmaniasis (25.91%) controlled at T1 and T2 all were positive for each tests used in this study. Whereas, the patients with cutaneous leishmaniasis (74.89%) controlled at T1 and T2 all were negative for microscopic and serological tests and 10% were positive of PCR-blood. A new control will be done at 3 (T3) and 12 (T4) months following the end of the treatment. In a second phase, we will use appropriated pharmaceutical form of Evanta extract for treatment of leishmaniasis.

Acknowledgment: *This study was financed by Sweden Cooperation SIDA/SAREC Programme.

Immune Intervention

PP-721
The correlation of adenosine deaminase and dipeptidil peptidase IV at immunity development in rats

H. Haruryunyan[1], S. Mardanyan[1], Sv. Sharoyan[1], Al. Antonyan[1], An. Movsesyan[2] and Hv. Avagyan[3]
[1]Laboratory of Nucletides and Nucleosides, H. Buniatiani Institute of Biochenistry of Armenian NAS, Yerevan, Armenia, [2]Department of Biochemistry, Faculty of Biology, Yerevan State University, Yerevan, Armenia, [3]Faculty of Veterinary Medicine, Armenian Agricultural State Agrarian University, Yerevan, Armenia.
E-mail: hayk@web.am

It is established that DPPIV of rats does not bind ADA. At this, rat's blood plasma posses both the ADA and DPPIV activity. The goal of our investigation was to reveal probable correlations between these enzymes after initiation of primary immune response by human red blood cells intraperitoneal inoculation (109 erythrocytes per animal). We can divide the dynamic of studied enzymes during immune response development on four parts. First one, 0–3 days after immunization, is a period of pre-immune inflammation. It is characterized by positive correlation between ADA and DPPIV activities, picking at the day of immunization (0). It is followed by periods of specific immunity induction. The second part, 3–11 days after immunization, conditionally is called IgM period. During this, the picks of increased activities of both enzymes are detected, but showing negative correlation. The third part of enzymes dynamics, 11–26 days after immunization, might be called IgG period. The curves of ADA and DPPIV activity dependencies on time again have some maximal points, less expressed than the previous. The last detectible part is pronounced pick at 26th day after immunization. It is again characterized by the positive correlation of the enzymes activity, as was the first period. The interaction of these dynamics with immunity development is obvious. The investigations aimed to adjust the links of ADA and DPPIV enzymes with the immune system in rats are in further development.

PP-722
Protease inhibitor cystatin C is differentiation dependent in human dendritic cells

T. Zavasnik-Bergant[1], M. Bergant[2] and G. Griffiths[3]
[1]Department of Biochemistry and Molecular Biology, Jozef Stefan Institute, Ljubljana, Slovenia, [2]Tissue Typing Center, Blood Transfusion Center of Slovenia, Ljubljana, Slovenia [3]European Molecular Biology Laboratory (EMBL), Heidelberg, Germany.
E-mail: tinazavasnik@yahoo.co.uk

Dendritic cells (DC) possess a big capacity to elicit immune response. Their maturation occurs as they migrate to lymphoid organs, where they present captured antigens to T cells. The central role of endosomal/lysosomal proteolytic enzymes in generating antigenic peptides and controlling MHC II traffic defines these enzymes as an important area of investigation. The involvement of natural protease inhibitors has been supported by the study on the cystatin homologue, secreted from filiar parasite, which was shown to down-modulate MHC II-restricted antigen presentation. Our study has been focused on another cystatin homologue, human cystatin C. In order to modulate the proteolytic capacity of intracellular proteases in human DC, our first goal was to characterize the endogenous cystatin C during the differentiation of monocytes to immature DC and their further maturation with TNF-alpha. We showed that in immature DC cystatin C content was highly elevated compared to their precursors. The low content of cystatin C in monocytes resulted from lower expression, but not from its elevated secretion. Increased expression of cystatin C and high content in Golgi were observed in immature DC. The transport of cystatin C was shown from Golgi towards the cell membrane, where cystatin C accumulated in fully mature DC. Differentiation and maturation dependence of endogenous cystatin C supports its intracellular regulatory potential and further suggests it new role in Golgi of immature DC.

PP-723
Generation and characterization of fully human antibodies against orthopoxviruses

T. Yun[1], L. Shingarova[2] and N. Tikunova[1]
[1]State Research Center of Virology and Biotechnology "Vector", Koltsovo, Russia, [2]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia.
E-mail: yun_t@ngs.ru

Reemergence of monkeypox as a serious human disease in Africa have fuelled renewed interest in orthopoxviruses. Vaccinia virus (VACV) was used in the past as an effective vaccine against smallpox. Although VACV is generally safe vaccine, disseminated, life-threatening infections occur infrequently, especially in individuals with impaired immunity. Such complications can be treated by therapeutic administrations of human VACV immunoglobulin (VIG), which has some limitations. Recombinant fully human antibodies offer an obvious alternative to VIG and human Mabs obtained by traditional hybridoma technology. Specific single-chain antibodies were selected from phage display library of human scFvs using biopanning procedure against VACV. Vh and Vl domains of promising scFvs were used for generation of fully human antibodies. The HEK293T human cells have been co-transfected with the plasmids encoding heavy and light chains of human IgG1. Fully human Mabs were purified from culture supernatant by affine chromatography. Immunochemical properties of the antibodies obtained have been assayed by ELISA and Western-blot analysis using different orthopoxviruses. Affinity constants were determined using subsequent dilutions of antigens and antibodies in ELISA, and compared with parental scFv.

Affinity constants for the generated Mabs were 100 times higher in average, than for the parental scFvs.

PP-724
Development and characterization of monoclonal antibodies to VEGFR2

A. Basalp, F. Yucel, A. Kumbasar and K. Baysal
TUBITAK, Research Institute for Genetic Engineering and Biotechnology Gebze, Kocaeli, Turkey.
E-mail: basalp@gmbae.tubitak.gov.tr

Vascular endothelial growth factor receptor 2 (VEGFR2/KDR) plays an essential role in physiological and tumor-associated angiogenesis and regulates proliferation and migration of endothelial cells. In the present study, monoclonal antibodies to VEGFR2 were produced by conventional hybridoma technology and blocking effect on endothelial cell proliferation was investigated. BALB/c mice were immunized with 5 μg of recombinant human soluble VEGFR2/KDR Domain 1–7 produced as a glycosylated monomeric protein with a mass of approximately 116 kDa. After selecting of hybridomas, five clones were found to be specific for VEGFR2 as tested with ELISA and all of them were of the IgG1 isotype. Monoclonal antibodies were purified and also tested for their ability to inhibit VEGF induced proliferation of human vascular endothelial cells (HUVEC) *in vitro*. Cell proliferation was evaluated by the neutral red uptake and BrdU incorporation assays. At antibody concentrations up to 10 μg/ml, all five clones had no discernable effect on VEGF dependent cell proliferation, while 1 μg/ml control antibody 293 (R and D Systems) inhibited VEGF activity up to 60%. This result suggests that the antigen recognition site of these antibodies differs from VEGF-VEGFR2 interaction domains. Western blotting experiments following electrophoresis showed that all five antibodies recognized the recombinant human soluble VEGFR2/KDR Domain 1–7.

PP-725
SN polarizes Th1/Th2 responses via the inhibition of immunostimulatory function of dendritic cells

J. S. Lee[1], S. K. Kim[2], Y. I. Jeong[3], C. M. Lee[3], E. Y. Choi[3], M. S. Yoon[2], G. Y. Kim[4], H. Y. Chung[1] and **Y. M. Park**[3]
[1]College of Pharmacy, Pusan National University, Busan 609-735, Korea, [2]Department of Obstetrics and Gynecology, Pusan National University College of Medicine, Busan 602-739, Korea, [3]Department of Microbiology and Immunology and National Research Laboratory of Dendritic cells regulation, Pusan National University College of Medicine, Busan 602-739, Korea, [4]Faculty of Applied Marine Science, Cheju National University, Jeju-do 690-756, Korea. E-mail: immunpym@pusan.ac.kr

SN is the major active compound in silymarin. It has been shown to have anti-carcinogenic effects, and hepato-protective effects. However, the effects of SN on the maturation and immunostimmulatory function of dendritic cells (DC) largely remains unknown. In this study, we investigated whether SN can influence surface molecule expression, dextran uptake, cytokine production, capacity to induce T cell differentiation, and their underlying signalling pathways in murine bone marrow-derived DC. SN significantly suppressed CD80, CD86, MHC class I, and MHC class II expression in DC. They also showed impaired LPS-induced IL-12 expression in DC. SN-treated DC was highly efficient at Ag capture via mannose receptor-mediated endocytosis. SN inhibited LPS-induced MAPK activation and the nuclear translocation of NF-κB p65 subunit. In addition to, SN treated DC showed the impaired induction of Th1 responses and a normal cell mediated immune response. These findings provide new insight into the immunopharmacological role of SN in impacting on the DC. These novel findings open perspectives for the understanding of the immunopharmacological role of SN and therapeutic adjuvants for DC related acute and chronic diseases.

PP-726
Interleukin-10 and tumour necrosis factor-alpha promoter regions polymorphisms in rheumatoid arthritis patients

O. Ates[1], V. Hamuryudan[2] and **A. Topal Sarikaya**[1]
[1]Department of Molecular Biology and Genetics, Science Faculty, Istanbul University, Istanbul, Turke, [2]Department of Rheumotology, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey. E-mail: atopal@istanbul.edu.tr, vhamuryudan@yahoo.com, omerates27@yahoo.com

A differential response to cytokine stimulation can exert strong control on the Th1/Th2 balance and hence affect immune disease etiology such as rheumatoid arthritis. It has been known that cytokines are often encoded by highly polymorphic genes. This polymorphism may be responsible for observed inter-individual differences in cytokine production and may be one possible mechanism for the perturbation of the Th1/Th2 balance. We investigated IL10 -1082 G/A, -819C/T/ (IL10-592), and TNF-α-308 G/A gene polymorphisms in 103 patients with rheumatoid arthritis and 122 control subjects. We found no evidence of association between TNF-α-308 G/A, IL10-819C/T (IL10-592) gene polymorphisms and rheumatoid arthritis ($P = 0.127$ and $P = 0.086$). IL10 -1082 G/A polymorphism was significantly associated with rheumatoid arthritis when compared with healthy controls ($P = 0.003$). These findings suggest that IL10 -1082 G/A polymorphism influences the Th1/Th2 balance and hence can play role in rheumatoid arthritis etiology.

PP-727
Polymorphism of SLC11A1 gene confers susceptibility to systemic sclerosis

O. Ates[1], B. Musellim[2], G. Ongen[2] and A. Topal-Sarikaya[1]
[1]Department of Molecular Biology and Genetics, Istanbul University, Istanbul, Turkey, [2]Chest Disease Department, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey.
E-mail:omerates27@yahoo.com, atopal@istanbul.edu.tr, gulongen@mynet.com

Solute carrier 11a1 (SLC11A1; formerly NRAMP1, where NRAMP stands for natural resistance-associated macrophage protein) is a proton/bivalent cation antiporter that localizes to late endosomes/lysosomes. SLC11A1 regulates macrophage functions that are of potential importance in the induction and/or maintenance of autoimmune diseases such as Rheumatoid arthritis, Kawasaki, Crohn's disease. We investigated SLC11A1 gene as a candidate gene for genetic susceptibility to systemic sclerosis (SSc) which is a progressive collagen vascular disorder with unknown etiology. Three SLC11A1 gene polymorphisms (D543N, 1729 + 55del4 and INT4 (469 + 14G/C) were analysed in a case-control study of 50 SSc patients with lung involvement and 122 control subjects. We found no evidence of association between D543N, 1729 + 55del4 polymorphisms and systemic sclerosis ($P = 0.249$). Linkage disequilibria could be found D543N and 1729 + 55del4. INT4 (469 + 14G/C) polymorphism was significantly associated with SSc when compared with healthy controls ($P = 0.03$). These findings suggest that INT4

(469 + 14G/C) of SLC11A1 influences the susceptibility to systemic sclerosis.

PP-728
Mature dendritic cells and antigen acquisition/ presentation

S. Henri, C. Siret, M. Mello, P. Machy and L. Leserman
Centre d'Immunologie de Marseille-Luminy.
E-mail: leserman@ciml.univ-mrs.fr

Studies using monocyte or bone-marrow-derived dendritic cells (DC) report DC express most class II molecules at the cell surface and downregulate pinocytosis after maturation *in vitro*. It is expected they have reduced capacity to take up and present antigen (Ag). What is the situation for DC in lymph nodes or in peripheral tissues? To determine how their state of maturity affects the capacity of these DC to acquire and present Ag to T cells we have performed experiments using different DC populations purified from lymph nodes as well as skin explant DC as Ag presenting cells. We measured proliferation of Ag-specific transgenic CD4 T cells *in vitro* and the CD4-dependent induction of cross presentation to Ag-specific CD8 T cells. There was little or no reduction in the capacity of DC purified from lymph nodes to acquire and present liposome associated Ag after overnight incubation, a situation resulting in upregulation of maturation markers. This acquisition and presentation was further unmodified by treatment with LPS *in vivo* before Ag exposure *in vitro*. As for bone marrow derived DC, all CD11c + DC populations isolated from lymph nodes express the IgG FcR and were much more efficient for presentation of antigen to CD4 + cells when the antigen was in IgG-opsonized liposomes. We conclude that DC maturation does not prevent Ag acquisition and presentation *in vitro* and suggest that targeted Ag may continue to be effective *in vivo* whatever the extent of DC maturity.

PP-729
Properties of fragments derived from a monoclonal antibody against prion protein displaying therapeutic interest

C. Alexandrenne[1], S. Padiolleau-Lefèvre[1], F. Dkhissi[2], G. Clément[3], C. Blache[2], C. Créminon[1], J. Grassi[1], J. Y. Couraud[2], D. Boquet[1] and A. Wijkhuisen[2]
[1]*Commissariat à l'Energie Atomique (CEA) Saclay. Service de Pharmacologie et d'Immunologie. Bat 136. 91191 Gif sur Yvette Cedex,* [2]*Equipe CEA- Université Paris VII UFR de Biologie, Tour54. 75005 Paris Cedex 05,* [3]*Laboratoire associé INRA-CEA d'immunoallergie alimentaire, Saclay. SPI, Bat 136. 91191 Gif sur Yvette Cedex. E-mail: wijkhuis@dsvidf.cea.fr*

Prion diseases are fatal neurodegenerative infectious disorders characterized by the accumulation of PrPSc, the abnormally folded isoform of the cellular prion protein (PrPc), which represents the major component of infectious prions. Currently, no effective treatment exists and novel therapeutic strategies have been developed in order to identify new compounds that could interfere with *in vivo* prion propagation. During last years, several studies have shown that anti-PrP antibodies could antagonize *in vitro* and *in vivo* prion propagation. In this context, we have produced two fragments derived from a monoclonal anti-PrP antibody, whose therapeutic effect on *in vitro* prion-infected neuroblastoma cells has been previously demonstrated. The Fab fragment has been obtained by papain digestion of whole antibody, whereas the scFv fragment has been produced by recombinant techniques. Using enzymoimmunoassay and surface plasmon resonance techniques, we have shown that Fab and scFv possess similar high affinity for recombinant human prion protein. Furthermore, we have shown that Fab and scFv were both able to specifically and in a dose dependent manner recognize the human PrPc expressed at the surface of a new stably transfected HEK 293 cells, which is a prerequisite for a therapeutic application. Data concerning the capacity of Fab and scFv to inhibit PrPsc replication in a cell culture model constitutively expressing murine PrPsc will be also presented.

PP-730
The association between cytotoxic T lymphocyte antigen-4 (CTLA-4) A49G polymorphism and autoimmune blood diseases

F. Akturk, **V.S. Hancer**, S. Guvenc and R. Diz-Kucukkaya
Division of Hematology, Department of Internal Medicine, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey.
E-mail: vshancer@yahoo.com

Decrease or loss of immune tolerance has an important role in the development of autoimmunity. Mechanisms of down regulation of T cell immunogenic response (TCR) by cytotoxic T lymphocyte antigen-4 (CTLA-4) may be due to competitive antagonism with CD28, increment of TCR stimulation threshold, limitation of T cell division capacity and activation of cell cycle arrest of T cells after antigenic stimulation. Recent studies showed that CTLA-4 molecule may have a great role in the development of autoimmune diseases and disorders. In human studies, CTLA-4 49th A/G polymorphism was found to be associated with autoimmune diseases. In the present study we have investigated CTLA-4 1. Exon 49 A/G polymorphism in 108 patients with autoimmune blood diseases grouped as immune trombocytopenic purpura (ITP, $n = 62$) and autoimmune hemolytic anemia (AIHA, $n = 46$). The frequency of CTLA-4 A49G polymorphism was also screened in 150 healthy subjects. No significant differences were found to be in terms of allele frequencies between patients and healthy controls. There were also no statistically significant differences between ITP, AIHA patients and healthy controls, regarding CTLA-4 1. exon 49 A/G polymorphism. Further studies with higher numbers of patients and simultaneous analysis of multiple gene variants conducted in genetically isolated populations may provide better results.

PP-731
Production and characterization of neutralizing single-chain antibodies against human VEGFR-2

B. Erdag, K. Balcioglu, **A. Ozdemir**, A. Kumbasar and K. Baysal
TUBITAK Research Institute for Genetic Engineering and Biotechnology, P.O.Box 21, 41470 Gebze, Kocaeli, Turkey.
E-mail: berrin@gmbae.tubitak.gov.tr

The human vascular endothelial growth factor (VEGF) and its receptor (VEGFR-2/KDR) play an essential role in angiogenesis which occur in normal growth and development as well as pathological conditions including tumor growth and rheumatoid arthritis. In the present study, a single-chain antibody phage display library was constructed from spleen cells of mice immunized with recombinant human soluble VEGFR2/KDR Domain 1–7 to obtain antibodies that block VEGF binding to VEGFR-2. Hence, VEGFR-2 signaling pathway is a major target for therapeutic applications. Two specific single-chain antibodies recognizing the human VEGFR-2 were selected and diversity analysis of these clones was performed by BstNI fingerprinting and nucleotide sequencing. The scFvs were expressed in soluble form and

the specificity of the interactions between the TALON purified scFvs and VEGFR-2 was confirmed by ELISA. The ability of these scFvs to block VEGF signaling pathway was investigated by HUVE cell proliferation assays. Both single chain antibodies (clone 1.3 and 2.6) inhibited human umbilical vein endothelial cell (HUVEC) proliferation *in vitro*, up to 50 and 61% respectively while VEGF neutralizing antibody (MAb293) exhibited between 53–58% inhibition. Pending further characterizations by *in vivo* experiments, HUVE cell proliferation assay results suggest that these two scFvs are potential candidates to be developed as therapeutic anti-angiogenic agents.

PP-732
Production of monoclonal antibodies against vascular endothelial growth factor

F. Yucel, S. Ozturk, A. Kumbasar, M. Serhatli, B. Erdag, K. Balcioglu, A. Basalp and K. Baysal
TUBITAK – Research Institute for Genetic Engineering and Biotechnology TUBITAK Gebze Yerleskesi P.O. Box 21 41470 Gebze – Kocaeli / Turkey E-mail: fatma@gmbae.tubitak.gov.tr

Vascular endothelial growth factor (VEGF) is a multi-functional cytokine that stimulates endothelial cell proliferation and angiogenesis. Antibodies that block the interaction of VEGF with its receptor may be used as therapeutic agents in various pathological states. In this study, monoclonal antibodies against VEGF were developed and characterized. BALB/c mice were immunized with human VEGF produced in *E. coli* (bhVEGF). Spleen and lymph nodes were used as a source of high-titer antibody producing lymphocytes and fused with myeloma cells of F0 origin separately. A total of 86 clones were obtained. ELISA tests showed that clones 5B2 and 2A7, both obtained form lymph nodes, produce antibody with the highest specificity for bhVEGF. The isotypes of these clones were found to be of IgG2a (5B2) and IgG1 (2A7); they did not exhibit any cross-reaction with related proteins. Western blotting experiments showed that both antibodies recognized recombinant human VEGF of eukaryotic origin. These antibodies were then tested for their ability to inhibit proliferation of human umbilical vein endothelial cells (HUVEC) *in vitro*. HUVEC cell proliferation was assessed by the Neutral Red uptake assay. The antibodies did not have an effect on VEGF dependent cell proliferation, while a function-blocking antibody MAb293 at a concentration of 1 μg/ml inhibited VEGF induced HUVEC proliferation up to 70%. These antibodies may be utilized in Western blot and immunohistochemical analyses.

PP-733
The possible association between systemic sclerosis and FokI, BsmI, TaqI polymorphisms in VDR

O. Ates[1], **B. Dolek**[2], S. Guz-Eroglu[2], B. Musellim[3], G. Ongen[3] and A. Topal-Sarıkaya[3]
[1]Department of Molecular Biology and Genetics, Istanbul University, Istanbul, Turkey, [2]Department of Molecular Genetics, Duzen Laboratory, Istanbul, Turkey, [3]Chest Disease Department, Cerrahpasa Medical Faculty, Istanbul, Turkey.
E-mail: bilgen@duzen.com.tr, omerates27@yahoo.com, atopal@edu.tr

Systemic sclerosis (SSc) is an autoimmune disorder of unknown etiology characterized by severe and often progressive cutaneous and visceral fibrosis, pronounced alterations in the microvasculature, numerous cellular and humoral immune abnormalities. SSc is similar to other rheumatic and autoimmune disease as a multifactorial disease, possibly triggered by environmental factors in a genetically predisposed host. We aimed to determine possible association between SSc and vitamin D receptor (VDR) polymorphisms such as FokI, BsmI, and TaqI. Polymerase chain reaction (PCR) based restriction enzyme analysis (REA) was carried out to screen these polymorphisms. We analysed these polymorphisms in 50 SSc patients with lung involvement and 80 healthy control subjects. We found no association evidence between SSc and FokI, TaqI ($P = 0.248$, $P = 0.189$, respectively). BsmI polymorphism studies are still in process.

PP-734
Avian collectins: protectors against infections in birds?

A. Hogenkamp[2], M. van Eijk[2], A. van Dijk[2], A. van Asten[1], E. Veldhuizen[2] and **H. Haagsman**[2]
[1]Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands, [2]Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.
E-mail: A.Hogenkamp@vet.uu.nl

Collectins play a key role in innate immunity. These pattern-recognition molecules have been shown capable of neutralizing various pathogens, including influenza A virus. In avians, the only collectin described thus far is chicken mannan binding lectin. In this study, we sought to identify other collectins in chicken by searching the EST database using the amino acid sequences of known collectins, and related the results to the chicken genome. Three chicken collectins were found and designated chicken Collectin 1 (cCL-1), chicken Collectin 2 (cCL-2), and chicken Collectin 3 (cCL-3), which resemble the mammalian proteins Collectin Liver 1, Collectin 11 and Collectin Placenta 1, respectively. Additionally, a lectin resembling Surfactant Protein A (SP-A) was found. Lacking the collagen-like domain characteristic for collectins, it was named chicken Lung Lectin (cLL). An extensive tissue distribution analysis showed that cCL-1, cCL-2 and cCL-3 are expressed in a wide range of tissues throughout the digestive, reproductive and lymphatic system. Similar to SP-A, cLL is mainly localized in tissues associated with the respiratory system. The newly found chicken collectins could have a significant role in avian immunity. Work is currently in progress to produce these lectins in order to study their characteristics. A better understanding of the role of these proteins may ultimately lead to strategies to reduce infectious diseases in poultry and zoonotic diseases.

PP-735
ASC is important for innate immunity against listeria monocytogenes *in vivo*

N. Ozoren[1], J. Masumoto[1], L. Franchi[1], T. Kanneganti[1], M. Body-Malapel[1], I. Erturk[1], R. Jagirdar[1], L. Zhu[1], N. Inohara[1], J. Bertin[2], A. Coyle[2], E. Grant[2] and G. Nunez[1]
[1]Department of Pathology, University of Michigan, Ann Arbor, MI-48109, USA, [2]Millennium Pharmaceuticals, Inc., Cambridge, MA-02139, USA. E-mail: nesrin.ozoren@boun.edu.tr

ASC is a critical adaptor for mammalian inflammasome assembly and apoptosis induction. We studied the role of ASC in the host defense against the intracellular pathogen Listeria monocytogenes using ASC-deficient mice. ASC was found to be essential for the secretion of IL-1β/IL-18, but dispensable for IL-6 and TNFα in macrophages infected with Listeria. Activation of caspase-1 was abolished in ASC-deficient macrophages, whereas activation of NF-κB and p38 was unaffected. Analysis of Listeria mutants

revealed that cytosolic invasion was required for ASC-dependent IL-1β secretion, consistent with a critical role for cytosolic signaling in the activation of caspase-1. Furthermore, ASC-deficient mice were more susceptible to Listeria infection *in vivo*. The lower survival rate of ASC-deficient mice appears to be due to inefficiency in clearing Listeria from their livers and spleens.

PP-736
Performance of different DNA extraction systems from sputum samples: a preliminary study

P. Yurdakul[1], K. Ergünay[1], **E. Buber**[2] and B. Şener[1]
[1]*Department of Clinical Microbiology, Faculty of Medicine, Hacettepe University, Ankara, Turkey,* [2]*Department of Biochemistry, Faculty of Medicine, Hacettepe University, Ankara, Turkey. E-mail: ebuber@hacettepe.edu.tr*

Molecular techniques have been widely used in clinical pathology laboratories for the diagnosis of infectious diseases. In this report we aimed to compare performances of different DNA extraction systems from sputum. A total of 20 fresh sputum samples that have been collected for routine bacteriological analysis were included in the study. After sputolysin treatment, each sample was divided into four 500 µl aliquots. The samples were then spiked with *B. cepacia* (Neqas quality control strain 7394) for a final amount of 107 to 103 cfu. Phenol chlorophorm method, CTAB method, a commercial spin column kit and simple boiling were employed for DNA extraction on a total of 80 samples. The amount of DNA and efficiency of extraction were evaluated by comparing absorbance values at 260 and 280 nm via spectrophotometry. Phenol chloroform extraction method displayed better DNA yields for most of the samples. For two samples spiked to a final amount of 105 and 104 cfu, CTAB method gave highest extracted DNA concentrations although this was not a consistent finding for other samples in the same group. Performance of spin column kit and simple boiling were overall lower compared to other systems. Depending on spectrophotometric results, phenol chloroform extraction method was found to be more suitable for DNA extraction from sputum samples.

PP-737
Tumor markers of potential clinical use in radioimmunotherapy

L. I. Brasoveanu[1], D. Hotnog[1], M. D. Simioana[1], L. Puiu[2] and M. C. Constantin[1]
[1]*Center of Immunology, Romanian Academy, Bucharest, Romania,* [2]*Institute of Oncology, Bucharest, Romania.*
E-mail: librasoveanu@rdslink.ro

Impossibility of eradication of all tumor cells seems to be the main obstacle against success of therapy in many cancers. Increase of replicative capacity, loss of cell adhesion and angiogenesis process represent aggravating factors of clinical evolution for cancer patients. Systemic administration of mAbs, targeted against tumor associated antigens (TAA) and labelled by isotopes might be used in radioimmunotherapy. Biomarkers like mucins Ep-CAM and MUC-1, a ligand of cell adhesion molecule ICAM-1, besides VEGF were taken into account as potential targets for radioimmunotherapy. The present work focused on evaluation by flow-cyometry of expression of several TAA like MUC-1, Ep-CAM and VEGF as compared to ICAM-1 in different cell cycle phases. Studies were performed on treated breast MCF-7, SK-BR-3, ovarian SK-OV-3, cervix HeLa and colon COLO 201 carcinoma cells by cytokines and cytotoxic drugs. Tumor cells are usually resistant to C-lysis induced by anti-TAA mAb due to the presence of membrane complement regulatory proteins (CRP). Therefore, gene expressions of ICAM-1, MUC-1 and CRP were evaluated by RT-PCR technique using specific primers. By using C-activating anti-TAA mAbs in combination with anti-CRP mAbs significant increases of lysis were observed. Percentages of C-lysis correlated with membrane expression and mRNA levels of CRP in tumor cell lines under study. Data obtained might ensure further innovative therapeutic approaches with potential application in cancer.

PP-738
Effects of rolipram on oxidative stress and inflammation in the early phase of *B. melitensis* infection

O. Aslantas[1], S. Erdogan[2] and S. Celik[2]
[1]*Department of Microbiology, Faculty of Veterinary Medicine,* [2]*Department of Biochemistry, Faculty of Veterinary Medicine, Mustafa Kemal University, 31034 Antakya, Turkey.*
E-mail: aslantas@mku.edu.tr

Effects of phosphodiesterase 4 (PDE4) inhibitor rolipram, intracellular cyclic AMP elevator agent, on malondialdehyde, nitric oxide (NO), superoxide dismutase activity, inducible nitric oxide synthase (iNOS), TNF-α, IFN-γ, and IL-10 mRNA transcriptions were investigated during the early phase of *B. melitensis* infection in rats. *Brucella* did significantly increase lipid peroxidation in plasma, liver and spleen and also NO content in the liver and spleen. Rolipram administration (1 mg/kg/day i.p., 3 days) gradually suppressed lipid peroxidation and NO formation to the basal level in plasma and spleen but slight decrease was observed in the liver. *Brucella* significantly decreased superoxide dismutase activity in the liver and spleen whereas rolipram restored the enzyme activity in the liver, but unchanged in spleen. Reverse transcriptase PCR (RT-PCR) analyses showed that *B. melitensis* do not alter TNF-α and IFN-γ transcriptions in the liver and spleen. The pathogen did not consistently induce iNOS mRNA transcriptions in animals housed even in the same group. IL-10 transcription was induced by rolipram in spleen but not in the liver. As a result, PDE4 inhibitor which elevates intracellular cyclic AMP level suppressed lipid peroxidation and NO concentration increased by *B. melitensis*. Moreover, rolipram induces anti-inflammatory cytokine IL-10 transcription in spleen although tissue dependent manner.

PP-739
Effect of aluminum on TNF-α secretion from murine RAW264.7 cells for endotoxin detection in hepatitis B vaccines

C. Y. Park, S. S. Lee and **D. K. Rhee**
College of Pharmacy, SungKyunKwan University, Su-Won, South Korea. E-mail: dkrhee@skku.edu

The rabbit pyrogen test and Limulus amoebocyte lysate (LAL) assay have been used to detect endotoxins present in vaccines. Currently, the rabbit pyrogen test is used to detect endotoxins in hepatitis B (HB) vaccines, even though the HB surface protein, which is the active ingredient, is over-expressed in and purified from eukaryotic cells that lack these endotoxins. Aluminum hydroxide in the HB vaccine can interfere with the LAL assay. In contrast, macrophages can detect the endotoxin as well as other pyrogens, and secrete TNF-α. Therefore, this study was undertaken to examine the possibility of replacing the animal

tests with more efficient TNF-α secretion assay. With this in mind, we determined if aluminum hydroxide in the HB vaccines affects the TNF-α secretion assay. HB vaccines and the HB protein solutions spiked with lipopolysaccharide (LPS) produced the same level of dose-dependent TNFα secretion and temperature increase in rabbits, indicating that aluminum hydroxide in the HB vaccine does not interfere with the pyrogenic response in rabbits, nor does it interfere with TNF-α secretion. In addition, the TNF-α assay was found to be more sensitive than the LAL assay, and correlated well with the pyrogen test and the LAL assay. These results suggest that the TNF-α assay in RAW264.7 cells is a good substitute for the current pyrogen assays that are used for detecting LPS in HB vaccines as well as in other vaccines containing aluminum.

PP-740
Increased serum neopterin levels in Familial Mediterranean Fever

U. Kisa[1], S. Guliter[2], O. Kisa[3], U. Musabak[4] and A. Tuzun[5]
[1]Department of Biochemistry, School of Medicine, University of Kirikkale, Turkey, [2]Department of Gastroenterology, School of Medicine, University of Kirikkale, Turkey, [3]Department of Microbiology, Gulhane Military Medical Academy, Turkey, [4]Department of Immunology, Gulhane Military Medical Academy, Turkey, [5]Department of Gastroenterology, Gulhane Military Medical Academy, Turkey. E-mail: uclerkisa@hotmail.com

Familial Mediterranean Fever (FMF)is an autosomal recessive inherited disorder characterized by periodic episodes of fever, polyserositis, arthritis, and skin lesions. FMF occurs predominantly in Sephardic Jews, Turks, Armenians, and Arabs. Neopterin is a pyrazino-pyrimidin compound, and is synthesized from guanosine triphospate (GTP) in macrophages and monocytes. Biosynthesis of neopterin requires multiple regulatory factors on monocyte/macrophage populations activated by interferon-γ. Neopterin concentration indicates cellular immune activation. The aim of the study was to evaluate the usefulness of neopterin in the clinical evaluation of FMF patients. Twenty one patients with FMF and 43 healthy persons were enrolled in the study. Serum levels of neopterin were measured by an Enzyme-Linked Immunoassay(ELISA); CRP, ASO, and RF levels by turbidimetric methods with an autoanalyser. Blood was drawn from patients with FMF during attack-free period, attacks, and 1 week after an attack. The mean levels of neopterin and CRP were 7.16 ± 1.91 pg/ml and 0.31 ± 0.11 mg/dl, 21.49 ± 7.14 pg/ml and 13.54 ± 10.2 mg/dl, 14.03 ± 4.02 pg/ml and 4.24 ± 5.31 mg/dl in patients with FMF on attack-free period, attack, and 1 week after an attack, respectively. The mean serum neopterin level of control patients was 4.10 ± 1.82. ASO and RF levels were found in normal ranges in after attack period. The levels of neopterin in FMF patients were significantly higher than those of controls ($P < 0.0001$). Neopterin and CRP levels were also significantly higher in FMF patients on attack than those of FMF patients on attack-free period and 1 week after the attack ($P < 0.001$, $P < 0.0001$ respectively). This study is the first with respect to investigating neopterin levels in FMF. Our findings confirm that neopterin may play a role in pathogenesis of FMF. On the other hand, it can be used as an activation marker in FMF.

New Vaccines

PP-741
Carbohydrate-based vaccines against malaria and leishmaniasis: ventures of chemistry and biology

X. Liu[1], F. Kamena[1], **G. Pluschke**[2] and P. Seeberger[1]
[1]Laboratory for Organic Chemistry, Swiss Federal Institute of Technology (ETH) Zürich Wolfgang-Pauli Strasse 10, 8093 Zürich, Switzerland, [2]Swiss Tropical Institute Socinstrasse 57 4002 Basel, Switzerland. E-mail: xliu@org.chem.ethz.ch

Cell surface glycoconjugates serve as specific markers on particular subsets of cells including cancer cells, bacteria, viruses and parasites. These disease-specific carbohydrates have attracted the attention of immunologists as antigens for the creation of novel vaccines. In recent years, the field of carbohydrate-based vaccines has witnessed dramatic strides as the methods for the synthesis of highly complex oligosaccharides have significantly improved. Described is the chemical syntheses of series of antigenic oligosaccharides, including glycosylphosphatidylinositols (GPIs) and lipophosphoglycans (LPGs) expressed on the cell surfaces of the parasites responsible for malaria and leishmaniasis respectively. These oligosaccharides were subsequently conjugated to either carrier proteins or virosomes and were evaluated as potential vaccines against malaria and leishmaniasis in the rodent models. The immunological data on the vaccination studies will be presented in details.

PP-742
Developing an immunotherapy for relapse prevention in cocaine abuse

W. Ball[1], S. Paula[1], M. Tabet[1], **M. Norman**[2] and A. Norman[2]
[1]Department of Pharmacology and Cell Biophysics, [2]Department of Psychiatry, University of Cincinnati, College of Medicine, Ohio, USA. E-mail: William.Ball@uc.edu

Passive immunization to cocaine may be a therapeutic alternative to the thus far unsuccessful development of pharmacological agents as a treatment for cocaine abuse. A successful immunotherapy requires an antibody with a high affinity for cocaine but not its major inactive metabolites [benzoylecgonine (BE) and ecgoninemethylester (EME)]. In addition, the antibody needs to be non-immunogenic since this treatment requires the antibody to be in circulation for an extended period. In our laboratory, we have used a novel transgenic mouse strain and hybridoma technology to produce a predominantly human sequence anti-cocaine monoclonal antibody (mAb), designated as 2E2. Radioligand and competition binding studies have shown 2E2 to have a high affinity for cocaine (Kd = 4 nM) and a 10- and 1500-fold lower affinity for BE and EME, respectively. Next, a 3D structure-activity relationship (3D-QSAR) model of 2E2 interactions with cocaine was achieved by determining mAb binding to a collection of cocaine analogues. Finally, the *in vivo* administration of 2E2 in mice has been found to effect a dramatic change in cocaine

distribution pharmacokinetics. A 26-fold increase and 78% decrease in plasma and brain cocaine concentrations (AUC), respectively, without alteration of cocaine's rate of elimination was achieved with stoichiometric levels of 2E2. Thus, 2E2 is predicted to have a significant clinical efficacy for reducing the probability of cocaine-induced relapse.

PP-743
Water-soluble polymeric bioconjugates of hepatitis B surface polypeptide antigens and their immunogenicity

Z. Mustafaeva Akdeste
Bioengineering, Yildiz Technical University, Istanbul, Turkey.
E-mail: zakdeste@yildiz.edu.tr

We have recently developed new approaches for obtaining highly immunogenic peptide conjugates: synthetic polyelectrolytes (PE) were used for the conjugation with peptide molecules in which PE carry out the carrier and adjuvant roles simultaneously. In this study, four epitopes of antigenic parts of surface antigen of Hepatitis B virus (2–16, 22–35, 95–109 and 115–129 of the s gene.) had been synthesized. The synthesis of peptides was performed by Explorer PLS $\reg$ Automated Microwave Synthesis Workstation (CEM). Peptide conjugates of synthetic anionic polyelectrolytes (copolymers of acrylic acid and *N*-vinylpyrrolidone) were synthesized by carbodiimide condensation following the modification procedures described early. Composition and structure of bioconjugates were characterized by HPLC (Shimadzu), NanoSPR-3, Zetasizer Nano ZS, Steady State Fluorescence Spectrometer QM-4 and Viscotek TDA 302 size exclusion chromatography. It was obtained that a single immunization of mice with PE-peptide conjugates without classical adjuvant increased the primary and secondary peptide-specific immune response to HBsAg. Moreover, these conjugates possess own selectivity for recognizing the antibody in blood sera of hepatitis virus injected people.

PP-744
Cooperative conjugation of peptide epitopes of VP1 protein of foot-and-mouth disease virus with anionic polyelectrolytes

B. Mansour, Z. Mustafaeva Akdeste and M. Mustafaev Akdeste
Bioengineering, Yildiz Technical University, Istanbul, Turkey.
E-mail: bmansur@yildiz.edu.tr

Recently, we report a novel approach to a totally synthetic vaccine which consists of a FMDV VP1 peptides, prepared by covalent conjugation of peptide biomolecules with membrane active carbochain Polelectrolytes (PEs). In the present study, peptide epitopes of VP1 protein both 135-161 (P1) amino acid residues (Ser-Lys-Tyr-Ser-Thr-Thr-Gly-Glu-Arg-Thr-Arg-Thr-Arg-Gly-Asp-Leu-Gly-Ala-Leu-Ala-Ala-Arg-Val-Ala-Thr-Gln-Leu-Pro-Ala) and triptophan (Trp) containing 135–161 amino acid residues(P2) were synthesized by using the solid-phase methods. The interaction of P1 and P2 peptides with copolimers of acrylic acid and *N*-vinylpyrolidone was investigated at different ratio of components (nPeptide/nPolymer, and pH value of solution by using different physicochemical analyses methods (HPLC; VÍSCOTEK-with Ultra-viole Visible, Refractive Index, Light Scattering, and Viscosity Quadruple Detector Systems; Fluorescence Spectroscopy. It was found that in general case the system PE-P1 (P2) is characterized by a bimodal distribution of polymer and peptide components of mixtures on chromatograms. Peptide oligomers bind to polymer macromolecules by cooperative mechanism: all added peptide molecules is strongly bound by the polyanione and the existence of the free polymer chains in the system under these conditions unambiguously indicates a non-random distribution of the peptide molecules between the anionic PEs. The dynamics of peptide-specific antibody formation induced by these conjugates was investigated.

Rational Drug Design

PP-745
Modulation of multidrug resistance in paclitaxel and vincristine resistant MCF-7 cell lines

M. Demirel Kars[1], Ö. Darcansoy İşeri[1], U. Gündüz[1], A. U. Ural[2] and **J. Molnár**[3]
[1]*Department of Biological Sciences, Middle East Technical University, Ankara, Turkey,* [2]*Department of Hematology, School of Medicine, Gülhane Military Academy, Ankara, Turkey,* [3]*Department of Medical Microbiology and Immunobiology, Faculty of Medicine, University of Szeged, Szeged, Hungary.*
E-mail: meltemd@metu.edu.tr

Resistance developed to broad spectrum of chemotherapeutic agents during cancer chemotherapy is named as multiple drug resistance (MDR).MDR phenotype acquired by drug responsive patients remains a major hindrance to efficient chemotherapy. P-gp over expression is one form of MDR phenotype. Decrease in cellular caspase-3 activity, increase in bcl-2 expression level and generation of mutations in genes encoding drug target proteins are some other known mechanisms of drug resistance. Aim of this study is, investigation of the molecular mechanisms of acquired drug resistance and reversal of MDR in MCF-7 mammary carcinoma cell lines. Sensitive parental MCF-7 cells were subjected to anticancer agents paclitaxel and vincristine by stepwise increasing drug concentrations and resistant sublines were developed. The results demonstrate that the resistant sublines are P-gp and MRP1 positive and that paclitaxel and vincristine resistant MCF-7 cell lines acquired an other form of drug resistance phenotype, which is decrease in caspase-3 activity. The extent of resistance was also determined by MTT cytotoxicity tests.IC50 values of paclitaxel and vincristine for MCF-7 cell lines were determined. Finally the drug resistance that was due to the expression of P-gp in MCF-7 sublines was modulated by various compounds such as phenothiazines, cinnamylidenes and silicon compounds. Determination of effective MDR modulators will lead to development of new strategies to overcome drug resistance.

PP-746
In vitro opioid properties of Tyr-Pro related peptides-from Tyr-Pro to endomorphins

E. Kató[1], M. Al-Khrasani[1], E. Szemenyei[2], G. Tóth[2] and A. Z. Rónai[1]
[1]*Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Semmelweis University, Budapest, Hungary,* [2]*Isotope Laboratory, Institute of Biochemistry, HAS Biological Research Center, Szeged, Hungary. E-mail: kato@pharma.sote.hu*

The history of natural opioid peptides bearing the N-terminal Tyr-Pro motif culminated in the discovery of endomorphins

(Tyr-Pro-Trp-Phe-NH2, EMo-1 and Tyr-Pro-Phe-Phe-NH2, EMo-2) in 1997. Our study was aimed at characterizing the opioid properties of (a) Tyr-Pro-related di- tri- and tetrapeptides and of (b) synthetic endomorphin analogs in isolated organs. Tyr-Pro-OH (10^{-3} M), Tyr-Pro-NH2, Tyr-Pro-diketopiperazine and the post-proline cleaving dipeptidyl-aminopeptidase EC 3.4.14.5 inhibitor Ile-Pro-Ile (10^{-4} M each) were devoid of opioid activity in the field-stimulated mouse vas deferens bioassay. Weak opioid agonism (30% maximal inhibition, IC30 = 290 μM) appeared first in Tyr-Pro-Phe-OH. There was moderate agonism in Tyr-Pro-Phe-NH2 (IC50 = 20.2 μM) and the casomorphin-related morphiceptin (Tyr-Pro-Phe-Pro-NH2, IC50 = 1.45 μM). Tyr-Pro-Phe-Phe-OH had modest agonism (IC50 = 5.8 μM) whereas EMo-1 and -2 displayed agonism in the low nanomolar range (IC50 = 23.2 and 17.2 nM, respectively). C-terminal amides were preferential agonists at the mu-opioid receptor type whereas carboxylic derivatives were mixed delta/mu agonists. Of the synthetic analogs, 2'6'-dimethyltyrosine (Dmt) 1-endomorphins were prominently potent agonists, endomorphins with C-terminal alcoholic (-ol) function were strong agonists. EMo-1, its –ol derivative, Dmt-EMo-1 and EMo-2-ol were partial agonists, morphiceptin and EMo-2 were closer to full agonism.

PP-747
Analgesic effect of Tyr-Pro-related peptides with or without EC 3.4.14.5 inhibitor

M. Al-Khrasani[1], F. Erdo[2], J. I. Székely[3], J. Timár[1], Zs. Gyarmati[1], E. Szemenyei[4], G. Tóth[4], E. Kató[1] and A. Z. Rónai[1]

[1]*Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Semmelweis University, Budapest, Hungary,* [2]*Department of Pharmacology, IVAX Drug Research Institute Ltd, Budapest, Hungary,* [3]*Institute for Drug Research, Budapest, Hungary,* [4]*Institute of Biochemistry, HAS Biological Research Center, Szeged, Hungary. E-mail: Alkhrasani@freemail.hu*

Tyr-Pro-OH, Tyr-Pro-Phe-NH2, morphiceptin (Tyr-Pro-Phe-Pro-NH2), endomorphin-1 (EMo-1, Tyr-Pro-Trp-Phe-NH2) and EMo-2 (Tyr-Pro-Phe-Phe-NH2) and the post-proline cleaving dipeptidyl-aminopeptidase EC 3.4.14.5 inhibitor Ile-Pro-Ile (IPI) caused a naloxone-reversible (i.e. opioid receptor-mediated) prolongation of tail-flick latency in rats, when injected intracerebroventricularly. The analgesic ED50 values were 5.14 nmol/rat for morphiceptin, 7.90 nmol/rat for endomorphin-1 and 295 nmol/rat for IPI; the ED50 of morphine, given by the same route, is 3.14 nmol/rat. 50% analgesia could not be attained by Tyr-Pro-OH and Tyr-Pro-Phe-NH2 at 100–1,000 nmol/rat and by EMo-2 at 30 nmol/rat. Since Tyr-Pro-OH and IPI are completely devoid of opioid agonist ('morphine-like') activity *in vitro*, Tyr-Pro-Phe-NH2 and morphiceptin are rather weak agonists (micromolar range) whereas EMo-1 and -2 are strong, nearly equipotent agonists *in vitro* (low nanomolar range, for details see poster by Kató et al.), it can be concluded that (a) Tyr-Pro-OH and IPI are likely to act indirectly *in vivo*. The former as a possible biosynthetic precursor to endomorphins (see Rónai et al., oral presentation) the latter by inhibiting the biodegradation of a Tyr-Pro-related analgesic brain peptide, and (b) the supraspinal analgesic actions of the two endomorphins and of morphiceptin are entirely different. Surprisingly, IPI neither potentiated nor prolonged the analgesic effect of EMo-1 or Tyr-Pro-Phe-NH2.

PP-748
Sensitivity of influenza virus RNA to artificial ribonucleases: cleavage within bulge-loops

I. Kunetsova, M. Zenkova and V. Vlassov

Institute of Chemical Biology and Fundamental Medicine, Lavrentev av. 8, Novosibirsk, 630090 Russia. E-mail: irakuzn@yahoo.com

We systematically investigated sensitivity of RNA bulge-loops of different size and sequence to cleavage by imidazole buffer, short cationic peptides mimicking RNAse A active centre, hydrophobic compounds containing 1, 4-diazabicyclo[2.2.2]octain bearing lipophilic substituents and RNase A. The bulges were created in 96-nt fragment of influenza virus M2 RNA (M2-96 RNA) by hybridization with specially designed deoxyribooligonucleotides. Phosphodiester bonds within artificial bulges of different size except for the one-member bulges were readily cleaved by all RNA-cleaving agents mentioned above. We have found that maximal sensitivity to cleavage is observed within 4 and 7-member bulge-loops. Imidazole buffer, short cationic peptides and RNase A cleaved phosphodiester bonds within bulges with efficiency similar to that of the phosphodiester bonds within the rest of M2-96 RNA. In the case of 4-diazabicyclo[2.2.2]octain containing compounds 75% of the cleavage products resulted from the attack of phosphodiester bonds within 7-member bulge. Thus an enhanced RNA cleavage at the selected bulge-loop by a synthetic ribonuclease not covalently bound to the bulge-forming oligonucleotide was achieved.

Acknowledgment: This work was supported by RAS program 'Molecular and Cellular Biology', 'Gene-targeted biologically active compounds as antiviral and anticancer drags', RFBR (02-04-4866).

PP-749
Immunological analysis of active site loop of lactate dehydrogenase from two *Plasmodium* species

V. Celik, D. Sadak and D. Turgut-Balik

Department of Biology, University of Firat Elazig, Turkey. E-mail: venharcelik@gmail.com

Lactate dehydrogenase from human malaria parasites has been targeted for a new and effective antimalarial drug design studies. Active site loop of this enzyme has been determined by crystal structure studies from both *Plasmodium falciparum* and *Plasmodium vivax* and shown to be an ideal site for the drug design studies. Five amino acids were removed from this site from both *Plasmodium vivax* and *Plasmodium falciparum* LDH's to determine the stability of the protein in the lack of these essential residues which are not present in their equivalent in human. Only one amino acid was removed at each step. Expression of the mutant proteins was poor. Therefore overproduced mutant proteins were successfully detected by Western blotting using an antibody raised against *P. falciparum* LDH.

PP-750
A facile and effective synthesis of dinucleotide 5'-triphosphates

T. Abramova, S. Vasilyeva, I. Serpokrylova and V. Silnikov

Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia. E-mail: abramova@niboch.nsc.ru

Nucleoside 5'-triphosphates have important therapeutic and diagnostic applications. Wide varieties of them are commercially available. On the contrary, 5'-triphosphates of di- and oligonucle-

otides have had limited usage as scientific tools so far. Developed in early 90's, SELEX (Selective Evolution of Ligands by Exponential enrichment) technique and aptamers are now powerful methods which need as much functional diversity of nucleoside triphosphates as possible. Using of 5'-triphosphate dinucleotides as substrates in template dependent enzymatic DNA synthesis may provide additional possibilities for functional diversification of aptamers [1]. We report a successful synthetic procedure for conversion of 5'-monophosphorylated 2'-deoxydinucleotides to their 5'-triphosphate derivatives with excellent yield. Activation of terminal phosphate group was achieved under the Mukayama conditions in the presence of nucleophilic catalyst. The reaction conditions (solvent, counter ions, activation time, and reagent excess) were optimized for all 16 dinucleotides. The purification of the 5'-triphosphate oligonucleotide derivatives was performed by two sequential anion exchange chromatographies, 10–40 mg of each triphosphate was obtained. The structure of the products was confirmed by ^{1}H and ^{31}P NMR and mass spectroscopy.

Acknowledgment: This work was supported by Russian Fund for Fundamental Research, Grant No 04-03-32490.

Reference:

1. Kless H. WO 01/16366, 2001; (CA 134, 217979).

PP-751
Genetic determinants of sensitivity to statin-induced apoptosis in multiple myeloma cells

W. W. Wong[1], **J. W. Clendening**[1], A. Martirosyan[2], P. C. Boutros[1], C. Bros[2], F. Khosravi[2], A. K. Stewart[3], I. Jurisica[1], P. L. Bergsagel[4] and L. Z. Penn[1]
[1]Medical Biophysics, University of Toronto, Toronto, Canada, [2]Cellular and Molecular Biology, Ontario Cancer Institute, Toronto, Canada, [3]McLaughlin Centre for Molecular Medicine, University of Toronto, Toronto, Canada, [4]Medicine and Pathology, Cornell University, NY, USA.
E-mail: jclenden@uhnres.utoronto.ca

Statins are commonly used to control hypercholesterolemia by inhibiting 3-hydroxymethylglutaryl coenzyme A reductase (HMGCR), the rate-limiting enzyme of the mevalonate pathway. A subset of tumour types has been shown to undergo apoptosis upon statin treatment. While multiple myeloma (MM) tumours are included in this group, the molecular characteristics conferring sensitivity are unknown. To gain insight into these molecular characteristics, we screened a large panel of MM cell lines with known genetic abnormalities. Approximately 50% of the cell lines were sensitive to statin-induced apoptosis while the remaining were resistant despite statin uptake. Moreover, sensitivity to statin-induced apoptosis in MM cells associated with translocation of 4; 14 which harbors fibroblast growth factor receptor 3/MMSET. Those cell lines sensitive to statin-induced apoptosis could be rescued by the addition of mevalonate, geranylgeranyl pyrophosphate, and partially by farnesyl pyrophosphate. Surprisingly, ectopic expression of prenylated GTPases of the Ras and Rho family did not affect sensitivity to statin-induced apoptosis. Expression profiling comparing resistant and sensitive MM cells upon statin treatment indicated resistant cells do not transcriptionally respond to lovastatin. The role this feedback response may hold in regulating statin-induced apoptosis will be explored. Taken together, our studies suggest a potential therapeutic agent for MM patients harboring 4; 14 translocations.

PP-752
Purification and analysis of lactate dehydrogenase from *Plasmodium vivax*

A. Aslan, V. Celik and D. Turgut-Balik
Department of Biology, University of Firat Faculty of Arts and Sciences, 23169, Elazig, Turkey. E-mail: dilekbalik@gmail.com

Drug resistance of *Plasmodium* to antimalarials is increasing throughout the world. This emphasises a need to develop novel, alternative drugs. In this study; gene encoding lactate dehydrogenase was amplified from *Plasmodium vivax* by adding six histidines to the C-terminal of the enzyme and the protein was expressed in *E. coli*. The overproduced protein was then purified by using Ni-NTA Spin kit and Ni-NTA agarose and analysed. An antibody produced against PfLDH was tested against pure PvLDH protein. It was shown that two proteins were similar enough that PvLDH reacted with PfLDH antibody. It is expected that the drug design studies applied on PfLDH could also be applicable on PvLDH.

PP-753
Insertion of five amino acids in the active site loop is essential for the stability of PvLDH protein

D. Sadak, V. Celik and D. Turgut-Balik
Department of Biology, University of Firat Faculty of Arts and Sciences, 23169, Elazig, Turkey. E-mail: dilekbalik@gmail.com

Despite more than a century of efforts to eradicate or control malaria, it remains one of the most important diseases in the developing world. It kills over a million each year, and some 3.2 billion people living in 107 countries or territories are at risk. Malaria is running out of control in part because many malarial parasites are now resistant to currently available antimalarial drugs. This necessitates development of new antimalarials that have different mode of action. Lactate dehydrogenase was targetted from Plasmodium vivax in this study. Active site loop of the enzyme has been determined by crystal structure studies and shown to be an ideal site for the drug design studies. Some protein engineering studies were carried out to understand the role of active site loop, extended by five amino acids, on the stability of the PvLDH protein. It was shown that the protein tolerates shortening the loop by two residues, but it becomes inactive when more than two amino acids were removed. Deletion of the five extra residues abolishes enzymatic activity because of rest of the active site loop being disrupted. All these results indicates that appropriate length of this loop must be required for catalysis.

PP-754
Structural and pharmacological studies on alpha-2 adrenoceptors

H. Xhaard and M. S. Johnson
Structural Bioinformatics Laboratory, Biochemistry and Pharmacy, Åbo, Finland. E-mail: hxhaard@abo.fi

Alpha-2 adrenoceptors (alpha-2 ARs) belong to the rhodopsin-like superfamily of G-protein coupled receptors (GPCRs) characterized by seven transmembrane helices. Human have three alpha-2 ARs, named subtypes A, B and C. Through their interactions with naturally occurring ligands, adrenaline and noradrenaline, alpha-2 ARs mediate a variety of physiological effects like anaesthesia, vasodilatation, vasoconstriction, bradycardia and mood effects. Thus, they are key targets for pharmaceutical development. We used structure modeling and ligand docking to derive three-dimensional models of ARs in complex with a set known drugs, agonists and antagonists. Maps predicting loca-

tions favorable for interactions were computed using a novel method and used as mean to validate the complexes. Model structures were also assessed in the light of wet-lab experiments conducted in a collaborating experimental group, at the department of Pharmacology and Clinical Pharmacology, Turku, Finland, and of data already available in the literature. Comparison of the model structures in the light of pharmacological data, e.g. ligand binding constants, provide insight into the binding specificity of drugs for the different alpha-2 AR subtypes.

References:

Xhaard, Rantanen et al. J Med Chem 2006 (in press).
Xhaard, Nyrönen et al. J Struct Biol 2005; 150: 126–143.
Ruuskanen, Xhaard et al. Mol Biol Evol 2004; 21: 14–28.
Ruuskanen, Laurila, Xhaard et al. Brit. J Pharm. 2005, 144: 165–177.

PP-755
Derivatives of RGD and KGD analogues with resveratrol. Synthesis and biological evaluation

C. Koutsas[1], P. E. Makris[2] and M. Liakopoulou-Kyriakides[1]
[1]Department of Chemical Engineering, [2]Department of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece. E-mail: markyr@eng.auth.gr

Activation of platelets by an agonist conformationally activates glycoprotein IIb/IIIa (GPIIb/IIIa), the platelet receptor for fibrinogen. The minimum sequence on fibrinogen recognized by GPIIb/IIIa is ArgGlyAsp (RGD) and it has been observed that specificity of the ligant for the GPIIb/IIIa is improved by conversion of the RGD to a KGD sequence. Furthermore, 3, 4?, 5-trihydroxy-trans-stilbene (resveratrol), a phytoalexin found in grapes, inhibits platelet aggregation. In a previous study we attempted to conjugate a RGD derivative [Ac-Arg(Pbf)GlyAsp(tBu)-OH] with resveratrol. Continuing our study we investigated the conjugation of more RGD and KGD derivatives with resveratrol in order to determine whether more potent inhibitors of platelet aggregation are obtained. The peptides were synthesized by solid phase technique, using the 2-chlorotrityl-chloride resin as a stationary phase, by the method of carbodiimides. Coupling reactions took place in solution through the protected forms of the peptides [Fmoc-Arg(Pbf)GlyAsp(tBu)-OH, Ac-Arg(Pbf)GlyAsp(tBu)-OH and Fmoc-Lys(Boc)GlyAsp(tBu)-OH] using DCC (*N*, *N*-dicyclohexylcarbodiimide) or DCC/DMAP (4-Dimethylaminopyridine) as coupling reagents. The products were purified by reversed phase HPLC and identified by MS. The stability of the compounds was investigated against deprotection conditions and their antiplatelet activity was examined *in vitro*.

Acknowledgements: HERACLETUS program financed from EPEAEK and Ministry of Education.

PP-756
The preparation of orthogonally protected basic amino acids for the semisynthesis of human insulin analogues

J. Jezek[1], **T. Barth**[2], H. Tallima[3] and J. Barthova[4]
[1]Department of Biol.Chem., Institute of Organic Chemistry and Biochemistry, Prague 6, Czech Republic, [2]Department of Biol. Active Peptides, Czech Society for Biochemistry and Mol. Biology, Prague 6, Czech Republic, [3]Department of Zoology, Cairo University, Cairo, Egypt, [4]Department of Biochemistry, Charles University, Prague 2, Czech Republic. E-mail: barthtom@yahoo.com

Already 25 years ago, we described the use of phenylacetyl group (Phac) for the protection of epsilon amino group of L-lysine in vasopressin analogue. Phac group on L-lysine in the position 8 of the peptide chain was successfully eliminated by the enzyme penicillinamidohydrolase. The enzymic reaction led to the hormonally active deamino[8-L-lysine] vasopressin. Since then hundreds of papers revealed the feasibility of this approach. The release of Phac group runs under mild conditions. We present the synthesis of orthogonally protected L-lysine and L-ornithine. The Cu^{2+} complexes of lysine or ornithine were prepared and isolated. The complexes were treated with phenylacetyl chloride and the product was filtered off and washed with cold water. Phac derivatives of lysine or ornithine were treated with di-tert-butyl dicarbonate under alkaline pH.Products were precipitated by acification and extracted with EtOAc. Fmoc derivatives of Phac-lysine and Phac-ornithine were obtained by treatment with Fmoc-ONSu. The reaction mixture was acidified and products isolated by extraction with EtOAc. The purity of products BocLys(Phac)OH, FmocLys(Phac)OH, BocOrn(Phac)OH and FmocOrrn(Phac)OH was checked by TLC, HPLC and MS. The modified amino acids were used for the construction of peptide chains (analogues of B-chain of human insulin).The final products were deblocked by penicillinamidohydrolase treatment.

Acknowledgment: Supported by funds of Academy of Sciences of the Czech Republic (Project IBS 4055303).

PP-757
An improved technique for preparation of doxorubicin loaded PLGA microspheres for drug release studies

M. Demirel Kars, Ö. Darcansoy İşeri, B. Küçük, P. Şen and **U. Gündüz**
Department of Biological Sciences, Middle East Technical University, Ankara, Turkey. E-mail: meltemd@metu.edu.tr

Controlled release of anticancer drugs is one of the fast developing chemotherapy strategies. Microspheres made of poly(lactic-co-glycolic acid) (PLGA) are biocompatible and biodegradable, rendering them a promising tool in the context of controlled drug delivery. Doxorubicin is a topoisomerase inhibitor and it is widely used in treatment of several cancers. Doxorubicin loaded PLGA microspheres are promising delivery systems, which let doxorubicin to stay in the blood much longer and to be slowly released at the site of target tumor tissue. In this study, sterile doxorubicin loaded PLGA microspheres were prepared by an improved experimental approach without the requirement of additional post sterilization techniques, their cytotoxicity degrees and drug release profiles were determined. Doxorubicin loaded microspheres were prepared using the oil-in-water double emulsion technique under aseptic conditions. Cytotoxicity of the microspheres was tested on MCF-7 breast carcinoma cell line by XTT assay. Drug release profile from drug-loaded microspheres was determined by dialysis and spectrophotometric measurements. Release profile appeared to consist of two components with an initial rapid release followed by a slower exponential stage. Doxorubicin encapsulation efficiency reached more than 80% efficiency.

PP-758
Structure based design of glutamate racemase inhibitors from computational and experimental chemical library screening

M. E. Johnson, H. Yu, M. May, S. Mehboob, D. C. Mulhearn, A. D. Mesecar and B. D. Santarsiero
Center for Pharmaceutical Biotechnology, University of Illinois at Chicago, Chicago, IL, USA. E-mail: mjohnson@uic.edu

With the accelerating incidence of bacterial resistance to all of the major classes of antibiotics currently in clinical use and the

potential of selecting antibiotic-resistant bacterial pathogens, there is increasing need for new antibiotic therapies that target new mechanisms for inhibiting bacterial growth. For *B. antracis*, there are two isoforms of the glutamate racemase enzyme that converts L-Glu to D-Glu for subsequent incorporation into the cell wall and spore capsule. We have recently solved the crystal structure of the RacE2 isozyme that appears essential for *B. anthracis* in complex with D-Glu. We have used computational approaches to screen several chemical library databases against the RacE2 structure, and will describe the comparative results of the computational screening against experimental screening of an approximately 60 000 compound in-house chemical library. We find an experimental hit rate of less than 1%, with several compounds acting as non-specific inhibitors. Parallel structure-based design has focused on strategies for targeting the catalytic thiols. Mass spectrometry results suggest an intriguing selectivity mechanism based on adjacent Cys/Tyr residue pairs.

Acknowledgment: Supported in part by NIH grant U19 AI56575.

PP-759
Computational optimization of anti-cd40l antibody 5c8

A. Lugovskoy[1], K. Simon[1], M. Jarpe[1], A. Sjodin-Boriack[1], Y. Li[1], J. Ferrant-Orgettas[1], A. Saeed-Kothe[1], J. Eldredge[1], G. Farrington[1], F. Taylor[1] and H. van Vlijmen[2]
[1]Research, Biogen Idec Inc., Cambridge, MA, USA [2]Research, Tibotec, Mechelen, Belgium.
E-mail: alexey.lugovskoy@biogenidec.com

Antibodies are key components of the adaptive immune system and are well-established protein therapeutic agents. Typically high-affinity antibodies are obtained by immunization of rodent species or by screening of libraries. Here we report the successful application of structure-based computational protein design to optimization of anti-CD40L antibody 5c8. By rationally making five point mutations in a Fab fragment of an antibody we improved its *in vitro* affinity by over 25 fold. The derived higher affinity monovalent Fab mutant was equipotent to a full-length bivalent antibody in blocking CD40L signaling in cells. The enhanced affinity mutants were structurally characterized by X-ray crystallography, and the predicted basis for the improved affinity was confirmed. We further used computational structure-based modeling techniques to generate antibody variants with altered affinity towards FcgammaR and FcRn receptors. Our results suggest the emerging utility of computational methods for rational engineering of antibodies.

PP-760
Synthesis of a wide variety modified 5′-triphosphates of dinucleotides

S. Vasileva, V. Silnikov and T. Abramova
Institute of Chemical Biology and Fundamental Medicine, Lavrentev av. 8, Novosibirsk, 630090 Russia.
E-mail: Vasilieva_2001@mail.ru

The technique, which was cointed SELEX (Systematic Evolution of Ligands by Exponential Enrichment), and aptamers, require a plenty functional nucleoside triphosphates. Despite of the immense potential use of simple aptamers, there is a clear internet drawback in the design due to relatively poor functional space, and limited vocabulary of only four building blocks. The use of various 5'-triphosphate dinucleotides allows to expand opportunities of these methods. Our aim was the efficient preparation of the dinucleotide 5'-triphosphates carrying various amino acid residues joined to heterocyclic bases. For this purpose appropriately protected dinucleotides, bearing aminocontaining linkers in different positions of heterocyclic bases were synthesized. Triphosphates were prepared by modified Ludwig's method [1, 2] with high yields. A number of compounds mimicking different amino acids (Trp, Tyr, Glu, Cys, Lys, Val, Arg, Met, Tre, Gln, Phe) were attached to amino linker of triphosphates. The purification of products was performed by HPLC-reverse phase method. The structure of the products was confirmed by ^{1}H and ^{31}P NMR and mass spectroscopy.

Acknowledgment: This work was supported by Russian Fund for Fundamental Research, Grant N 04-03-32490.

References:

1. Ludwig J. Acta Biochim Biophys Acad Sci Hung 1981; 16: 131.
2. Abramova TV, Bakharev PA, Vasilyeva SV, Silnikov VN. Tetrahedron Lett 2004; 45: 4361–4364.

PP-761
Toxins and aquaporins

K. Jeyaseelan, A. Armugam, D. C. I. Koh and S. C. Chai
Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, 8 Medical Drive, Singapore 117597. E-mail: bchjeya@nus.edu.sg

Animal venoms contain mixtures of various polypeptides. Some of which have been shown to be useful in the development of drugs such as anti-coagulant drugs, painkillers, anti-hypertensive and anti-tumour drugs and even drugs for cosmetic surgery. Recently we have been exploring the possibility of finding modulators of aquaporins among scorpion and snake venoms using microarray and real-time PCR. Interestingly, snake venoms have been found to contain both inhibitors as well as activators to water channels AQP1, 2, 4, 5, 8 and 9. Scorpion venom however, showed the presence of inhibitors to aquaporins 1, 4 and 9 as well as to a potassium channel, Kir4.1. These findings may be useful in developing peptides that regulate movement of water in biological systems especially in diseases where water homeostasis is impaired.

PP-762
In vitro DNA binding mode analysis of some benzimidazole and thiazolidindion retinoids

Y. Musdal and A. K. Piskin
Biochemistry Department, Faculty of Medicine, Hacettepe University, Ankara, Turkey. E-mail: ymusdal@hacettepe.edu.tr

A great number of antitumoral and antimicrobial drugs exert their effects via DNA binding. Efficacy of numerous retinoids as potential cancer drugs is extensively analysed. Binding of seven retinoids containing benzimidazole or thiazolidindion rings to DNA is analysed. Genomic and plasmid DNAs are incubated with retinoids and analysed by agarose electrophoresis and PAGE. Nucleotides consisting of AT or GC and double strands consisting of AT octamers are used in the detection of sequence selectivity. DNA binding of EtBr, DAPI and ATRA is analysed and compared to those of retinoids. It is determined that a decrease in the density or disappearance of bands indicates binding. In the analysis of sequence specificity 200 ng nucleotides run in 20% PAGE was able to demonstrate gel retardation due to DNA binding. It is concluded that DNA binding mode of benzimidazole retinoids resembles to that of DAPI and may involve attachment to the grooves, whereas thiazolidindion retinoids bind DNA more strongly in a similar manner with EtBr and ATRA, either by intercalation or DNA breakage. No sequence specificity for any of the compounds was detected. These findings may suggest that these retinoids may be considered as potential antitumor or antimicrobial drugs.

Drug Targeting

PP-763
Preparation and characterization of biodegradable microparticles for anticancer – drug release

G. Eyövge[1], U. Gündüz[1], G. Gündüz[2] and **E. Güç**[1]
[1]*Biology Department, Middle East Technical University, Ankara, Turkey,* [2]*Chemical Engineering Department, Middle East Technical University, Ankara, Turkey. E-mail: ufukg@metu.edu*

In recent years several type of polymeric materials are used for controlled release of anticancer drugs which provide improvement in treatment efficiency, reduction in systemic toxicity, and prevention of drug resistance. Biodegradable polymers are especially preferred. Poly (lactide), poly (glycolide), and copolymers are commonly used. In this study biodegradable copolymer of poly (D, L-lactide-co-glycolide) microspheres are prepared and characterized for controlled release of 5- fluorouracil, methotrexate, and tamoxifen which are commonly used in cancer chemotherapy. Two different techniques are used to prepare empty and drug loaded microparticles and, morphology, size, drug content and drug release rates are examined.

PP-764
Cyclodextrins in drug delivery: ATP synthesis inhibition by alpha- and beta-CD but not gamma-CD

M. Fragata[1] and **S. Dudekula**[2]
[1]*Université du Québec à Trois-Rivières, Département de chimie-biologie, Trois-Rivières, Québec, G9A 5H7, Canada,* [2]*Center for Cellular and Molecular Biology, Habsiguda, Hyderabad, Andhrapradesh, 500007 India.*
E-mail: fragata@uqtr.ca

The cyclodextrins (CD) are a class of cyclic oligosaccharides containing 6 to 8 D-glucose residues linked by alpha-1, 4 glycosidic bonds. These substances have the ability of binding to lipids, proteins, DNA or RNA by encapsulating chemical groups of small size. Because of these properties the CDs were widely used in the food and pharmaceutical industries, and applied often as carriers in drug delivery [see recent review in AAPS PharmSciTech 2005; 6 (2), 43] to enhance, for example, drug absorption in the gastrointestinal mucosa (CRC Crit. Rev. Ther. Drug Carrier Syst. 1987, 3, 1). In the present work, we investigate the effect of alpha-, beta- and gamma-CDs on the ATP synthesis in isolated thylakoid membranes using the luciferin-luciferase luminescence assay. We showed that alpha- and beta-CDs, but not gamma-CD, inhibit the ATP synthesis in the thylakoid membrane. This effect is observed at concentrations as low as 4 mM (alpha-CD) and 2 mM (beta-CD). Contrarily, similar gamma-CD concentrations stimulate the ATP synthesis. First, we conclude that alpha- and beta-CDs, but not gamma-CD, uncouple the electron transport and the ATP synthesis in the thylakoid membrane. Secondly, if this effect is also observed in animal mitochondrial membranes, we suggest that gamma-CD should be used in drug delivery instead of alpha- and beta-CDs on account of possible harmful physiological effects. This latter question is under study.

Acknowledgment: This work is supported by the NSERC Canada.

PP-765
Xray structure of Cisplatin-protein interactions: selective platination of His 19 in superoxide dismutase

A. Casini, L. Messori, V. Calderone and S. Mangani
Department of Chemistry, University of Florence, Italy.
E-mail: angela.casini@unifi.it

Cisplatin is a leading anticancer drug. [1] The fact that DNA represents the probable target for this metallodrug has prompted a huge number of studies on cisplatin-DNA interactions [2]; in contrast, the interactions of anticancer platinum drugs with proteins have received, until now, far less attention. Thus, it is of great interest, in our opinion, to determine the nature of the platinum-containing molecular fragments that are bound to proteins, their exact location, their strength of binding, the reversibility of the interactions. While a few solution studies on the interactions of cisplatin and analogues with selected proteins have appeared through the years, we noticed a substantial lack of crystallographic information for protein adducts with platinum drugs. These arguments led us to perform new crystallographic investigations of cisplatin derivatives of proteins in order to give a detailed structural description of the nature of platinum-protein interactions. Here we present the Xray diffraction results for the adduct of the bovine erythrocyte copper, zinc superoxide dismutase with cisplatin [3].

1. Lippert B. Cisplatin: chemistry and biochemistry of a leading anticancer drug, Wiley VCH 1999.
2. (a).Brabec V. Prog Nucleic Acid Res Mol Biol 2002; 71: 1–68; (b) Reedijk J. PNAS 2003; 100 (7): 3611–3616.
3. Calderone et al. Angewandte Chemie Int Ed (in press).

PP-766
Amitriptyline, amodiaquine and metoprine interactions with rat kidney histamine *N*-methyltransferase

S. Rajtar and T. Irman-Florjanc
Institute of Pharmacology and Experimental Toxicology, Faculty of Medicine, Ljubljana, Slovenia.
E-mail: simona.rajtar@mf.uni-lj.si

Histamine *N*-methyltransferase (HNMT), a histamine degrading enzyme, is found in high concentrations in mammal kidney, liver, colon and central nervous system. Previously we have shown that amitriptyline (AMI), tricyclic antidepressant, in concentrations 1–100 μM, increases rat kidney HNMT activity up to 67%. The aim of this study is to investigate the AMI binding site on rat HNMT, which could be responsible for the increase of enzymatic activity. We used two HNMT inhibitors, metoprine (MET) and amodiaquine (AMO) with known binding sites and examined whether AMI competes with them for the same sites. We did *in vitro* experiments on rat kidney homogenates. Samples were first incubated with saline (control) or 1, 30, 50, 100 μM AMI, then with 10, 100 nM, 10 and 30 μM MET or AMO. The enzymatic activity of HNMT was determined by radiometric assay. HNMT activity was compared with HNMT activity measured without any active substance added. Incubations of rat kidney homogenates with AMI followed by AMO (100 nM, 10 and 30 μM) resulted in inhibition of HNMT activity. When incubation was performed with AMI and MET (10 or 100 nM), HNMT activity was increased up to 74%. However, at higher MET concentrations the increase in HNMT activity appeared at lesser

extent, indicating the competition for the same binding site. The results suggest that AMI might have different binding site on rat kidney HNMT from AMO, while AMI and MET might compete for the same binding site on HNMT.

PP-767
Blockade of neurokinin 1 receptor attenuates CC and CXC chemokine production in acute pancreatitis

J. Sun and M. Bhatia
Department of Pharmacology, National University of Singapore, Singapore. E-mail: phcsj@nus.edu.sg

The neuropeptide substance P (SP) and its receptor neurokinin 1 receptor (NK-1R) play a key role in the pathogenesis of acute pancreatitis (AP) and associated lung injury. The present study investigated the involvement of CC and CXC chemokines in SP/NK-1R-related pathogenesis of this condition. In a mouse model of caerulein-induced acute pancreatitis, a selective NK-1R antagonist CP-96 345 was employed to block the interaction of SP and NK-1R. The temporal and dose-related effects of caerulein hyperstimulation and CP-96 345 treatment on various CC and CXC chemokine expression were examined. The results showed that MCP-1, MIP-1alpha and MIP-2 were early mediators upregulated in both the pancreas and lungs after AP induction, whereas RANTES was a later mediator found induced only in the pancreas. Treatment of CP-96 345 significantly suppressed caerulein-induced increase in chemokine mRNA and protein expression. Additionally, immunohistochemical staining revealed that in the pancreas chemokines were localized to pancreatic acinar cells and the infiltrating leukocytes, while in the lungs they were expressed by alveolar macrophages, epithelial and endothelial cells. We therefore identified chemokines as important mediators in SP/NK-1R-related pathway in the pathogenesis of AP. SP, probably acting via NK-1R on the chemokine-producing cells in the pancreas and lungs, stimulates the production of chemokines that aggravate local pancreatic damage and its systemic sequelae.

PP-768
The role of hydrogen sulfide in cecal ligation and puncture induced sepsis in the mouse

H. L. Zhang, L. Zhi, P. K. Moore and **M. Bhatia**
Department of Pharmacology, National University of Singapore, Singapore. E-mail: g0403081@nus.edu.sg

Endogenous hydrogen sulfide (H_2S) is naturally synthesized by various mammalian cell types in a reaction catalyzed by cystathionine-γ-lyase (CSE) and/or cystathionine-β-synthase (CBS). Lately, several studies have suggested that H_2S functions as a vasodilator and neurotransmitter. However, so far little is known about its role in systemic inflammation. The study was aimed to investigate the role of endogenous H_2S in cecal ligation and puncture (CLP) induced sepsis. Swiss mice ($n = 12$ in each group) were subjected to CLP and treated with either saline (i.p.), DL-propargylglycine (PAG, 50 mg/kg i.p., CSE inhibitor) or NaHS (10 mg/kg, i.p., H2S donor). CLP induced sepsis significantly increased both plasma H_2S concentration and liver H_2S synthesis as compared with sham operated animals. Induction of sepsis resulted in a significant up-regulation of CSE mRNA in liver. In contrast, prophylactic and therapeutic administration of PAG significantly reduced the level of chemokines and cytokines in lung, liver and plasma as well as myeloperoxidase activity in lung and liver. Lung permeability, plasma aminotransferase activity and mortality after induction of sepsis were decreased due to PAG treatment. Injection of NaHS, significantly aggravated sepsis associated systemic inflammation. Therefore, the effect of inhibition of H_2S formation and administration of NaHS suggests that H_2S plays a pro-inflammatory role in regulating the severity of sepsis and associated organ injury.

PP-769
Activation and desensitization of leptin receptors on Caco2 cells: consequences for PepT-1

P. Hindlet[1], M. Buyse[1], A. Bado[2] and R. Farinotti[1]
[1]Clinical Pharmacy Department, Faculty of Pharmacy, Paris-11 University, Chatenay-Malabryt, France, [2]Inserm U410, Faculty of Medecine Xavier-Bichat, Paris-7 University, Paris, France. E-mail: patrickhindlet@yahoo.fr

Leptin is involved in the central regulation of food intake. Recently we showed *in vivo* that leptin is also implicated in nutrient absorption via PepT-1, the oligopeptides transporter. In this study, we analysed the regulation of PepT-1 by leptin *in vitro* on Caco2 human cell model. Cells were grown on Transwell$\reg$ plates and treated for 7 days with 0.2 nM (mimicking normoleptinemia) or 1 nM (mimicking hyperleptinemia) on apical or basolateral or apical + basolateral sides. PepT-1 activity was then measured by monitoring cephalexin transport, a typical substrate for PepT-1. PepT-1 expression was studied by western-blot analysis. Treatment with leptin (0.2 nM) on apical, basolatéral and apical + basolateral sides upregulated PepT-1 activity (x 2.5, 2 and 2 respectively) and protein expression (x 2, 2.5, 3 respectively) compared to control non-treated cells ($P < 0.05$). Interestingly, treatment with higher concentration of leptin (1 nM) failed to increase PepT-1 activity or protein expression ($P > 0.05$ versus control), though exhibiting Caco2 desensitization. Time-dependant protein expression analysis showed that upregulation gradually occurred for 0.2 nM leptin treatment from 24 h to 72 h (x 1.7 to 2 respectively), whereas 1 nM leptin induced high activation at 24 h (x 2), followed by desensitization at 72 h. Altogether, these data confirmed our previous findings concerning implication of leptin on PepT-1 regulation. Moreover, we showed that hyperleptinemia rapidly led to Caco2 desensitization.

PP-770
The role of nuclear proteins in the binding of anthracycline antibiotics, daunomycin and idarubicin, to chromatin

Z. Zahraie and A. Rabbani
Department of Biochemistry, Institute of Biochemistry and Biophysics, Tehran, Iran. E-mail: zahraie@ibb.ut.ac.ir

Studies on the interaction of anthracycline antibiotics with DNA indicate that DNA is the main target. However in chromatin, the binding of histones and nonhistone proteins to DNA probably influence the binding accessibility of DNA to drugs. In the present study, the nuclei were isolated from rat liver and digested with micrococcal nuclease and fractionated into S1 and EDTA soluble (S2) fractions. The fractions were then exposed to various concentrations of daunomycin and idarubicin and analysed by SDS-polyacrylamide gel electrophoresis, immunobloting and UV/Vis spectroscopy. Both SDS gels and immunobloting against histones and HMG proteins antisera reveal that although various concentrations of drugs did not alter the protein content of S1 fraction, gradual increase in drug concentration reduced H1 and core histone proteins contents of S2 fractions, thus at high concentration of drugs (drug/DNA 1:1 and 1:2), histone proteins were disappeared. The results of UV/Vis spectroscopy showed

that in the drug treated samples, most of the drug had bound to chromatin and coprecipitated with it during centrifugation. The results suggest that interaction of daunomycin or idarubicin with chromatin, changes chromatin structure in a dose dependent manner, thus at lower concentration of drugs, it can possibly release some histone and nonhistone proteins but at higher concentration, crosslinks histones to DNA or histone to histone and inhibit releasing of them from chromatin.

PP-771
In vitro anticancer and anti-proliferative effects of *Rapana thomasiana* hemocyanin

A. Patronov[1], K. Idakieva[1], D. Dundarova[2], M. Apostolova[3] and **P. Genova**[2]

[1]Bulgarian Academy of Sciences, Institute of Organic Chemistry, Acad. G. Bonchev str., bl. 9, 1113 Sofia, Bulgaria, [2]Laboratory of Cell Culture, Department of Virology, National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria, [3]Medical and Biological Research Laboratory, Institute of Molecular Biology, Bulgarian Academy of Sciences, Acad. G. Bontchev Str., bl 21, Sofia 1113, Bulgaria. E-mail: petia.d.genova@abv.bg

Many of the marine hemocyanins (Hc) are well known as biologically active substances. Previously published data have shown that *Rapana thomasiana* hemocyanin (RtH) is a mixture of two isoforms. There are no data concerning the biomedical activities of native RtH and its functional subunits. The aim of this study was to investigate the cytotoxic and anti-proliferative effects of Hc isolated from the hemolymph of the Black sea mollusk *Rapana thomasiana*. Six cell lines were used in our experiments – five human cancer cell lines (SiHa- cervical squamous carcinoma, CaOV- ovarian adenocarcinoma, Mia PaCa - pancreatic carcinoma, RD- rhabdomyosarcoma, EJ- urinary bladder carcinoma) and one nontumor human lung line (Lep). Following RtH treatment cell viability was evaluated at 24 h and 48 h by two methods. Cytotoxic and cytostatic effect of RtH was compared to the effect of Tamoxifen (commercially available anticancer drug). The results showed that tumor cell lines were more sensitive to the application of RtH compared to the effect on nontumor Lep cells. Significant cell growth inhibition ($P < 0.05$) was observed in three of the five cell lines – CaOV, SiHa and EJ, tested at both time treatment intervals. The cervical cell line – SiHa exhibited a mean growth inhibition and cytopatic effect (range 37 to 59%) at 48 h, whereas the ovarian cell – CaOV had a range of 4 to 44% at these same concentrations. In conclusion we might suggest the native RtH could have a potential anti-tumor activity.

PP-772
Tropolone derivatives as inhibitors of HCV NTPase/helicase

A. Najda[1], K. Kopanska[1], A. Boguszewska-Chachulska[2], M. Krawczyk[2], A. Stankiewicz-Drogon[2], I. Zhukov[3], T. Kulikowski[1] and M. Bretner[1]

[1]Laboratory of Antimetabolites, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5A, 02-106 Warsaw, Poland, [2]Department of Protein Biosynthesis, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5A, 02-106, Warsaw, Poland, [3]Laboratory of Biological NMR, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5A, 02-106 Warsaw, Poland. E-mail: andzelikanajda@ibb.waw.pl

Hepatitis C virus (HCV) is one of the most infectious pathogens since over 170 million people worldwide are infected. In the majority of cases (85%) the HCV infection develops into chronic hepatitis and furthermore into liver cancer. Current therapy (based on the administration of PEG-interferon plus ribavirin) is effective only in 30–50% of cases. So, there is an urgent need to develop new anti – HCV agents. To achieve this goal we focused on one of the best characterized and indispensable for viral life cycle enzymes of the HCV virus, namely NS3 NTPase/RNA helicase. Screening for potential HCV helicase inhibitors revealed the inhibitory activity of the seven membered aromatic ring compounds - tropolones. We synthesized some tropolone analogues (e.g. bromo, morpholinomethylene). The influence of new derivatives on the unwinding activity of the NTPase/helicase of HCV was investigated. Also some studies to explain mechanism of action of tropolone derivatives were undertaken including NMR experiments and DNA melting studies. Some compounds inhibited 50% of the helicase activity at concentrations about 20 μM in the fluorometric test, and exerted low toxicity in a yeast-based toxicity assay.

Acknowledgment: This work was supported by the grant 2PO5A 038 29.

PP-773
Interaction of erythromycin with *E. coli* ribosomes under physiological ionic conditions

A. D. Petropoulos and D. L. Kalpaxis

Laboratory of Biochemistry, School of Medicine, University of Patras, Patras, Greece. E-mail: alexp@med.upatras.gr

Most of the kinetic and crystallographic studies concerning the binding of erythromycin to ribosomes have been conducted so far under conventional ionic conditions (10 mM Mg^{2+}, monovalent ions). Nevertheless, early binding studies pointed out that, in addition to these ions, polyamines are also essential for establishing an optimum ionic environment for ribosomal activity. This prompted us to re-examine the interaction of erythromycin with *Escherichia coli* ribosomes, by utilizing a series of polyamine buffers. We found that, at 4.5 mM Mg^{2+} and 150 mM NH_4^+, erythromycin (E) interacts with translating ribosomes (C) to form an encounter complex CE, which then undergoes a slow isomerization to a tighter complex C*E. Increase of Mg^{2+} affects negatively both the CE and C*E formation. Similarly, polyamines disfavor erythromycin binding to ribosomes by increasing the dissociation constant of the encounter complex CE and by lowering the rate constant of C*E formation. In contrast, they have no effect on the stability of C*E complex. Footprinting analysis demonstrated that the protection pattern of CE and C*E complexes differs and depends on the presence of polyamines. In addition, cross-linking experiments revealed that polyamines link adjacently to the binding site of the drug in the ribosome. These observations tempted us to suggest that polyamines bound at the vicinity of the erythromycin binding-site hinder drug interaction with the ribosome.

PP-774
Effect of cp96 345 treatment on the expression of adhesion molecules in acute pancreatitis

H. Y. Lau and **M. Bhatia**

Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore. E-mail: mbhatia@nus.edu.sg

In this study, we investigated the effect of treatment with specific neurokinin-1 receptor antagonist, CP96 345, on the regulation of ICAM-1, VCAM-1, E-selectin and P-selectin expression during acute pancreatitis (AP). AP was induced in male balb/C mice

(20–25 g) by the administration of 10 consecutive hourly intraperitoneal (i.p.) injections of 50 μg/kg caerulein. In the antagonist treatment groups, CP96 345 was administered at a single dose of 2.5 mg/kg i.p. either 30 min before (prophylactic) or 1 h after (therapeutic) the first caerulein injection. One hour after the last caerulein injection, the animals were sacrificed by pentobarbitone overdose, and the lungs and pancreas were isolated for RNA extraction and RT-PCR; or immunohistochemical staining. The mRNA expression of the four adhesion molecules was upregulated in the pancreas during AP. Treatment with CP96 345 effectively reduced the mRNA expression of P-selectin and E-selectin, but not ICAM-1 and VCAM-1. In the lungs, ICAM-1, E-selectin and P-selectin mRNA expression was increased during AP. The antagonist suppressed the elevation. Pulmonary VCAM-1 expression was not affected during AP. Similar expression pattern was seen in the immunohistochemical stainings. In conclusion, differential regulation of the expression of adhesion molecules in the pancreas and lungs was observed. These data provide an important information regarding the regulation of adhesion molecule expression during AP.

PP-775
One molecule of azithromycin binds to *Escherichia coli* ribosomes, via a two-step mechanism

E. C. Kouvela, A. D. Petropoulos and D. L. Kalpaxis
Laboratory of Biochemistry, School of Medicine, University of Patras. E-mail: kkouv@upatras.gr

Azithromycin is a derivative of erythromycin with improved activity against Gram-negative bacteria. Although exhibiting a marginal inhibition effect on peptidyltransferase activity, it strongly inhibits the protein synthesis by blocking the progression of the nascent peptide towards the exit tunnel. However, crystallographic data regarding the number of azithromycin molecules bound to ribosomal subunits of *Deinococcus radiodurans* compared to *Haloarcula marismortui* have raised questions about the specificity of binding. Kinetic analysis based on the ability of azithromycin to compete with tylosin, an antibiotic strongly inhibiting the puromycin reaction, revealed that azithromycin (A) and *E. coli* translating ribosomes (C) interact in a molar ratio 1 : 1 to form the encounter complex CA, which is then isomerized slowly to a tighter complex C*A. Changes in the ionic environment (Mg^{2+} ions, polyamines, etc) influence the formation and stability of CA and C*A complexes, but do not alter the number of the drug molecules bound per ribosome. This suggests that, at least for *E. coli* and *H. marismortui*, only one molecule of azithromycin binds per ribosome, independently of the ionic conditions. Therefore, the deviating pattern of azithromycin binding to *D. radiodurans* ribosomes must be attributed to species-specific differences in the rRNA and ribosomal proteins constituting the drug-binding site rather than to differences resulting from the high-salt requirement of archaea.

PP-776
Antinociception activity in rat estrous cycle stages after morphine treatment

K. Wroczynska and H. Makulska-Nowak
Pharmacy, Pharmacodynamic Institute, Warsaw, Poland. E-mail: kwroczynska@wp.pl

Antinociception activity in rat's estrous cycle stages after morphine treatment. Pain sensitivity and response to antinociceptive treatment are different according to sex and estrous cycle stage. Previous studies suggest that sex differences in morphine antinociception in rodents might be attributed to the activation of gonadal hormones. The goal of present studies was to compare antinociceptive effect of rat's stages of estrous cycle using mechanical noxious stimuli. These findings were obtained in female rats: normotensive Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) strain. A strong correlation in antinociception in four estrous cycle stages between SHR and WKY observed after subcutaneous administration of morphine. It was found that the same dose of mu agonist morphine is more effective in metestrus and diestrus phases and less in estrus during ovulation period. These findings might explain the opposite available data. Thus present results may have an implication for the usage of mu opioids in the clinical settings, especially in pain treatment such as cancer pain and postoperative pain.

PP-777
Protective role of BX471, a non-peptide CCR1 antagonist, in acute pancreatitis and sepsis

M. He[1], **R. Horuk**[2] and M. Bhatia[1]
[1]Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, [2]Department of Immunology, Berlex Biosciences, Richmond, Canada. E-mail: mbhatia@nus.edu.sg

Chemokines and their receptors play a key role in the pathogenesis of several acute inflammatory diseases including acute pancreatitis and sepsis. BX471 is a potent non-peptide CCR1 (CC chemokine receptor-1) antagonist in both human and mouse. The aim of the present study was to evaluate the effect of prophylactic and therapeutic treatment with BX471 on experimental acute pancreatitis and sepsis in the mouse and to investigate the underlying mechanisms. In acute pancreatitis induced by caerulein hyperstimulation, treatment with BX471 significantly protected mice against lung injury associated with caerulein induced pancreatitis by attenuating MPO (myeloperoxidase) activity, an indicator of neutrophil sequestration, in both lungs and pancreas and attenuating lung morphological changes in histological sections. In sepsis induced by cecal ligation and puncture, treatment with BX471 significantly protected mice against lung, liver and kidney damage by attenuating MPO activity in lung and liver and attenuating morphological changes in lung, liver and kidney in histological sections. In both models blocking CCR1 by BX471 led to a down-regulation of intercellular adhesion molecule-1 expression in specific organs compared with vehicle-treated controls. These findings suggest that interfering with neutrophil migration and activation by targeting CCR1 may represent a promising strategy to prevent disease progression in both acute pancreatitis and sepsis.

PP-778
Inhibition of Japanese encephalitis virus RNA replication by genome-targeted cell-penetrating peptide nucleic acids

J. -S. Yoo, J. -Y. Kim, I. -J. Jeon, J. -H. Kim and **J. -W. Oh**
Department of Biotechnology, Yonsei University, Seoul 120-749, Korea. E-mail: jwoh@yonsei.ac.kr

Japanese encephalitis virus (JEV) is a member of *Flaviviridae* family and a cause of viral encephalitis. Despite the major clinical impact of JEV, no specific and effective antiviral drugs are available to this virus. Peptide nucleic acid (PNA) is a synthetic oligonucleotide, in which the phosphodiester backbone of DNA/RNA is replaced with a polyamine-(2-aminoethyl) glycine skeleton. In this study, we investigated the antiviral effect of the PNAs targeted to the JEV *cis*-acting RNA essential for RNA

synthesis initiation. JEV genome is an approximately 11-kb single-stranded positive-sense RNA that has a cap structure at its 5' terminus but lacks a poly(A) tail at its 3'-terminus. The coding region of the genome is flanked by 5'- and 3'-untranslated region (UTR). The 3'-UTRs on both plus- and minus-strand JEV genome serve as important *cis*-acting elements required for the replication of the viral genome. *In vitro* RNA-dependent RNA polymerase assays using recombinant JEV NS5 proteins in the presence of the PNAs targeted to the JEV 3'-UTR 83-nt showed a dose-dependent RNA synthesis inhibition. Delivery of the inhibitory PNAs to the JEV-infected cells by conjugating them to various cell-penetrating peptides suppressed JEV replication. Our results showed a sequence specific inhibition of JEV replication by antisense PNAs, suggesting the possible application of PNA as a novel anti-JEV agent.

PP-779
Activity differences of two aminoglycoside antibiotics in yeast ribosomal function uncovered by mutations in 18S rRNA

S. Tselika and **D. Synetos**
Department of Biochemistry, School of Medicine, University of Patras, Patras 26110, Greece E-mail: stselika@med.upatras.gr

The C1409-G1491 base pair of 16S rRNA is important for high affinity binding of aminoglycosides. Eukaryotes, with an adenine in position 1491, are naturally resistant to these drugs. U1495 is a conserved nucleotide and mutation U1495C confers hygromycin resistance in Tetrahymena thermophila. We investigated the effects of mutations A1491G (rdn15) and U1495C (rdnhyg1) in helix 44 of yeast 18S rRNA. Neither mutation affected the catalytic activity of the ribosome. However, both caused translational infidelity, rdn15 to a higher and rdnhyg1 to a lesser degree. With the aid of these mutations we examined the differences between paromomycin and tobramycin, which bind to the same region of 16S rRNA but, while paromomycin is active also in eukaryotes, tobramycin is not. We confirm that rdn15 and, to a lesser extent, rdnhyg1 mutants are sensitive to paromomycin but both are unaffected by tobramycin. Sensitivity to paromomycin was followed by increased translational infidelity *in vitro* since, in its presence, the error frequency was increased from 25 to 338 errors per 1000 codons in rdn15 ribosomes and from 8 to 22 errors in rdnhyg1 ribosomes. Notably, the error frequencies of both strains were also increased by tobramycin, indicating that the lack of effect of this antibiotic in eukaryotes may arise from differences in its accessibility of 18S rRNA.

Acknowledgment: Supported by a grant from the General Secretariat of Research and Technology, Ministry of Development of Greece (624/PENED01).

PP-780
High throughput affinity ranking of antibodies using surface plasmon resonance microarrays

D. Wassaf, D. Martik, D. Sexton, Q. Wul, J. Cosic, C. Pazmani, S. Hogan, A. Nixon, J. Jaques, K. Kopacz, R. Ladner, Q. Nguyen and M. Toews
Dyax Corporation, Cambridge, MA, USA.
E-mail: dwassaf@dyax.com

A method was developed to rapidly identify high affinity human antibodies from phage display library selection outputs. It combines high throughput Fab fragment expression and purification with surface plasmon resonance (SPR) microarrays to determine kinetic constants (kon and koff) for 96 different Fab fragments in a single experiment. Fabs against human tissue kallikrein 1 (hK1, KLK1 gene product) were discovered by phage display, expressed in *E. coli* in batches of 96 and purified using protein A PhyTip columns. Kinetic constants were obtained for 191 unique anti-hK1 Fabs using the Flexchip SPR microarray device. The highest affinity Fabs discovered had dissociation constants less than 1 nM. The described SPR method was also used to categorize Fabs according to their ability to recognize an apparent active site epitope. The ability to rapidly determine the affinities of hundreds of antibodies significantly accelerates the discovery of high affinity antibody leads.

PP-781
Prediction of volatile anesthetics binding sites in proteins

J. H. Streiff[1], T. W. Allen[1], E. Atanasova[2], N. Juranic[3], K. A. Jones[1], A. R. Penheiter[1] and **S. Macura**[4]
[1]Department of Anesthesiology, [2]Department of Molecular Pharmacology and Experimental Therapeutics, [3]Department of Biochemistry and Molecular Biology, [4]Department of Biochemistry and Molecular Biology, Mayo Clinic, Mayo College of Medicine, Rochester, MN, USA. E-mail: macura@mayo.edu

Computational methods designed to predict and visualize ligand protein binding interactions were used to characterize volatile anesthetic (VA) binding sites and unoccupied pockets within the known structures of VAs bound to serum albumin, luciferase and apoferritin. We found that both the number of protein atoms and methyl hydrogen, which are within approximately 8 Å of a potential ligand binding site, are significantly greater in protein pockets where VAs bind. This computational approach was applied to structures of calmodulin (CaM), which have not been determined in complex with a VA. It predicted that VAs bind to $[Ca^{2+}]_4$-CaM, but not to apo-CaM, which we confirmed with isothermal titration calorimetry. The VA binding sites predicted for the structures of $[Ca^{2+}]_4$-CaM are located in hydrophobic pockets that form when the Ca^{2+} binding sites in CaM are saturated. The binding of VAs to these hydrophobic pockets is supported by evidence that halothane predominantly makes contact with aliphatic resonances in $[Ca^{2+}]_4$-CaM (Nuclear Overhauser effect) and increases the Ca^{2+} affinity of CaM (fluorescence spectroscopy). Our computational analysis and experiments indicate that binding of VA to proteins is consistent with the hydrophobic effect and the Myers-Overton rule.

PP-782
Cloning and expression of human CAI in *E. coli*

H. Yildirim[1], F. Kockar[1], M. Aydin[1] and O. Arslan[2]
[1]Department of Biology, Faculty of Science and Literature, Balikesir University Balikesir, Turkey, [2]Department of Chemistry, Faculty of Science and Literature, Balikesir University, Balikesir, Turkey. E-mail: hbozkurt@balikesir.edu.tr

The carbonic anhydrases (CAs, EC 4.2.1.1) are ubiquitous metaloenzymes which present in procaryotes and eukaryotes and encoded by three distinct evolutionary unrelated gene families: α- CAs (in vertebrates, bacteria, algae and cytoplasm of green plants), β- CAs (predominantly in bacteria, algae and chloroplasts of both mono- and dicotyledones) and γ-CAs (mainly in archae and some bacteria). In higher vertebrates, including humans 14 α-CA isozymes or CA-related proteins (CARPs) have been described with very different subcellular localizations and tissue distributions. CAI is an important enzyme associated with many processes such as CO2 transport as HCO3-, acid- base homeostasis, ion transport, formation of aqueous humour and

gastric juice and syntheses of urea, glucose and fatty acids. The aim of the study is to change some important amino acids in human CAI gene that are important for inhibitors by site-directed mutagenesis and the mutant gene would be expressed in *E. coli*. The effect of different inhibitors on mutant protein would also be investigated. Firstly, hCAI gene was cloned into an expression vector. Specifically, total RNA was isolated from K562 cell line and subsequently cDNA was prepared with RT-PCR strategy. hCAI gene was amplified by gene specific primers designed to open reading frame of hCAI gene and then cloned into pET21 expression vector. IPTG induced expression conditions of hCAI were optimized *E. coli*.

PP-783
Site-directed mutagenesis of Asn67/Ile67 in human CAII and expression of mutant protein in *E. coli*

S. Aydogan[1], F. Kockar[1], S. Sinan[1] and O. Arslan[2]
[1]*Department of Biology, Balıkesir University Faculty of Science and Literature, Balıkesir, Turkey,* [2]*Department of Chemistry, Balıkesir University Faculty of Science and Literature, Balıkesir, Turkey. E-mail: sumeyye_ayd@hotmail.com*

Carbonic anhydrases (CAs, EC 4.2.1.1) are a family of enzymes which catalyse the reversible reaction from H_2O and CO_2 to HCO_3- ions. The catalytic mechanism of CAII has been studied in particular detail. The zinc ion is located in cone-shaped cavity and coordinated to three histidine residues and a solvent molecule. Inhibitors bind at or near the metal center guided by a hydrogen-bonded system comprising Glu-106 and Thr-199. The hCAII enzyme is the target for drugs, such as acetazolamide, methazolamide and dichlorphenamide for the treatment of glaucoma. However, since the specificity of sulphonamides against CA enzymes that consist of 14 isoforms is low, the adverse effect could be encountered during the treatment of the related diseases. Therefore, it is important to elucidate the inhibition mechanism of the enzyme in order to develop more specific novel inhibitors. Thus, the aim of the study is to identify some amino acids that may be involved in catalytic centre. Site directed mutagenesis has been performed using PCR based strategy replacing Asn67 to a hydrophobic amino acid, Isoleucin. The mutant gene has been confirmed by the DNA sequencing. The expression of the mutant protein was performed in *E. coli* with the optimized conditions by inducing IPTG and it was purified with affinity chromatography. The purity of the proteins has been checked with SDS gel electrophoresis. The activity and inhibition manner of the mutant enzyme was compared to the wild type enzyme.

PP-784
Hypouricemia in individuals admitted to an outpatient clinic

G. Buğdaycı, E. Serin and Ö. Şahin
Department of Biochemistry, Izzet Baysal Faculty of Medicine, Abant Izzet Baysal University, Bolu, Turkey.
E-Mail: gbugdayci@yahoo.com

Hypouricemia is a sensitive indicator of decreased production or increased renal excretion of uric acid, suggesting an underlying pathological condition such as minimal renal tubular dysfunction. The aim of this study is to examine the prevalence of hypouricemia in individuals admitted to our outpatient clinic and identify the underlying causes and pathogenetic mechanisms and any association of hypouricemia and uricosuria with other tubular defects. Hypouricemia is defined as serum urate level of less than 2.5 mg/dl. A total of 3257 serum urate measurements performed between October 2005–January 2006 in Izzet Baysal Medical School Hospital were included in this study. Seventy four patients were detected as having hypouricemia with a prevalence of 2.27% for patients, respectively. Fractional excretion uric acid (FEUA) levels above 10% were considered pathological. There was an inverse correlation between FEUA and uric acid levels (r = -0.49 P < 0.0001). The most common cause of hypouricemia were found to be drugs affecting homeostasis (n = 40). Low protein diet and heavy black tea drinking were also strongly associated with FEUA. Eleven patients with hypouricemia showed one or more other manifestations of proximal tubular damage, such as glucosuria, phosphaturia and kaliuria. Hypouricemia caused by inappropriate uricosuria is not rare in outpaients. Hypouricemia is related to underlying diseases, and may be associated with other abnormalities of proximal tubular function.

PP-785
Inhibition of DPPIV by flavonoids as an alternative strategy for the treatment of type 2 diabetes

A. Antonyan[1], G. Lupidi[2], E. Camaioni[3], S. Mardanyan[1] and S. Sharoyan[1]
[1]*Laboratory of Nucleotides and Nucleosides, H. Buniatian Institute of Biochemistry of Arm. NAS, Yerevan, Armenia,* [2]*Department of Biology M. C. A., University of Camerino, Camerino, Italy,* [3]*Deptartment of Chemistry and Drug Technology, University of Perugia, Perugia, Italy. E-mail: alvardbio@yahoo.co.uk*

The incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are important in blood glucose regulation. However, both incretin hormones are rapidly degraded by the enzyme dipeptidyl peptidase IV (DPPIV). One of the approaches to overcome this problem is the using of DPPIV inhibitors. At present, some DPPIV inhibitors (valine pirrolidide, ile-thiazolidine, etc.) are used in treatment of type 2 diabetes. To elucidate the influence of some flavonoids presented in plants commonly used in food and/or traditional folk medicine we testified the inhibiting activity of different synthesized flavonoids on DPPIV highly purified from bovine kidney. Using the spectrophotometric method we determined the character of inhibition and IC50 for myricetin, fisitin, kaempferol, quercetin, resveratrol, genistein, EGCG, luteolin and crysin. It was demonstrated that luteolin and crysin do not inhibit the enzyme. The characters of inhibition by the others are competitive with IC50 in μmolar range. Myricetin demonstrated the most effective inhibiting activity. Additionally, we estimated the values of Kd for the interaction of DPPIV and above mentioned flavonoids spectrofluorimetrically. The obtained data allowed us to perform molecular modeling of DPPIV and flavonoids interaction and predict the amino acids involved in that. On the base of these data, the flavonoids more effective in the treatment of type 2 diabetes mellitus could be selected.

PP-786
Effects of *Nigella sativa* and its major constituent, thymoquinone on ethanol induced gastric mucosal damage

M. Kanter[1], O. Coskun[1] and **H. Uysal**[2]
[1]*Department of Histology-Embryology, Faculty of Medicine, Trakya University, Edirne, Turkey,* [2]*Department of Biochemistry, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey. E-mail: huysal@veterinary.ankara.edu.tr*

The aim of this study was to assess the possible protective effects of *Nigella sativa* (NS) and its constituent, thymoquinone (TQ) on ethanol-induced gastric mucosal damage in an experimental

model. Forty male rats aged 4 months were divided equally into four groups; the control group received physiologic saline (10 ml/kg) and the ethanol group had taken 1 ml (per rat) absolute alcohol by gavage. The third and fourth groups also received NS (500 mg/kg) and TQ (10 mg/kg) by gavage 1 h before alcohol administration, respectively. Gastric damage was confirmed histomorphometrically by significant increases in the number of mast cells (MC) and gastric erosions in ethanol treated rats. The NS treatment significantly decreased the number of MC and reduced the area of gastric erosions. Likewise, TQ treatment was also able to reduce the number of MC and the gravity of gastric mucosal lesions, but to lesser extent compared to NS. Gastric tissue histamine levels and myeloperoxidase activities were found to be increased in ethanol treated rats, and NS or TQ treatment reversed these increases. Results obtained from this study suggest that both drugs, particularly NS could partly protect gastric mucosa from acute alcohol-induced mucosal injury, and these gastroprotective effects could be due to their antiperoxidative, antioxidant and antihistaminic effects.

PP-787
3-FABS: a versatile NMR-based functional screening method

M. Veronesi, D. Caronni and N. Mongelli
Chemistry Department, Nerviano Medical Sciences S.r.l., Nerviano, Milano, Italy. E-mail: marina.veronesi@nervianoms.com

3-FABS (three Fluorine Atoms for Biochemical Screening) is a versatile, reliable NMR-based functional screening method for the identification of enzyme inhibitors and for the accurate measurement of their potency. The method can be applied in a primary screening for the identification of hits against a pharmaceutical relevant target and in the hit to lead phase optimization process. The focus of this poster is to provide a comprehensive insight into the 3-FABS methodology and some of its applications to the detection of inhibitors of different drug targets. The method requires the labeling of the substrate with a CF3 moiety, located either near or far from the modification site. 19F- NMR spectroscopy is then used to detect and quantify the signals of the substrate and of the product of the enzymatic reaction; the possibility of monitoring both signals in a non-destructive way allows one to derive properties of complex enzymatic reactions and mechanisms of inhibition. A significant advantage of this methodology is the possibility of directly monitoring the real concentration, stability and solubility of the screened compounds and therefore determine their real strength. Other applications of 3-FABS include the identification of new drug targets and the creation of the selectivity profile of an inhibitor toward different enzymes of the same class. The speed and easy set-up of 3-FABS can have a major impact in the drug discovery process for discovering new clinical candidates.

PP-788
The effects of compound of C1, indomethacin, nimesulide and rofecoxib on COX and NO, *in vivo* and *in vitro*

B. Demircan[1], Y. N. Sahin[1], H. Suleyman[2], H. Aksoy[1] and **I. Gul**[3]
[1]Biochemistry, Medical School, Erzurum, Turkey, [2]Pharmacology, Medical School, Erzurum, Turkey, [3]Pharmaceutical Chemistry, Faculty of Pharmacy, Erzurum, Turkey.
E-mail: ynsahin@atauni.edu.tr

The aim of this study was to investigate the effects of 3-benzoyl-1-ethyl-4-phenyl-4-piperidinol-hydrochloride (C1), which is a structural and also non-classical isomer of bis Mannich base B1, on cyclooxygenases (COX) activities and nitric oxide (NO) levels in 48 rats with inflammation by using carrageenan-induced paw edema and to compare its effect with other NSAIDs. C1, at the doses of 50 100 and 200 mg/kg, significantly decreased COX-1 and COX-2 activities as compared with the control group, whereas it significantly reduced NO levels only at 50 mg/kg dose. While the inhibitory effect of nimesulide on COX-1 and COX-2 activities were insignificantly less than that of C1 at all doses, this effect for NO levels was insignificantly more than that of C1. C1, at 200 mg/kg dose, significantly inhibited COX-1 and COX-2 activities in comparison to rofecoxib, but its effect on NO was not significantly different from rofecoxib. NO levels were higher in the rats given C1, at doses of 50 and 100 mg/kg, than rofecoxib-given ones. Indomethacin significantly reduced both COX-1 and COX-2 activities and NO levels compared to C1 at doses of 50 100 mg/kg. In conclusion, it might be claimed that C1 has an antiinflammatory effect, and its COX-2 selectivity is stronger than indomethacin and nimesulide but weaker than rofecoxib. In addition to COX inhibition, the role of NFIkappaB and other transcription factors should be investigated to clarify the mechanisms of antiinflammatory effect of C1.

PP-789
Purification of human erythrocytes 6PGDH enzyme, research the effects of some drugs on enzyme activity *in vitro, in vivo*

F. Ozabacigil[1], S. Beydemir[2], **N. Bakan**[1], K. Gumustekin[3] and M. Ciftci[2]
[1]Biochemistry, Medical School, Erzurum, Turkey, [2]Biochemistry, Faculty of Arts and Sciences, Erzurum, Turkey, [3]Department of Physiology, Medical School, Erzurum, Turkey.
E-mail: fatmanur25@yahoo.com

In this study, 6-phosphogluconate dehydrogenase was purified in human erythrocytes. This process was carried out by the preparation of hemolysate, precipitation by $(NH_4)_2SO_4$ and 2', 5'-ADP Sepharose 4B affinity chromatography. The degree of purity of the enzyme was determined with SDS-PAGE electrophoresis. The effects of some drugs on the enzyme were investigated *in vivo* and *in vitro*. Human erythrocyte 6PGD was purified in 742-fold in the end of all purification process. The recovery of 6PGD was 50%, and its specific activity was 0.46 U/mg in erythrocytes. Enzyme activity was spectrophotometrically measured. Fluorouracil, cisplatin, menadione sodium bisulfide, piroxicam, tenoxicam, ketoprofen and metilergobazin maleat inhibited the enzyme activity in *in vitro* conditions, while adrenalin, midazolam, phentanyl, dexametasone sodium fosfat and pentoxifyllin did not have any effect on enzyme activity. I50 values of the drugs inhibiting *in vitro* were determined. For the drugs having low I50 values (fluorouracil, cisplatin, menadione sodium bisulfide, piroxicam and ketoprofen), *in vivo* studies were performed in New Zeland-albino rabbits. In the evaluation of the *in vivo* effects of the drugs on 6PGD activity; it was observed that at the first hour fluorouracil, ketoprofen and piroxicam; at the third hour fluorouracil, ketoprofen, piroxicam, menadione sodium bisulfide and cisplatin; and at the fifth hour fluorouracil and cisplatin significantly inhibited 6PGD activity.

PP-790
L-carnitine infusions suppress serum C-reactive protein in hemodialysis patients

M. Duranay[1], H. Akay[1], **F. M. Yılmaz**[2], M. Şeneş[2], N. Tekeli[1] and D. Yücel[2]
[1]*Dialysis Unit of Ankara Education and Research Hospital, Ministry of Health, Ankara,* [2]*Clinical Biochemistry Laboratory of Ankara Education and Research Hospital, Ministry of Health, Ankara. E-mail: doyucel@yahoo.com*

Carnitine loss through dialysis membranes is shown to be related with the lack of carnitine in long term hemodialysis patients. It has been previously reported that hemodialysis patients might have benefited from carnitine supplementation, but data in this field has not been consistent. A total of 21 chronic hemodialysis patients maintaining carnitine supplementation and 21 controls (hemodialysis patients not receiving carnitine) were included in the study. L-carnitine was used intravenously three times a week after each hemodialysis session, at 20 mg/kg dose. CRP, lipid profile, nutritional parameters and required recombinant human erythropoietin (rHuEPO) doses were determined at the baseline, after 3- and 6-months of the treatment and compared with the control group. CRP levels were decreased significantly in carnitine group in contrast to the increase in control group. Transferrin and albumin levels were raised in carnitine group. rHuEPO requirements were decreased in both of the groups. The decrease in the rHuEPO requirement was statistically more significant in carnitine group. In conclusion, there was a significant benefit of L-carnitine on CRP, nutritional parameters and rHuEPO requirement.

PP-791
Characterization of a bipartite-equivalent nuclear localization signal of a cytotoxic ribonuclease variant

M. Rodríguez[1], A. Benito[1], P. Tubert[1], J. Castro[1], M. Ribó[1], B. Beaumelle[2] and M. Vilanova[1]
[1]*Laboratori d'Enginyeria de Proteïnes, Departament de Biologia, Facultat de Ciències, Universitat de Girona, Campus de Montilivi, 17071 Girona, Spain,* [2]*UMR 5539 CNRS, Dept Biologie-Santé, Université Montpellier II, 34095 MONTPELLIER CEDEX 05, France. E-mail: marc.ribo@udg.es*

Nuclear import of proteins is determined by specific signals that allow them to bind to receptors that mediate their energy-dependent transport through the nuclear pore. These signals are termed nuclear localization signals (NLS) and do not constitute a specific consensus sequence. Among them, the most characterized corresponds to monopartite and bipartite nuclear localization signals which interact with the importin α/β heterodimer. We previously described a cytotoxic variant of human pancreatic-ribonuclease that is actively transported into the nucleus, providing a new strategy to design cytotoxic ribonucleases. Here we show that this protein interacts with importin α through different basic residues including Lys1 and the arginine clusters 31–33 and 89–91. Although these residues are scattered along the sequence they are in close proximity in the three-dimensional structure of the protein and their topological disposition strongly resembles that of a classical bipartite nuclear localization signal. The results have implications for the identification of NLS in nuclear proteins and prove the high versatility of importin α when recognizing a nuclear signal.

Acknowledgment: This work was supported by grants from the Ministerio de Ciencia y Tecnologia (BMC2003-08485-CO2-02, HF2000-0017) and Generalitat de Catalunya (SGR2000-64 and SGR2001-00196).

Environmental Biomonitoring and Health

PP-792
The level of environmental estrogens, CYP polymorphisms and CYP expression in breast cancer patients

M. Ociepa-Zawal and W. H. Trzeciak
Department of Biochemistry and Molecular Biology, Poznan University of Medical Sciences, Poznan, Poland.
E-mail: mociepa@am.poznan.pl

The hypothesis that environmental estrogens (EE) are breast cancer risk factors is based on the assumption that they disturb, via AhR and PXR receptors, the clearance of estrogens by affecting cytochromes P4501A1, P450 1B1 and P4503A4, constituents of the enzyme complexes, involved in steroid hydroxylation. It is also postulated that polymorphisms of CYP genes, encoding cytochromes variants with changed enzymatic activity, may play a role in breast carcinogenesis. The main aim of the project was to study a relation between breast cancer, exposure to EE, CYP polymorphisms and CYP, AhR and PXR expression in breast cancer tissue. 71 patients and 23 controls were included to the study. The project was approved by the local Ethical Committee. The concentrations of EE in adipose tissue, measured by GLC-MS, did not differ between patients and controls, but women with higher EE concentration and additional risk factors more frequently developed estrogen-independent cancer. Although the frequency of CYP polymorphisms, examined by PCR-RFLP, was similar in both groups, CYP1A1*2A and CYP1A1*2C polymorphisms were more frequent in younger patients. The CYP1A1 expression in breast cancer, determined by real-time PCR, was higher, while the expression of CYP1B1 was lower, in the patients with high AhR expression. The CYP1B1 level correlated with stage of disease. The results show that both EE and CYP polymorphisms, together with other risk factors, may influence at breast cancer.

PP-792a
Application of *Escherichia coli* to monitoring the toxicity of pesticides in the environment

J. J. Aaron[1], S. Trajkovska[2] and M. Petrovska[3]
[1]*ITODYS University Paris 7, Paris, France,* [2]*Department of Madical Biochemistry, Medical Faculty, Skopje,* [3]*Department of Microbiology, Medical Faculty, Skopje Macedonia.*
E-mail: jeanjacquesaaron@yahoo.com

Pesticides are widely used in modern agriculture to protect crops, but they also constitute important organic pollutants of the environment. The goal of this work was to develop a method of evaluation of the acute toxicity response of pesticides by using *Escherichia coli* as a biosensor. We modified the Microbial ATP

Kit-BioThema by means of firefly bioluminescence, which allows one to measure the intracellular adenosine triphosphate (ATP) concentration, in order to evaluate the viability of bacterial cells. In our procedure, a suspension of *Escherichia coli* cultures, characterised by DO = 0.410 at 600 nm, was exposed independently to four pesticides, including fenoxaprop-p-ethyl (FPE), diclofop-methyl (DCM), 2, 4-dichlorophenoxy acetic acid (2, 4-D), and metsulphuron methyl (MSM) at concentrations of 5.0×10^{-4} and 3.3×10^{-4} mol/l. After an exposure time of 20 min, the toxic effect of pesticides was monitored. In these conditions, it was found that, at these respective concentrations, 99.46% and 35.14% bacteria were killed by FPE, 79.03% and 19.03% by DCM, 43.47% and 37.50% by 2, 4-D, and 32.18% and 18.47% by MSM. Therefore, *Escherichia coli* can be considered to be a good biosensor for the determination of the toxicity of the pesticides in the environment.

PP-793
The effects of different doses of acetylsalicylic acid on proliferation of lymphocyte cell culture

T. Dujic, A. Causevic and M. Malenica
Department of Biochemistry and Clinical Analysis, Faculty of Pharmacy, University of Sarajevo, Bosnia and Herzegovina.
E-mail: tanja-dujic@hotmail.com

Numerous are the studies related to the effects of acetylsalicilic acid in different cell cultures. This drug and salicilates in general cause the inhibition of homocysteine synthesis in mononuclear cells of human peripheral blood, suggesting possible similar effects *in vivo*. It can also cause inhibition of enzymes IkB kinase-beta, neutrofile NADPH oxidase, inhibition of HIV 1 virus replication and activation of caspases leading to induction of apoptosis. Therefore, it was of interest to examine possible effects of different therapeutic doses of acetylsalicylic acid on proliferation of lymhocytes in the cell culture, having in mind the fact that some of the studies reported earlier implicated suppression of lymphocyte transformation. In this work, micronucleus citoholasin test was used as mutagenesis test in order to detect whether acetylsalicylic acid (aspirin tablets, Bayer, Pharma) applied in tested doses of 1 mmol/l and 30 μmol/l can induce the formation of small fragments of DNA in the cytoplasm of lymphocyte cells in the interphase. Index of lymphocyte cell division was calculated based on formula reported by Eastmond and Tucker in 1989. Our results show that two applied concentrations of aspirin do not cause major changes in the proliferation of normal lymphocytes. Although, some degree of necrosis and apoptosis is seen in lymphocyte cell culture, it is not related to the dose of aspirin applied in this study.

PP-794
The effects of low dose aluminum on hemorheological and hematological parameters in rats

S. Turgut, M. Bor-Küçükatay, G. Emmungil, P. Atsak and G. Turgut
Physiology Department of Medical Faculty, Pamukkale University, Denizli, Turkey. E-mail: sturgut@pau.edu.tr

Aluminum (Al) toxicity can induce several clinical disorders such as neurotoxicity, gastrointestinal toxicity, hepatotoxicity, bone diseases, and anemia. This study aimed at evaluating the possible effects of short term and low dose Al exposure on hemorheological and hematological parameters in rats. 14 young, male rats were divided into two groups: 1 mg/200 g body weight of Aluminum sulfate was injected intraperitoneally to the first group for 2 weeks, three times a week. The animals of the control group received only physiological saline solution during this period. At the end of the experimental period, anticoagulated blood samples were collected and hematological parameters were determined using an electronic hematology analyser. Red blood cell (RBC) deformability and aggregation were measured using an ektacytometer and plasma and whole blood viscosities were determined with a viscometer. Significant decreases in mean corpuscular volume, RBC deformability at low shear stress levels, the aggregation half time and the amplitude of aggregation and significant increments in whole blood viscosity at native and 40% Hematocrit of Al treated rats have been observed. In conclusion; low dose Al sulfate exposure for a short-time may be responsible for alterations in hemorheological properties of blood through a remarkable effect on RBC membrane mechanical properties. These alterations may also play an important role in the development of anemia in the Al treated animals.

PP-795
Cholinesterases as potential biomarkers in *Tubifex* sp.

A. Özdemir, A. Şen and M. Duran
Department of Biology, Faculty of Arts and Sciences, Pamukkale University, 20020 Denizli, Turkey.
E-mail: aozdemir@pamukkale.edu.tr

Cholinesterases (ChE, E.C.3.1.1.7) have been used for a long time as tools for determination of nerve agents and pesticides contamination. Whilst ChE have been extensively studied in vertebrates and insects, very few studies are available on ChE in annelids. *Tubifex* sp. worms are common in muddy sediments of rivers but many also occur in brackish water in estuaries, or in fully marine conditions. The present work investigates the possible use of ChE activity measurements in *Tubifex* in pollution monitoring. For this purpose, we have studied the *in vitro* and *in vivo* ChE sensitivity towards commonly used model insecticides. *Tubifex* worms were collected from low polluted site at the Sigma site of Menderes River (West of Turkey). For *in vivo* contamination studies including control, worms were exposed in plastic tanks containing mud and water collected at the same site. Stock solutions of pesticides, diluted in water were added to obtain the desired concentrations. This work remains preliminary but it has established the conditions for measuring ChE activity in *Tubifex* worms which are of interest in biomonitoring. The *in vitro* and *in vivo* sensitivities of the ChE activities of *Tubifex* worms toward well known pesticides are comparable to that reported for other species. Although further studies should be done and other contaminants tested, the studied *Tubifex* sp. can be considered as interesting 'sentinel' species in the monitoring of the water contamination by pesticides.

PP-796
Biomonitoring of the toxic pollutants by using mullet liver enzymes in the West Black Sea region of Turkey

A. Bozcaarmutlu[1], Z. Aygun[1], **C. Sapmaz**[1] and E. Arinç[2]
[1]Department of Chemistry, Institute of Natural and Applied Sciences, Abant Izzet Baysal University, Bolu, Turkey, [2]Biochemistry Graduate Programme, Middle East Technical University, Ankara, Turkey. E-mail: canansapmaz@gmail.com

In this study, CYP 1A associated 7-ethoxyresorufin O-deethylase (EROD) activities (indicative of exposure to toxic carcinogenic persistent organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) and polychlorobiphenyls (PCBs)) were measured

in liver microsomes of mullet (*Mugil soiuy*) in the West Black Sea Region of Turkey. Fish samples were collected by fish nets from four sites, Sakarya River, Melen Stream, Guluç Stream and Zonguldak Harbor, in August 2005. Fish were killed by decapitation and livers were removed and frozen immediately in liquid nitrogen. Liver microsomes were prepared by differential centrifugation. EROD activities were measured in liver microsomes by fluorimetric assay. EROD activities of mullet captured from the edge of Sakarya River, Melen Stream and Guluç Stream were 904 ± 684 pmol/min/mg protein (n = 10), 981 ± 627 pmol/min/mg protein (n = 12) and 2296 ± 794 pmol/min/mg protein (n = 13), respectively. Mullet collected from the Zonguldak harbor, a highly urbanized and industrial city in the West Black Sea Region, had EROD activity of 3232 ± 1362 pmol/min/mg protein (n = 7). The results of this study indicate that these sites in West Black Sea are highly contaminated with PAH and/or PCB type organic pollutants.

Acknowledgment: This study was supported by TUBITAK (Project No:104Y083).

PP-797
Screening of PAH/PCB type pollution along Izmir Bay by CYP1A levels and EROD activities of three different fish species

T. B. Tumer and E. Arinç
Biochemistry Graduate Programme, Middle East Technical University, Ankara, Turkey. E-mail: btugba@metu.edu.tr

Hepatic EROD activity and CYP1A levels in three feral fish species: leaping mullet as a pelagic, annular seabream as a benthopelagic and common sole as a benthic fish were used as biomarker for the assessment of PAH, PCB and dioxins type toxic carcinogenic persistent organic chemicals along the Izmir Bay, Turkey, in 2002 and 2003 following the operation of Great Canal Project. CYP1A protein levels were determined by Western blot analysis using a polyclonal antibody produced in our laboratory against purified leaping mullet liver cytochrome P4501A. Mullet sampled from three highly urbanized and industrial sites of the Inner Bay – Harbor, Üçkuyular and Pasaport- exhibited significantly induced CYP1A protein levels and highly increased EROD activities which were 104, 80 and 73 times higher than counterparts sampled from reference sites located at Outer Bay. Hepatic CYP1A protein levels and EROD activities of annular seabream caught along the varying degrees of pollution gradient at five sites in the Bay exhibited pollution correlated induction pattern. In addition hepatic CYP1A protein levels and EROD activities of sediment fish, common sole caught from six different sites indicated that sediment of the Inner Bay and some regions in the Outer Bay like mouth of Gediz River were contaminated with CYP1A inducing chemicals. The results showed contamination in the Bay was particularly serious and might pose a threat to the health of the marine inhabitants as well as human consumers.

PP-798
Ionizing radiation influence upon mitochondrial processes

A. I. Gritsuk and **A. N. Koval**
Biochemistry, Gomel State Medical University, Gomel, Belarus. E-mail: gritsuk@inbox.ru

The influence of incorporated radionuclides is mediated by impaired mitochondrial function in the cells. The most sensitive to this action we believe are the cells of the aerobic organs. The same tissues are damaged by hypoxia. This suggest the similar defense mechanisms in eukaryotes in response to low-energy state. And this mechanism is based on the free radical formation. We suppose that prolonged exposure to radiation especially internal one cause the exhaustion of antioxidant system, lowering of ATP formation following proton gradient drop to reduce peroxidation of the membrane structures. All these events reveal the evolutionary adaptation of ancestors of prokaryotes and mitochondria: this symbiosis was accompanied by developing of defense mechanisms against radical formation by hypoxia mitochondria, and apoptosis to eliminate the damaged organelles.

PP-799
Development of *in vitro* regeneration system for a metal accumulator plant *Brassica nigra*

Y. Yildizhan[1], U. Demirel[2] and **A. R. Memon**[1]
[1]TUBITAK, Inst of Genetic Engineering and Biotechnology, Gebze, Kocaeli, Turkey, [2]Faculty of Agriculture, Haran University, Sanliurfa, Turkey. E-mail: armemon@gmbae.tubitak.gov.tr

The use of metal accumulator plants to clean-up soil and water contaminated with toxic metals is the most rapidly developing, environmental friendly and cost-effective technology. In present study, *Brassica nigra* was found to be accumulator of Cu and Cd. Our main aim is to mass produce these plants for phytoremediation. In this study, we developed a simple and cost effective regeneration system for *Brassica nigra* for mass production. Thirty days old plants were divided into shoot, apex, and hypocotyls and these explants were cultured in MS media containing 20 g/l sucrose, 1 ml/l MS vitamin solution and with various combinations of plant growth regulators. After shoot formation, these plants were sub-cultured to five different MS media for root formation. Plants were transferred to the soil culture after root formation. The suspension cell culture was derived from callus generated from hypocotyls of *B. nigra* and final concentration of the cells were maintained as 2×10^6 cell/ml. After 24 hours growth, cells were exposed to CuSO4 for 72 hours and following concentrations were maintained: 0, 50, 100, 200, 500, 1000 μM. For determination of the metal accumulation capacity of whole plant, thirty days old plants were transferred to solution culture containing different Cu concentrations. After HNO3 and HClO4 acid mixture (5:2) digestion and metal accumulation capacity of the cells and whole plants were determined by atomic absorption spectrophotometer.

PP-800
Effects of mercury, cadmium and nickel ions on purified leaping mullet NADPH-cytochrome P450 reductase

A. Bozcaarmutlu[1] and E. Arinç[2]
[1]Department of Chemistry, Institute of Natural and Applied Sciences, Abant Izzet Baysal University, Bolu, Turkey, [2]Biochemistry Graduate Programme, Middle East Technical University, Ankara, Turkey. E-mail: bozcaarmutlu_a@ibu.edu.tr

Heavy metals including cadmium, nickel and mercury are known to be highly toxic to the living organisms. These metal ions exert their toxicity by binding of metal ions to the sulfhydryl group of the enzymes or by generation of reactive oxygen species or both. Cytochrome P450 reductase contains one essential cysteine residue at or near NADPH binding site. In the present study we measured in vitro effects of Hg, Cd and Ni on the activity of purified leaping mullet cytochrome P450 reductase to provide insight into how metal ions modulate the overall function of this enzyme. NADPH-cytochrome P450 reductase is an essential

component of cytochrome P450 monooxgenase system that metabolizes a variety of endogenous and xenobiotic compounds including steroids, drugs and carcinogens. NADPH-cytochrome P450 reductase was purified from leaping mullet in our laboratory. All of the metal ions caused inhibition of the enzyme activity, Hg exhibited much higher inhibitory effect at lower concentrations and it was evidently more potent inhibitor than others. IC50 values of Hg, Cd and Ni were calculated as 0.069, 33 and 143 μM, respectively. Furthermore, all three metals are noncompetitive inhibitors of NADPH cytochrome P450 reductase as analyzed by Lineweaver-Burk plot. The noncompetitive nature of binding of metals implies that these metal ions do not directly interact with the substrate binding site of cytochrome P450 reductase.

Acknowledgment: This study was supported by A.I.B.U (BAP-2005.03.03.238).

PP-801
Global research on *Helicobacter pylori*

F. M. İpek and B. Salih
Fatih Universitesi, Biyoloji Bolumu, Akcaburgaz Mevkii, 34900, Buyukcekmece, Istanbul/Turkey. E-mail: fmipek@yahoo.com

The isolation of Helicobacter pylori from the human stomach by the Nobel laureates Marshall and Warren in 1983 have really paved the way for the current understanding of the important role of this bacterium in peptic ulcer disease and gastric cancer. H. pylori infection is now recognized as one of the most common bacterial infections in humans with approximately half of the world populations are currently infected. A PubMed bibliographic database search conducted for the last 23 years revealed over 15 000 publications and continues to increase. Recent reports revealed that virulent H. pylori strains vary significantly in their geographic distribution, which was shown to have an effect on the severity of the disease and the clinical outcome. H. pylori strains from Europe and the US were found to possess genotypes (cagA,vacA) that are different from those from Asia and other developing countries. In addition, the incidence of duodenal ulcer, gastric ulcer and gastric cancer were reported to be more prevalent in developing countries than in developed countries. While the incidence of H. pylori infection and the severity of the disease are much higher in the developing countries, the developed countries are the one more heavily engaged in H.pylori research. We are writing this letter to bring the attention to the fact that since H. pylori virulent strains and the outcome of the disease varies in their geographic distributions and being more pronounced in the developing countries.

PP-802
Cell and tissue injury in analog microgravity

A. Sundaresan
Dept.of Biology, Texas Southern University, Houston, TX, USA. E-mail: sundaresana@TSU.edu

With human exploration and long-term space travel, the immune system of the astronaut has to be optimally maintained. Individual risks to organs, such as the heart, bone, muscle and the immune system occur in microgravity. In order to isolate biomarkers of organ injury in a validated '*in vivo*' analog microgravity model, the Antiorthostatic suspension murine model (AOS) was utilized. Mice matched for age were suspended antiorthostatically for one week, along with control 1 g (unit gravity) mice. They were sacrificed after one week of hind limb suspension. mRNA was collected and subjected to gene array analysis using the Affymetrix HG_U95 mouse gene array. Genes related to organ injury were analyzed. A five-fold increase in biomarkers of cardiovascular injury, such as PIGF (placental induced growth factor) was seen in AOS splenocytes (5−fold increase compared with 1 g housed mice-90% confidence interval). PIGF is a specific biomarker for cardiovascular injury in comparison to C-reactive protein. There was a six-fold increase in Syk (Spleen tyrosine kinase). Syk is expressed in B cells, T cells, and myeloid cells and is an important enzyme in inflammatory pathways relevant to respiratory diseases such as asthma. The careful assessment of adaptational responses in both human and closely related mammalian systems will help identify targets and propose interventions for space exploration and help in early detection of organ injury here on earth.

PP-803
Regulation of the diphenolase activity of a highly hydrophobic polyphenoloxidase from Blanca artichoke bracts

L. Sidrach[1], A. Mendez[2], J. N. Rodriguez-Lopez[1], F. Garcia-Canovas[1] and J. Tudela[1]
[1]*GENZ-Grupo de investigacion Enzimologia (http://www.um.es/genz), Departamento de Bioquimica y Biologia Molecular-A, Facultad de Quimica, Universidad de Murcia, E-30071, Murcia, Spain,* [2]*ARTBIOCHEM S.L., Archena, Murcia, Spain. E-mail: http://www.um.es/genz*

A highly hydrophobic isoform of tyrosinase or polyphenoloxidase (hhPPO, EC 1.14.18.1) from bracts of artichoke (*Cynara scolymus* L., v. Blanca) has been characterised. The isolation has involved plant tissue fragmentation, fractional precipitation with ammonium sulphate and concentration by tangential flow ultrafiltration. IEXC were on carboxymethylcellulose and diethylaminoethylcellulose, with increasing sodium chloride gradients. HIC was on phenylsepharose with decreasing sodium chloride gradient. The process has been checked with assays of PAGE and IEF. The diphenolase activity of hhPPO has been measured at neutral optimum pH with 3,4-DHPPA, in the presence of the chromophoric nucleophile MBTH. The effects of several experimental variables on the activity and stability of hhPPO have been studied. Thus, type of buffer, ionic strength, temperature, inhibitors, surfactants and organic solvents. Optimum assay conditions for this hhPPO isoform have been determined, with consideration of its possible biotechnological applications on analysis, degradation and synthesis of phenolic compounds.

Acknowledgment: The authors are grateful to ARTBIOCHEM S.L. by free samples of industrial wastes of artichoke. This work has been partially supported by grants from several Spanish organizations. (J.N.R.L. and J.T.) Projects 07-B102005/01-6464 and 00672/PI/04 Fundacion Seneca, CARM, Murcia. (A.M.) ARTBIOCHEM S.L. (L.S.) Doctorate fellowship AP-2001-0956, FPU-MEC, Madrid.

PP-804
Spectrophotometric determination of ascorbic acid with laccase oxidizing 2,6-dimethoxyphenol

M. Abad[1], V. Tomas[2], R. Martinez-Gutierrez[3], F. Garcia-Canovas[1] and J. Tudela[1]
[1]*GENZ-Grupo de investigacion Enzimologia (http://www.um.es/genz), Departamento de Bioquimica y Biologia Molecular-A, Facultad de Quimica, Universidad de Murcia, E-30071 Murcia, Spain,* [2]*Departamento de Quimica Analitica, Facultad de Quimica, Universidad de Murcia,* [3]*NOVOZYMES SPAIN S.A., Madrid, Spain. E-mail: http://www.um.es/genz*

Laccase (EC 1.10.3.1) is a four copper oxidase that catalyze the oxidation for molecular oxygen of phenols up to phenoxyl

radicals, which can evolve up to quinones. These are reduced by ascorbic acid (vitamin C) which oxidizes to dehydroascorbic acid. The spectrophotometric assays of laccase oxidizing 2,6-dimethoxyphenol in presence of ascorbic acid, they show a lag period proportional to the concentration of this vitamin. The assay conditions, reaction medium, concentration of enzyme and of chromogenic substrate have been optimized. A broad linear range with micromolar limits of detection and of quantification of ascorbic acid has been reached. The test is sensitive, precise and quick and requires low quantities of sample. The method is useful to evaluate the antioxidant activity of ascorbic acid, and for assays of quality control in drugs that contain vitamin C as active compound. The assay does not require electrochemical neither spectrofluorimetric instruments. The method is applicable on spectrophotometers (1 ml cuvette) and on microplate readers (96×250 µl well), which favours its mechanization for high throughput screen assays.

Acknowledgement: The authors are grateful to NOVOZYMES SPAIN S.A. by free samples of laccase. This work has been partially supported by grants from several Spanish organizations. (J.T.) Project 00672/PI/04 Fundacion Seneca, CARM, Murcia. (R.M.G.) NOVOZYMES SPAIN S.A. (M.A.) Doctorate fellowship BES-2004-3829, FPI-MEC, Madrid.

PP-805
Polyphenoloxidase affinity towards oxygen: determination from a conversion time method

R. Zamora[1], V. Tomas[2], R. Martinez-Gutierrez[3], F. Garcia-Canovas[1] and J. Tudela[1]

[1]GENZ-Grupo de investigacion Enzimologia (http://www.um.es/genz), Departamento de Bioquimica y Biologia Molecular-A, Facultad de Quimica, Universidad de Murcia, E-30071 Murcia, Spain, [2]Departamento de Quimica Analitica, Facultad de Quimica, Universidad de Murcia, [3]NOVOZYMES SPAIN S.A., Madrid, Spain. E-mail: http://www.um.es/genz

Tyrosinase or polyphenoloxidase (PPO, EC 1.14.18.1) takes part in the melanin biosynthesis in humans, animals, plants and microorganisms. The enzyme catalyzes the oxidation by molecular oxygen of monophenols and o-diphenols to o-quinones, which decompose by means of non-enzymatic reactions up to melanins. PPO has great affinity towards oxygen, thus it is difficult to determine the value of its Michaelis constant using steady state rate assays. An alternative method consists of the use of progress curves of assays with oxygen depletion. The analysis of the data points of a progress curve needs its complex fitting by non-linear regression to an implicit equation, the integrated equation of Michaelis. A simpler method is based on the derivation of the analytical expressions of the conversion time. There have been obtained linear and hyperbolic useful expressions for the determination of Michaelis constant. Besides, there have been established equations that allow predicting the duration of the reactions of substrate depletion. The method has been applied successfully for the determination of the Michaelis constant of PPO towards oxygen, in the presence of 4-tertbuthylcatechol as substrate.

Acknowledgement: This work has been partially supported by grants from several Spanish organizations. (J.T.) Project 00672/PI/04 Fundacion Seneca, CARM, Murcia. (R.M.G.) NOVOZYMES SPAIN S.A. (R.Z.) Doctorate fellowship AP-2004-5759, FPU-MEC, Madrid.

PP-806
Monitoring the effects of water pollution on *Cyprinus carpio* in Karakaya Dam Lake

R. E. Guler[1], A. Gungordu[2] and **M. Ozmen**[2]

[1]Department of Biological Sciences, Middle East Technical University, Ankara, Turkey, [2]Department of Biological Sciences, Inonu University, Malatya, Turkey. E-mail: elifg@metu.edu.tr

The effect of environmental pollution resulting from industrialization around Karakaya Dam Lake and domestic sevage and agricultural activities was investigated. The dominant fish species *Cyprinus carpio* was selected for this biomonitoring study. Brain AChE and hepatic CaE, LDH, AST, ACP, GST, activities were determined for carp collected at different seasons. Hepatic LDH activities from Boran station contaminated by discharging of domestic sevage and rain water containing pesticides in to the lake, from agricultural land during spring season, showed elevated LDH activity 104.5 n Mol/dak/mg t. protein about 73 times higher comparing to the summer season LDH activities. (1.43 ± 0.18 mol/dak/mg t.protein). The results were statistically significant $P < 0.05$. At Tecimli station mean AChE enzyme activity was 92.46 ± 7.73 U/l and AChE enzyme activities at Egribuk station was 87.75 ± 5.05 U/l during spring time. There was nearly 50% inhibition of AChE activity; 43.16 ± 4.89 U/l in Hasırcılar, 50.19 ± 6.08 U/l in Boran and 53.56 ± 4.24 U/l in Eğribük location. The inhibition observed on AChE enzyme activity might be result from OP and Carbamate pesticides.

Results showed that, Hepatic LDH, GST and brain AChE activities were found to be the most affected ones with environmental changes. Karakaya Dam Lake is highly polluted especially in Hasırcılar and Boran areas where level of pollution was determined to be significantly higher compared to other two areas.

PP-807
Choline-oxidase based biosensor for paraoxon determination

Sh. Sajjadi and H. Ghourchian

Department of Biophysics, Institute of Biochemistry and Biophysics, Tehran, Iran. E-mail: sajjadi@ibb.ut.ac.ir

A new amperometric method for the detection of paraoxon has been developed. The method is based on a Prussian-blue chemically modified screen-printed electrode coupled with choline-oxidase. The activity of choline-oxidase is inhibited in the presence of paraoxon1. A satisfactory detection limit for determination of inhibitor was achieved using the electrode containing low enzyme loading, and this was consequently used for construction of the biosensor. Analysis was carried out using choline as substrate. Thus, choline was oxidized by choline-oxidase and subsequently H2O2 was produced. Meanwhile, H2O2 was electrochemically detected at −0.05 V versus the internal screen-printed Ag pseudo-reference electrode. The decrease of substrate steady-state electric current as a result of enzyme inhibition owing to addition of paraoxon was used for evaluation of the detector. Based on this approach down to 2×10^{-7} M of paraoxon was detected.

Reference:

1. Tavakoli H, Ghourchian H, Moosavi-Movahedi AA and Chilak FC, Effects of Paraoxon and Ethylparathion on Choline Oxidase from Alcaligenes Species: Inhibition and Denaturation, Int. J. Biol. Macromol, 2005; 36: 318–323.

Food, Nutrition and Health: Nutrigenomics

PP-808
In vivo study of *Berberis vulgaris* on hepatocarcinogenesis rats

P. Hanachi[1], F. Yakhob[2], F. Othman[2] and G. Motalleb[2]
[1]*Department of Biomedical Science, Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400, UPM, Serdang, Selangor, Malaysia, Department of Reproductive Biomedicine & Stem Cell, Women Research Center, Al-Zahra University of Tehran, Iran,* [2]*Department of Biomedical Science, Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400, UPM, Serdang, Selangor, Malaysia Sciences, Selangor, Malaysia. E-mail: paricheher78@yahoo.com*

Berberis vulgaris is the most significant European representative of the Berberidaceae and has been used extensively as a medicinal plant in traditional medicine. The effect of *Berberis vulgaris* aqueous extract on hepatocarcinogenesis rats was studied to investigate the apoptosis and antioxidant elements properties. The Sprague dawley rats were randomly divided into two groups, normal and cancerous. Each group was divided into four groups. Each first group of normal and cancerous group act as normal and cancer control group while the others were treated with 25, 50 and 100 mg/kg of *Berberis vulgaris* extract. Microscopic observations of the TUNEL-positive apoptosis cells have shown significantly difference ($P < 0.05$) between cancer control and normal control group. The results indicate that increasing concentration of *Berberis vulgaris* aqueous extract in cancer treated groups showed an increasing significant different ($P < 0.05$) of TUNEL-positive apoptosis cells count compared to cancer control group. The level of antioxidant elements observed was variable. Sodium and chloride level were shown significantly different ($P < 0.05$) in cancer control group compared to normal control group. The results suggest that apoptosis level was increase by *Berberis vulgaris* extract concentration in cancerous groups. The *Berberis vulgaris* extract play a prominent role in promoting apoptosis upon the treatment and it is dose dependent.

PP-809
Induction of the human ABCC6 gene by flavonoids

M. Ratajewski[1], G. Bartosz[2] and L. Pulaski[1]
[1]*Laboratory of Transcriptional Regulation, Centre for Medical Biology PAS, Lodz, Poland,* [2]*Department of Molecular Biophysics, University of Lodz, Lodz Poland. E-mail: mratajewski@cbm.pan.pl*

Flavonoids are a widely distributed group of diphenolic compounds of plant origin. They are known in pharmacology for their antioxidant activity and various anti-proliferative effects in cancer cells. Some of them influence cell proliferation in a phytoestrogenic manner, while others have additional cellular activities such as regulation of cellular signaling pathways, regulation of the cell cycle and induction of apoptosis. ABCC6 is an active transporter from the ATP-binding cassette superfamily (known also as MRP6). Mutation in ABCC6 gene cause pseudoxanthoma elasticum (PXE) – a genetic disorder characterized by calcification and fragmentation of elastic fibers in the skin, the retina, and the cardiovascular system. The molecular etiology of this condition is unknown. The ABCC6 gene seems to be a constitutive gene and virtually no treatments are known which would significantly induce its expression. We demonstrate that some flavonoids (e.g. genistein), but not others (e.g. resveratrol), activate transcription of ABCC6 in a reporter gene assay and in real-time PCR measurements in HepG2 cell line. Thus, the molecular mechanism of flavonoid-mediated activation of ABCC6 expression is not related to their common chemical properties, but rather to their differential activity in cellular signaling pathways. Based on our results, we believe that some flavonoids could potentially be used to alleviate the condition of patients who have PXE caused by underexpression of ABCC6 gene.

PP-810
Pleiotropic effects of daidzein on the growth, differentiation, and apoptosis of neuroblastoma cells

F. H. Lo[1], **N. K.** Mak[2] and K. N. Leung[1]
[1]*Department of Biochemistry, The Chinese University of Hong Kong, Hong Kong, China,* [2]*Department of Biology, Hong Kong Baptist University, Hong Kong, China. E-mail: lofaihang@cuhk.edu.hk*

Daidzein and genistein are two major isoflavones found in soy beans and in other natural sources. The anti-tumor properties of genistein have been studied intensively over the years. However, the effects of daidzein on neuronal cancers and their action mechanisms remain less understood. Neuroblastoma (NB) is the commonest extracranial childhood solid tumor. Due to the neuronal origin of the NB cells, they have been used in studies of neuronal differentiation and anti-tumor mechanisms of chemotherapeutic agents. In this study, daidzein was found to exert an growth inhibitory effect on a panel of murine and human NB cell lines. It was shown that daidzein induced cell-cycle arrest at the G0/G1 phase on the murine NB Neuro-2a (BU-1) cells. Moreover, daidzein triggered apoptosis in the BU-1 cells, as evidenced by DNA fragmentation and a change in 7-actinomycin D staining property. In addition, daidzein was shown to induce neuronal differentiation of the BU-1 cells indicated by the enhanced neurite outgrowth, expression of neuronal differentiation marker microtubule-associated protein 2, and increased acetylcholine esterase activity. The daidzein-induced neurite outgrowth was demonstrated to be inhibited by estrogen receptor inhibitor ICI 182, 780. Collectively, our results suggest that daidzein exerts antitumor activities on the NB cells by inhibition of cell growth, induction of cell cycle arrest, and triggering of neuronal cell apoptosis and differentiation.

PP-811
Effect of diet oils on lipid levels of the brain of rats

S. Kurban, I. Mehmetoglu and G. Yilmaz
Department of Biochemistry, University of Selcuk, Meram Faculty of Medicine, Konya, Turkey. E-mail: kurbansevil@hotmail.com

We have investigated the effects of sunflower oil, olive oil, margarine, soybean oil and butter on cholesterol, triglyceride (TG) and phospholipid levels of the brain of rats. The groups were fed with a diet containing 15% of the oils for a period of 8 weeks. Then, the rats were decapitated, brain samples were removed and cholesterol, TG and phospholipid levels were measured as mg/gr.protein. The findings were as follows:

Brain cholesterol levels of the groups were changed in the following order, olive oil > sunflower oil > soybean oil > margarine > butter > control.
Brain TG levels of the groups were changed in the following order; olive oil > soybean oil > sunflower oil > butter > control > margarine.
Brain phospholipid levels of the groups were changed in the following order, olive oil > soybean oil > control > margarine > sunflower oil > butter.
All lipid parameters of the olive oil group were higher than the same parameters of the other groups ($P < 0.001$). Brain cholesterol and TG levels of all vegetable oil groups were significantly higher than the same parameters of the saturated oils groups. Brain cholesterol and phopholipid levels of the butter group were lower than those of other groups. Our findings show that brain lipid levels of rats are differently affected by various oils mechanism of which needs to be investigated. Especially, the differences between the saturated and unsaturated oils and those of the olive oil and butter groups draws further attention.

PP-812
The role of genistein in mammary gland carcinogenesis: a new approach using stable isotope (2H2O)-mass spectrometry technique

Y. Kim[1], H. Kim[2], **D. Park**[3], J. Lee[4], W. Jeong[5] and K. Jeong[5]
[1]Department of Home Economics Education, Kyungpook National University, [2]Major in Food & Nutrition, Mokpo National University, [3]Laboratory Animal Center, Seoul National University, [4]Bioanalysis & Biotransformation Research Center, Korea Institute of Science & Technology, [5]Department of Veterinary Pathology, College of Veterinary Medicine, Kyungpook National University. E-mail: yookim@mail.knu.ac.kr

We investigated the effects of genistein in mammary gland carcinogenesis by incorporation of 2H from ^2H_2O into the deoxyribose (dR) moiety of purine deoxyribonucleotides in dividing cells. Female Sprague–Dawley rats were fed estrogen free diet from 1 week before breeding through lactation. Female pups were assigned to the following groups: vehicle; genistein; estradiol benzoate. On postnatal days 16, 18 and 20, female pups were injected subcutaneously with 500 μg genistein/g body wt, 500 ng EB/g body wt or vehicle. At day 50 postpartum, half of each group was gavaged with 60 mg DMBA and all animals were labeled through oral administration of 4% 2H2O. Mammary epithelial cell proliferation was measured by enrichment (EM1) of dA from rats. DMBA groups showed higher fractional synthesis than non-DMBA groups. The group exposed to only genistein showed significantly lower EM1 (1.46 ± 0.87%) than those of control groups. BrdU staining revealed that genistein reduced proliferation of the MEC and the number of BrdU positive cells both in DMBA and non-DMBA groups. H&E staining of MEC also showed that the exposure to genistein decreased proliferation of the mammary epithelium. In conclusion, exposure to genistein in the prepubertal period inhibited mammary epithelial cell proliferation. The ^2H_2O labeling results were in good agreement with the results of BrdU incorporation and histomorphometry, which demonstrates that ^2H_2O labeling can be used as a tool to measure carcinogenesis.

PP-813
Extraction and identification of peonidin-3-galactoside in *Paeonia officinalis* L., (Paeoniaceae)

A. Uzunovic[1], A. Mehmedagic[1], J. Toromanovic[2], L. Sofic[3] and E. Sofic[2]
[1]Department of Biopharmaceutical-Technological Evaluation, Institute for Quality Control of Medicines, Sarajevo, Bosnia and Herzegovina, [2]Department of Chemistry, Faculty of Science, University of Sarajevo, Bosnia and Herzegovina, [3]Faculty of Pharmacy, Cekalusa 90, Sarajevo, Bosnia and Herzegovina. E-mail: alijauzunovic@gmail.com

It was developed simple extraction and identification method of peonidin-3-galactoside from red peony flowers and of malvidine from mallow flowers. Spectrum of extracted peonidin-3-galactosides and malvidin was recorded. Maximum absorption for peonidin-3-galactoside was at 517 nm and at 530 nm for malvidin, respectively. High performance liquid chromatography (HPLC) was performed with photodiode detector of extracted peonidin-3-galactoside and malvidin. Mobile phase was (water : methanol : acetonitril : formic acid : isopropyl alcohol) in proportion (75.5 : 12.0 : 6.0 : 5.0 : 1.5). Retention time for peonidin was 4 minutes and for peonidin-3-galactoside 6.5 minutes, while retention time for malvidin was 5 minutes and 8 minutes for malvidin-3-galactoside or malvidin-3-glucoside, respectively.

PP-814
Leptin, ghrelin, adiponectin and resistin concentrations in human breast milk

Y. Ozarda Ilcol and Z. B. Hizli
Department of Biochemistry, Uludag University Medical School, Bursa, Turkey. E-mail: yesim@uludag.edu.tr

Leptin, ghrelin, adiponectin and resistin have important roles in energy homeostasis, glucose and lipid metabolism, reproduction, cardiovascular function, and immunity. They are significantly regulated by nutritional status. The objectives of this study were to assess leptin, ghrelin, adiponectin and resistin concentrations in breast milk during the 180 days post-partum. Cross-sectional analysis of milk leptin, ghrelin, adiponectin and resistin from 60 women enrolled either in the first three days ($n = 10$, colostrum), days 4–14 ($n = 29$, transitional milk), days 15–90 ($n = 11$, mature milk) or days 91–180 ($n = 10$, late mature milk) postpartum. Milk leptin, ghrelin, adiponectin and resistin were measured by immunoradiometric assay. Leptin concentrations in colostrum were higher ($P < 0.05$) than in mature milk. Leptin concentrations in whole mature milk decreased with time and showed inverse relation ($r = -0.412$; $P < 0.001$) with the lactation days. Total ghrelin concentrations in transitional and mature milk were higher ($P < 0.01$) than in colostrum and showed positive relation ($r = 0.429$; $P < 0.05$) with the lactation days. Resistin concentrations in colostrum were higher ($P < 0.01$) than in transitional and mature milk and showed negative relation ($r = -0.597$; $P < 0.001$) with the lactation days. There was no correlation between lactation days and adiponectin concentrations. In conclusion, these hormones are present in breast milk, and their levels are affected differently by duration of lactation.

PP-815
The confirmation of the inhibition mechanism of 15-lipoxygenase by β-carotene

A. Serpen and V. Gökmen
Food Engineering, Hacettepe University, Ankara, Turkey.
E-mail: aserpen@hacettepe.edu.tr

Lipoxygenase, being found in essentially all higher plants and animals, is an iron-containing dioxygenase which catalyzes the oxidation of polyunsaturated fatty acids containing a cis, cis-1,4-pentadiene units to the conjugated cis, trans dienoic monohydroperoxides. The confirmation of the inhibition mechanism of 15-lipoxygenase (soybean type-1) by β-carotene was studied. It is confirmed by GC-MS analyzes that presence of β-carotene in the reaction mixture decreased or completely inhibited the activity of 15-lipoxygenase by means of protecting linoleic acid. The inhibitory effects of β-carotene into 15-lipoxygenase activity were determined using model solutions containing linoleic acid and enzyme in the presence and absence of β-carotene. β-Carotene directly influences the amount of enzyme in the reaction medium available for the catalytic conversion of linoleic acid into corresponding hydroperoxides. The results obtained here confirms the suggestion of reaction between β-carotene and linoleiyl radical at the beginning of the chain reaction which prevents the accumulation of peroxy forms. Since linoleiyl radical transforms back to its original form of linoleic acid by means of hydrogen transfer mechanism, the enzyme cannot complete the chain reaction and thus remains at inactive Fe (II) form. These findings contribute to therapeutic property of β-carotene in addition to its traditional role in preventing the auto-oxidation of lipids.

PP-816
Total content of phenols and anthocyanins in edible fruits and red wine

Z. Rimpapa[1], J. Toromanovic[2], I. Tahirovic[2] and E. Sofic[2]
[1]Faculty of Medicine, University of Sarajevo, Bosnia and Herzegovina, [2]Department of Chemistry, Faculty of Science, University of Sarajevo, Bosnia and Herzegovina.
E-mail: ida@bih.net.ba

Subject of this study was a total content of phenols and anthocyanins in some edible fruits and red wine. Content of phenols and anthocyanins was estimated by photometric method. Cyanidin-3-galactoside chloride served as a standard. It was estimated that total content of phenols in elderberry fruits was 12.7 mg/g, in cultivated bilberry 10.5 mg/g, in wild bilberry 9.3 mg/g, in cultivated cherry 8.8 mg/g , and in wild cherry fruits 7.2 mg/g, in cultivated raspberry had 7.1 mg/g, cultivated blackberry 5.9 mg/g, cultivated strawberry 3.5 mg/g, while sour cherry fruits from different locations had in average 2.4 mg/g. The lowest quantity of total phenols was in edible parts of melon, only 0.2 mg/g. Total phenols content in red wine was 28.8 mg/g. Total anthocyanins content in cultivated cherry was 6.8 mg/g, in elderberry fruits 6.7 mg/g and in cultivated bilberry 4.5 mg/g. Wild bilberry fruits from different locations had in average 3.5 mg/g, cultivated raspberries 3.2 mg/g, cultivated blackberries 2.1 mg/g, cherries from different locations 1.3 mg/g, wild blackberries 1.0 mg/g, cultivated strawberries 0.8 mg/g while melon fruit had no anthocyanins at all. Red wine had in total 1.1 mg/g of anthocyanins. Acidity was measured in macerate of edible fruits by direct insertion of electrode. pH value of macerated bilberry fruits was 2.99, blackberries 3.31, sour cherries 3.59, cherries 3.99, elderberries 4.44 and melon 6.19.

PP-818
Total content of phenols and anthocyanins in flowers of some plants

J. Toromanovic[1], S. Selman[1], S. Calkic[1], A. Sapcanin[1], Z. Rimpapa[2] and E. Sofic[1]
[1]Department of Chemistry, Faculty of Science, University of Sarajevo, Bosnia and Herzegovina, [2]Faculty of Medicine, University of Sarajevo, Bosnia and Herzegovina.
E-mail: jast@bih.net.ba

Subject of this study was a total content of phenols and anthocyanins in petals of some flowers. Petals of flowers were homogenised and then homogenates were centrifuged and supernatants were used for analysis. Total content of phenols and anthocyanins was estimated by photometric methods. Cyanidin-3-galactoside chloride served as a standard solution. Total phenols content was within the following range: cabbage rose flower had 22.7 mg/g, sweet violet 22.0 mg/g, rose 20.5 mg/g, Deptford pink and chicory 8.8 mg/g, viper's bugloss 8.5 mg/g, petunia had 8.2 mg/g. Peony flower from different locations had in average 7.7 mg/g. Total phenols content in wild pansy flowers was 7.8 mg/g, in dame's rocket 7.6 mg/g, in Japanese flowering quince 7.5 mg/g, in two-coloured petunia 6.6 mg/g, in bird vetch 5.3 mg/g, in mallow 3.8 mg/g, in bugleweed 3.3 mg/g and in Chinese wisteria 3.1 mg/g. Total anthocyanins content was the highest in rose flowers-8.5 mg/g, followed by 4.3 mg/g in peony, 2.3 mg/g in sweet violet, 1.3 mg/g in rose, 1.2 mg/g in petunia, 1.1 mg/g in Deptford pink. Cabbage rose flower had 0.8 mg/g of total anthocyanins, chicory 0.47 mg/g, bird vetch 0.37 mg/g, viper's bugloss 0.33 mg/g, bugleweed 0.16 mg/g, Japanese flowering quince 0.15 mg/g, mallow 0.14 mg/g and two-coloured petunia had 0.13 mg/g. Flower of Chinese wisteria had 0.11 mg/g of total anthocyanins, dame's rocket had 0.08 mg/g while wild pansy flower had only 0.01 mg/g.

PP-819
Influence of growth phase and zeolite on the sphingoid bases biosynthesis in waste brewer's yeast

I. Karmelić[1], F. Ivušić[2], S. Ribar[1], L. Feher Turković[1], V. Marić[2] and M. Mesarić[1]
[1]Department of Chemistry and Biochemistry, School of Medicine, Zagreb, Croatia, [2]Department of Biochemical Engineering, Faculty of Food Technology and Biotechnology, Zagreb, Croatia.
E-mail: ivana.karmelic@mef.hr

Sphingolipids are a group of lipids which are present in all eukaryotic cells, particularly in plasma membranes. The backbones of complex sphingolipids (sphingoid bases and ceramides) are important intracellular second messengers that play a role in the regulation of cell growth, differentiation and programmed cell death. They are not an essential food constituent, but recent studies point to their dietary significance, since their breakdown releases bioactive substances in the gastrointestinal tract. Increased uptake of food sphingolipids gives rise to reduced incidence of colon cancer and decreased concentration of plasma cholesterol. Due to its rich chemical composition, waste brewer's yeast is being increasingly used as additive and crude material in food processing. Our research is focused on how growth conditions and addition of natural zeolite clinoptilolite to the growth medium influence the concentrations of the individual sphingoid bases from the waste brewer's yeast. Total sphingoid bases were extracted according Riley at al. /O-Phthalaldehide derivatives of the sphingoid bases were prepared and analysed by HPLC. The results point out the following conclusions: waste brewer's yeast is a good source of sphingolipids and the predominant sphingoid

base is phytosphingosine. Growth phase has influence on the phytosphingosine concentration. The addition of zeolite to the growth medium caused an increase in the concentrations of analysed sphingoid bases.

PP-820
Antioxidant and antibacterial activity of *Artemisia annua* L. plant extracts and essential oil

S. Cavar, M. Maksimovic, D. Vidic and A. Paric
Faculty of Science, University of Sarajevo, Sarajevo, Bosnia and Herzegovina. E-mail: sanja_cavar@yahoo.com

The antioxidant properties of the plant extracts and essential oil of *Artemisia annua* L. were examined by two different spectrophotometric methods, the DPPH radical scavenging test and the reducing power test. The total phenolic, non-flavonoid and flavonoid content were also determined by Folin-Ciocalteu method. Growth inhibitory activity of the samples was analyzed against five bacteria: *Micrococcus luteus* (ATCC 4698), *Staphylococcus aureus* (ATCC 6538), *Salmonella goldcoast*, *Bacillus subtilis* (ATCC 6633) and *Pseudomonas aeruginosa* (ATCC 9027). The paper disc diffusion method was used. It was found that 80% ethanolic and water extracts have a significant antioxidant and antibacterial activity. Essential oil poses weak antioxidant activity but inhibit *M. luteus* and *P. aeruginosa* growth.

PP-821
Malnutrition, nutritional indices and metals in hair: a view from a different perspective

M. Donma[1] and **O. Donma**[2]
[1]*Department of Paediatrics, Suleymaniye Maternity and Children's Education and Research Hospital, Ministry of Health, Istanbul, Turkey,* [2]*Department of Biochemistry, Istanbul University Cerrahpasa Medical Faculty, Istanbul, Turkey.*
E-mail: mdonma@gmail.com

Malnutrition is globally the most important risk factor for illness and death, with millions of children affected. Apart from marasmus and kwashiorkor, deficiencies in trace elements are the main manifestations of malnutrition in developing countries. In this study, hair metal concentrations and some nutritional indices in children with protein-energy malnutrition (PEM) will be provided. Twenty and fourteen children with marasmus and kwashiorkor, respectively, were included into the study. Hair Zn, Cu and Mg levels were determined by AAS. The nutritional indices; weight/height, body mass index and ponderal index were calculated. The values were compared with those of twenty-two healthy children. Increased hair Zn and decreased hair Cu levels were detected in the group with PEM. The values for nutritional indices were depressed in PEM group. The elevated values of hair Zn, which impairs Cu retention, may be attributed to accumulation of Zn in hair whose growth was stunted as a result of severe deficiency. The findings are consistent with the fact that the pathologic features of PEM are still not fully understood. Multiple multinutrient interventions are important in populations with a high prevalence of malnutrition. Synergistic and antagonistic interactions of metals as well as the beneficial and adverse effects of phytonutrients have to be taken into consideration. The other factors associated with impaired growth in children should also be considered.

PP-822
Process for elimination of flatulence-inducing carbohydrates from legume seeds

R. Rakijian[1], J. Miller[2], K. DeLeon[1], R. C. Zane[1], J. R. Wilson[2] and **M. A. Payne**[1]
[1]*Chemistry and Biochemistry, La Sierra University, Riverside CA, USA,* [2]*Biology, La Sierra University, Riverside CA, USA.*
E-mail: mpayne@lasierra.edu

Flatulence in humans is most often the result of ingesting foods containing oligosaccharides raffinose, stachyose and verbascose: raffinose series oligosaccharides (RSO). These oligosaccharides, with fructose and sucrose, represent major reserves of soluble carbohydrates that are mobilized during seed germination. Humans lack enzymes that hydrolyze RSO to monosaccharides. Un-hydrolyzed RSO are fermented by microorganisms present in the colon and produce gases such as carbon dioxide, hydrogen, and methane that manifest as intestinal distress and flatulence. Here we examined the effects of saline treatment on RSO content in legumes during and before germination. Various legumes were imbibed and germinated from 24 to 48 h, at 25 to 30 degrees Celsius. Germinated and non-germinated seeds were soaked in increasing concentrations of sodium chloride. Sucrose and RSO were then measured over a time course. Both increasing salinity and treatment times correlated with decreasing RSO in germinated and non-germinated seeds. Optimal treatment conditions resulted in greater than 95% reduction of the RSO present in ungerminated seeds. The reduction in RSO caused by salinity treatment was more rapid in germinated seeds than in un-germinated seeds, likely related to changes in cellular structure during germination. The process developed in this study will allow the production of legumes for food, free of the adverse effects caused by RSO.

PP-823
Small heterodimer partner (SHP) is a key mediator of hipolipidemic action of grape seed procyanidins

J. M. del Bas, I. Quesada, J. Fernández-Larrea, A. Ardèvol, M. Blay, G. Pujadas, M. J. Salvadó, L. Arola and C. Bladé
Biochemistry Department, Rovira i Virgili University, Tarragona, Spain. E-mail: mariacinta.blade@urv.net

Procyanidins are polyphenolic compounds found in vegetables and derived foods. Grape seed procyanidins contained in red wine have shown different properties. In a previous work, we showed that a grape seed procyanidin extract (GSPE) lowered the atherosclerotic risk indexes in healthy rats, diminishing plasma apolipoprotein B levels. Parallely, the expression of liver small heterodimer partner (SHP) was upregulated. Since SHP is gaining relevance regarding lipid and lipoprotein metabolism control, this work was designed to study the role of this nuclear receptor in the hypolipidemic activity of procyanidins. With this regard, HepG2 cells were treated with GSPE. Dose-response experiments showed a dose-dependent upregulation of SHP, correlated with the inhibition of apolipoprotein B release to the media. To assess the putative link between SHP upregulation and the inhibition of apolipoprotein B secretion, HepG2 cells were transfected with a siRNA against SHP or scramble RNA for control cells. The results show that in SHP knocked down cells, the activity of GSPE is reduced. On the other hand, to gain insight into the mechanisms involved in the GSPE activity, the expression of different genes was followed in time. The results showed a significant upregulation of SHP 90 minutes after treatment, followed by the inhibition of MTTP. All together, these

results point out that SHP is a key mediator of the hypolipidemic activity of GSPE.

PP-824
Human salivary alkaline phosphatase

M. J. Martins, R. Negrão, E. Keating and I. Azevedo
Department of Biochemistry, Faculty of Medicine (U38-FCT), University of Porto, 4200-319 Porto, Portugal.
E-mail: mmartins@med.up.pt

Alkaline phosphatase (ALP; EC 3.1.3.1) represents an ubiquitous family of ecto-phosphomonoesterases. ALP isoenzymes can be released into biological fluids according to physiological and pathological conditions. Our aim was to quantify human salivary ALP and to study its in vitro modulation by a specific inhibitor of the tissue-nonspecific ALP isoenzyme, levamisole, by widely consumed beverages, apple juice and black tea (with or without glucose), and by a glucose control solution. Gender, blood phenotype, smoking habits, contraceptive use, dental caries and week of menstrual cycle were considered in results analysis. ALP activity was determined at pH 10.4 using p-nitrophenylphosphate as substrate. There were no differences in ALP activity among the groups considered. Forty four to 76% of salivary ALP was inhibited by levamisole; the inhibition was higher in male than in female group. Tea activated ALP in blood type O and in male (all men, non smoking men and men without caries) groups but inhibited ALP in female group (all women, non smoking women, women without caries, non-contraceptive women, 2nd and 4th week cycle women). Glucose activated ALP in groups activated by tea as well as in 1st week cycle women, but inhibited ALP in 2nd week cycle women. Apple juice inhibited ALP in all groups but had no effect in those activated by tea. We show that human salivary ALP may be influenced by physiological and exogenous parameters. These findings may help to clarify ALP physiology.

PP-825
Biological activity of hydrophilic bee extract

K. Krzymiński[1], N. Kadnikova[2], A. Roshal[3], L. Tynynyka[4], O. Synchykova[5] and **B. Sandomirsky**[4]
[1]Faculty of Chemistry, University of Gdansk, Gdansk, Poland, [2]Cryoprotectants Department, Institute for Problems of Cryobiology & Cryomedicine of National Academy of Sciences of Ukraine, Kharkov, Ukraine, [3]Department of Physical Organic Chemistry, Institute of Chemistry at Kharkov National University, Kharkov, Ukraine, [4]Department of Experimental Cryomedicine, Institute for Problems of Cryobiology & Cryomedicine of National Academy of Sciences of Ukraine, Kharkov, Ukraine, [5]Department of Cell Cryophysiology, Institute for Problems of Cryobiology & Cryomedicine of National Academy of Sciences of Ukraine, Kharkov, Ukraine.
E-mail: cryo@online.kharkov.ua

Hydrophilic extract derived from bee body was used in experiments. Investigation of qualitative and quantitative composition of the extract was performed with spectral and chromatographic methods. Anti-oxidative activity was assessed by hemiluminometric method using acridine acid ether- hydrogen peroxide system. Saccharomyces cerevisiae cells were grown in a complete liquid nutritive medium comprised various concentrations of the extract under study. Accumulation of biological mass was accomplished during 6 and 8 h at 30 °C with continuous aeration. Number of seeded and grown yeast was found with standard method of calculating macrocolonies formed on agarized media. Examining of yeast generative activity (ability of cells to form a certain number of daughter cells within a certain time period) was performed with counting small cells in a sample under microscope. When culturing Saccharomyces cerevisiae cell population with adding mentioned above extract there was noted a growth stimulation and statistically significant rise in generative activity of yeast. Biological effect of extract is probably stipulated with a high content in it of free amino acids, low molecular peptides, and flavonoids. Their content made 1.3–1.9%, that testifies to a perspective of apiextract obtaining as a source of biologically active substances.

PP-826
Antioxidant potential of whey protein fractions

T. Bayram[1], M. Pekmez[2], E. Önay-Uçar[2], N. Arda[2] and **A. S. Yalçın**[1]
[1]Department of Biochemistry, School of Medicine, Marmara University, 34668 Haydarpasa, Istanbul, Turkey, [2]Department of Molecular Biology and Genetics, Faculty of Science, Istanbul University, 34118 Vezneciler, Istanbul, Turkey.
E-mail: asyalcin@marmara.edu.tr

Milk contains all the essential nutrients as well as vitamins, trace elements, enzymes and proteins. The liquid part of clotted milk is called milk serum or whey. The soluble proteins in whey are composed of major (β-lactoglobulin, α-lactalbumin, serum albumin, immunoglobulins and glycomacropeptides) and minor (lactoferrin, lactoperoxidase, lysozyme) fractions. In recent years multiple therapeutic effects of whey proteins have been reported. We have evaluated the antioxidant potential of whey in an *in vitro* system using 1,1-diphenylpicryl- hydrazyl (DPPH). Whey solution exhibited antioxidant potential at a concentration range of 4–6 g/l. We have further studied the antioxidant potential of individual whey proteins after separating them by differential ultrafiltration using membranes with different MW cut-off values. Three different fractions were studied: F1 contained proteins with MW higher than 100 kDa, F2 contained proteins with MW between 30–100 kDa and F3 contained proteins with MW lower than 30 kDa. Our results showed that F3 had the highest antioxidant potential compared to the other two fractions (F1 and F2). Further characterization of the individual proteins in F3 was carried by polyacrylamide gel electrophoresis and showed the presence of β-lactoglobulin and α-lactalbumin in this fraction.

Acknowledgment: Supported by Turkish Scientific and Technical Research Council (Project no: 104S507).

PP-827
Effects of *Angelica gigas* and its biologically active compounds on the gene expression profiles in SH-SY5Y human neuroblastoma cells as analyzed by high density oligoucleotide microarrays

Y. W. Choi[1], J. M. Kim[2], S. H. Sung[3] and Y. C. Kim[4]
[1]Dept. of Bioscience and biotechnology, Sejong University, Seoul, Korea, [2]Dept. of Bioscience and biotechnology, Sejong University, Seoul, Korea, [3]Institutue for Life Science, Elcom Science Co., Seoul, Korea, [4]College of Pharmacy, Seoul National University, Seoul, Korea. E-mail: nglee@sejong.ac.kr

Angelica gigas Nakai has been used in traditional Korean folk medicine. The extract of *Angelica gigas* and its major coumarin constituents have been reported to have an neuroprotective activity as well as antitumor, antiamnestic and antibacterial activities. In this study, we evaluated the neuroprotective effects of *Angelica gigas* in SH-SY5Y human neuroblastoma cells treated with a neurotoxin MPP+. We then examined differential gene expression profiles of SH-SY5Y cells in response to MPP+ and the

compounds using oligonucleotide microarrays that consisted of more than 20 000 human genes. SH-SY5Y cells were treated with the extract or compounds for 24 h and exposed to 1 mM MPP+ for 1 h or 24 h. The genes showing altered expression were confirmed by real time PCR analysis. The data obtained in this study should expand our knowledge on the MPP+-mediated neurotoxicity and provide molecular mechanisms of the neuroprotective activity by *Angelica gigas* and its major coumarin constituents.

PP-828
Genetically modified (GM) soybean content in Japanese food products

H. Hisashi, **H. Hisaya** and H. Hiroyuki
Department of Health Science Musashigaoka Collage Minami-Yoshimi 111-1, Yoshimi, Hiki, Saitama 355-0154 Japan. E-mail: ofhmyoga@musashigaoka.ac.jp

The herbicide tolerant GM soybean, Roundup Ready, which shows glyphosate resistant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) activity was developed in 1996 and has been distributed worldwide. Approximate 3.2 million tons of these soybeans are imported to Japan from USA in a year and processed into various traditional Japanese foods, such as tofu, natto, kinako etc. Therefore, soybeans are one of the most important crops supplying proteins and lipids. Most of the imported soybeans are distributed with IP handling but unintentional mixing of GM soybean to non-GM seems to be inevitable. Seventy-two soybean products commercially available in Japan were analyzed using PCR method. Soybean DNAs were extracted by the CTAB method and subjected to PCR analysis to amplify GM-specific 121 bp region designated RRS. Intrinsic soybean lectin 1 gene was used as a positive control. It was shown that GM-positive signals were detected in 89.7% of soybean products. The result of real-time PCR indicated that 0.05–0.1% of GM soybean was unintentionally mixed into non-GM soybean products. The mixing ratio seems rather lower than the permitted mixing level in Japan (up to 5%). However, these results provide significant meaning for Japanese. Consumers should pay much more attention to the unintentional mixing of GM soybean into foods since as much as 1 million tons of soybeans are consumed as foods each year. It is suggested that Japanese consume one thousand tons of GM soybeans each year.

PP-829
In vivo assessment of *Urtica urens* supplementation on rat xenobiotic metabolizing enzymes

A. Sen, H. H. Agus, M. Bayav and H. Sevim
Pamukkale University, Faculty of Arts & Sciences, Department of Biology, 20020 Denizli, Turkey. E-mail: sena@pamukkale.edu.tr

Interest in medical herbal products is tremendously increasing. Present study explores the in vivo effects of annual nettle (*Urtica urens*) on CYPs and GST. Nettles have a long history of use in the home as a herbal remedy. Male Wistar rats were treated with nettle seed extract, 200 mg/kg, i.p. daily, for four consecutive days. Aminopyrene N-demethylase (AND), aniline 4-hydroxylase (AH), benzyloxyresorufin O-deethylase (BROD), caffeine N-demethylase (CND), coumarin hydroxylase (CH), erythromycin N-demethylase (END), ethoxyresorufin O-deethylase (EROD), methoxyresorufin O-deethylase (MROD), N-nitrosodimethylamine N-demethylase (NDMA-ND), penthoxyresorufin O-deethylase (PROD), p-nitrophenylhydroxylase (PNPH) and glutathione S-transferase (GST) activities were determined in control and treated rats. Crude nettle seed oil showed the highest inhibition in CND (50%), followed by AND and NDMA-ND (32%) and the highest induction in END (95%) and AH (27%). Data of the presented study clearly suggest that herbal products containing nettle may have the potential to inhibit or induce the metabolism of certain co-administered drugs. As indicated by these *in vivo* data, nettle preparations contain constituents inhibiting or activating the activities of major drug metabolizing enzymes; interactions with drugs whose route of elimination is mainly via CYPs therefore possible. As a result, the obtained results should merit further investigations to clarify their clinical relevance.

PP-830
Omega-3 fatty acid and breast cancer: possible involvement of voltage-gated sodium channel

B. Isbilen[1], S. P. Fraser[2] and M. B. A. Djamgoz[2]
[1]Biochemistry Laboratory, Canakkale State Hospital, Canakkale, Turkey, [2]Division of Cell & Molecular Biology, Imperial College London, London, UK. E-mail: drisbilen@yahoo.com

Purpose: Docosahexaenoic acid (DHA) is a biologically significant member of omega-3 polyunsaturated fatty acid (ω-PUFA) family. The major source of DHA is marine food. There is a positive correlation between marine food intake and reduced incidence of various cancers. Several *in vivo* and *in vitro* studies have reported inhibitory effects of DHA, but the precise mechanism(s) underlying the anti-cancer effects of ω-3 PUFAs are not well known. A potentially novel mode of action is ion channels. The aims of the study were to determine the effects of DHA on the voltage-gated Na channel (VGSC) membrane current; mRNA, protein expression and in vitro migration.
Methods: The voltage-gated Na channel (VGSC) signalling was investigated in MDA-MB-231 cells by patch clamp recording. The contribution of VGSC activity to metastasis was evaluated by transwell migration assay. mRNA and protein expression were achieved using RT-PCR, immunocytochemistry and Western blot. Results: DHA reduced the VGSC currents and the migration of breast cancer cells. 0.5 and 5 μM doses of DHA were exhibited lower mRNA and protein expression of neonatal Nav1.5. Conclusions: DHA has a selective action upon the strongly metastatic MDA-MB-231 cells, consistently decreasing the VGSC signalling and its protein/gene expression levels. Also, DHA produced an anti-migratory effect, contributing VGSC activity. In conclusion, DHA is involved in metastasis by reducing migration via inhibition of VGSC expression and activity.

PP-831
Antioxidative action of royal jelly in yeast *Saccharomyces cerevisiae*

P. Jamnik[1], D. Goranovič[1] and P. Raspor[1]
[1]Food Science and Technology Department, Biotechnical Faculty, Ljubljana, Slovenia. E-mail: polona.jamnik@bf.uni-lj.si

Royal jelly is a bee product, secreted from the hypopharingeal and mandibular glands of nurse bees (Apis mellifera). There are many reports on pharmacological activities of royal jelly in experimental animals, but there a few about its antioxidative properties, especially tested *in vivo*. The aim of the work was to investigate the effect of royal jelly on intracellular oxidation and protein profile in the yeast Saccharomyces cerevisiae as a model organism. Yeast Saccharomyces cerevisiae was cultivated in YEPD medium with different concentrations of royal jelly like 1, 2 and 5 g/l. After incubation cells were centrifuged and then

intracellular oxidation was measured using oxidative sensitive probe 2'7'-dichlorofluorescein and protein profile of cell extract was analyzed by 2-D electrophoresis. Results showed that royal jelly decreased intracellular oxidation, specifically in concentration of 5 g/l and caused changes in protein expression. We showed that antioxidative role of royal jelly in the cell is not only related to scavenging of reactive oxygen species, but also to proteome changes indicating indirectly its antioxidative action.

PP-832
Highly acidic phytase from *Aspergillus niger* NCIM 563 under submerged fermentation

S. K. Soni and J. M. Khire
NCIM Resource Center, National Chemical Laboratory, Pune-411008. E-mail: suttan_7779@yahoo.com

Phytase is one of the important animal feed supplement enzyme as monogastric animals are unable to utilize phosphorus present in the feed as it is in the form of phytic acid. Thermotolerant fungus, *Aspergillus niger* NCIM 563, was found to produce novel, highly acidic extracellular phytase at 30 °C under submerged fermentation conditions. Maximum phytase activity (41.47 IU/ml at pH 2.5 and 10.71 IU/ml at pH 4.0) was obtained when dextrin was used as carbon source along with glucose and sodium nitrate as nitrogen source. Optimum pH and pH stability studies indicate possibility of two different phytases viz. active at pH 2.5 and 4.0. The enzyme showing pH optimum 2.5 was stable in the pH range 1.5 to 3.5 while other showing optimum pH 4.0 was stable in the wider pH range, 2.0 to 7.0. However both the enzymes show temperature optima at 60 °C only. Nearly 13 times increase in phytase activity (at pH 2.5 and 4.0) was observed when phosphate in the form of Di hydrogen potassium phosphate (4 mg %) was added in the fermentation medium. Preliminary biochemical characterization and zymograms of phytase produced during solid state and sub merged fermentation by *A. niger* NCIM 563 it is observed that both of these phytases are different. HPLC Analysis of complete phytate degradation reaction mixture showed myo-inositol as the main product. As shown in literature myo-inositol plays a major role in many cell signaling pathways.

PP-833
The effects of some vegetable oils on indomethacine-induced gastric damages and carrageenan-induced paw odema

F. Odabasoglu[1], M. Halici[1], H. Suleyman[2], Y. Bayir[1] and **A. Cakir**[3]
[1]Biochemistry Department, Ataturk University-Faculty Of Pharmacy, Erzurum, Turkey, [2]Pharmacology Department, Ataturk University-Faculty Of Medicine, Erzurum, Turkey, [3]Chemistry Department, Ataturk University-Kazim Karabekir Education Faculty, Erzurum, Turkey. E-mail: fodabasoglu@yahoo.com

Non Steroidal Anti Inflammatory Drugs (NSAIDs) are widely used in the treatment of fever, pain and inflammation. Indomethacine (IND) is one of the potent NSAIDs. However, IND and other NSAIDs have some side effects, especially on the gastrointestinal tracts. Vegetable oils, extracted from a variety of fruits, seeds, and nuts are commonly used for human consumption but are also used in animal feed, for medicinal purposes, and for certain technical applications. In this study, the gastroprotective effects of olive oil, sunflower oil and corn oil on IND-induced gastric damages in rats was studied. Our results show that all of the oils significantly reduced the gastric damages caused by IND ($P < 0.05$). The oils and IND were also tested with Carrageenan-induced paw edema to determine their effects on inflammation process in rats. The results show that sunflower and corn oils have moderate anti-inflammatory activity as compared with control group. However, olive oil did not exhibit a significant anti-inflammatory activity. As expected, IND strongly reduced the paw edema. To determine the effects of these oils on the anti-inflammatory activity of IND, these vegetable oils were also individually administrated with IND. It is interesting to find that these oils did not exhibit any reduce on the anti-inflammatory activity of IND. The present results indicated that IND shall not have any side effects on the gastrointestinal tract when it is used together with vegetable oils.

PP-834
Hydroxyl radical scavenging antioxidant activity assay using a modified CUPRAC method

B. Bektaşoğlu, S. E. Çelik, M. Özyürek, K. Güçlü and R. Apak
Department of Chemistry, Istanbul University, Istanbul, Turkey. E-mail: burcubek@hotmail.com

Reactive oxygen species (ROS) such as superoxide anion, hydroxyl (*OH), peroxyl, and alkoxyl radicals may attack biological macromolecules giving rise to oxidative stress-originated diseases. Since *OH is very short-lived, secondary products resulting from *OH attack to various probes are measured. Although the measurement of aromatic hydroxylation is more specific than the low-yield TBARS test, it requires sophisticated instrumentation. As a more convenient and less costly alternative, we used p-aminobenzoate, 2,4- and 3,5-dimethoxybenzoate probes for detecting hydroxyl radicals generated from an equivalent mixture of Fe (II) + EDTA with hydrogen peroxide. The produced hydroxyl radicals attacked both the probe and the water-soluble antioxidants in 37 °C-incubated solutions for 2 h. The CUPRAC (i.e., our original method for total antioxidant capacity assay) absorbance of the ethylacetate extract due to the probe decreased in the presence of *OH scavengers, the difference being proportional to the scavenging ability of the tested compound. The second-order rate constants of the scavengers were determined by competition kinetics. The 3,5-dimethoxybenzoate was the best probe in terms of linearity and sensitivity. Dithionite, thiourea, and formate were shown by the modified CUPRAC to be more effective scavengers than mannitol, dimethylsulfoxide, and ethanol, as in the TBARS assay.

PP-835
Mutagenic and carcinogenic heterocyclic aromatic amines

F. Oz and M. Kaya
Food Engineering Department, Agriculture Faculty, Ataturk University, Erzurum, Turkey. E-mail: fatihoz@atauni.edu.tr

Heterocyclic aromatic amines (HAAs) are formed during cooking of proteinaceous foods such as meat and fish. They are, therefore, widely consumed by humans. On the basis of results from long-term animal studies, several of HAAs are considered mutagenic and carcinogenic. Compared to other known food mutagens such as aflatoxin B1 and benzo[a]pyrene HAA's have been shown to be 100 and 2000 folds more mutagenic, respectively. The International Agency for Research and Cancer, IARC, judged the heterocyclic amines MeIQ, MeIQx and PhIP to be possible human carcinogens and IQ as probably carcinogenic. To date, more than 20 different heterocyclic aromatic amines have been identified at ng/g level in cooked foods. They are formed from the precursors creatine, amino acids and reducing sugar in complex reactions at high temperatures. The competent

authorities in most Western countries recommend minimizing HAAs occurrence. Thus, it is important to learn more about the formation and inhibition of these compounds in cooked foods. Their occurrence, mutagenicity, carcinogenicity, exposure and minimizing ways are briefly presented in this review.

PP-836
Kinetics and thermostability of catalase activity in *Helianthus tuberosus* L. tubers

M.-Z. Keyhani
Islamic Azad University, Shahr-e-Rey, and Laboratory for Life Sciences, Tehran, Iran. E-mail: keyhanius2002@yahoo.com

Helianthus tuberosus L. (Jerusalem artichoke) is grown for its edible tuberous roots as well as its pretty yellow flowers. The high insulin content of the tubers gives them beneficial properties such as lowering the blood cholesterol and sugar levels, reducing low density lipoproteins and triglycerides and helping in the prevention of heart diseases. Investigations on the biochemical processes taking place in the tubers have been very limited so far. In this work, the presence of catalase, an important antioxidant defense enzyme that catalyzes H2O2 dismutation, was investigated in *Helianthus tuberosus* L. tubers extract prepared by homogenization in phosphate buffer 0.01 M, pH 7, and centrifugation 10 min at 3 000 g and 30 min at 35 000 g. Catalase activity, measured in the presence of H2O2 by following the decrease in absorption at 240 nm, was detected in the extract. The activity was optimum at pH 7.0, with Km = 10 mM, Vmax = 3.6 mM/min/mg prot and catalytic efficiency = 0.36/min/mg prot. Substrate inhibition (50%) was observed at 100 mM H2O2. KCN and azide inhibited the catalase activity with IC50 values of 0.02 and 0.002 mM, respectively. Thermostability studies showed a maximum catalase activity after preincubation at 30 °C, 50% decrease after preincubation at 50 °C, 92% and 100% decreases after preincubation respectively at 65 °C and 70 °C. Arrhenius plot showed transition temperatures of 40 °C and 50 °C.

PP-837
In vitro investigation of some plant growth regulators on antioxidant enzyme activities

S. Beyaztas, N. Gencer, O. Ozensoy and O. Arslan
Balikesir University Science and Art Faculty Department of Chemistry Biochemistry division 10100 Balikesir/Turkey.
E-mail: beyaztas@balikesir.edu.tr

In the development technology, growth population is one of the main problem to find new sources for nutrition. Using plant growth regulators was one of the solution on this problem. In our country and also in the world, there are so many studies about plant growth regulators (PGR) on human health however there is no evidence about the effects of antioxidant enzyme activities such catalase (CAT), polyphenoloxidase (PPO), superoxidedismutase (SOD) glutation peroxidase (GP) and glutation reductase (GR) on PGRs, thus our goal is to dedicate the antioxidant activity relations with PGRs in this present study. According to the results, PPO and CAT is inhibited with gibberellic acid and indole-3 butiric acid.

PP-838
Effect of conjugated linoleic acid (CLA) supplementation with exercise on body composition and blood lipid profile

S. B. Bulut[1], **R. C. Colak**[1], H. T. Turnagol[1] and S. B. Baseskioglu[2]
[1]School of Sport Sciences and Technology, Hacettepe University, Ankara Turkey, [2]Department of Nutrition and Dietetics, Hacettepe University, Ankara, Turkey. E-mail: slmbulut@hacettepe.edu.tr

The purpose of this study is to investigate effects of Conjugated Linoleic Acid (CLA) supplementation with standardized exercise program on body composition, blood lipid profile, plasma glucose and insulin concentration. In this study 18 male volunteer attended who was sedentary, age 23.8 ± 3.5 years, height 175.1 ± 4.5 cm, weight 82.8 ± 8.4 kg and BMI < 30. This study was performed double blind placebo controlled design and 18 male volunteer was divided two groups (CLA + Exercise and Placebo + Exercise). CLA + Exercise and Placebo + Exercise groups took daily 3 g CLA or 3 g placebo during 30 days. Both groups did exercise with bicycle ergometer (Monarg 834E) 3 times a week during one month and each season lasted 30–40 min. Intensity of exercise was performed 50% peak VO2R. Test subjects food intake, peak VO2, anthropometric properties, serum lipid profile, glucose and insulin concentration measured before and after the trial. After the study both groups waist and hip girth, insulin, insulin resistance and Placebo + Exercise group serum glucose values were decreased significantly ($P < 0.05$). Consequently, extent of this study no important effect of CLA is observed on blood lipid profile, body composition, glucose and insulin values in addition to exercise. However, studies in the future which exercise intensity, volume, frequency and duration is adjusted differently, could be shed light on the subject more clearly.

Acknowledgment: Supported by: TUBİTAK (Extent of the 104S499 (SBAG-2964) numbered project).

Other

PP-839
Changes of calcium, phosphorus and trace elements iron, copper, magnesium concentrations in amniotic fluid with increasing gestational age

A. Kale[1], E. Kale[2], S. Batum[2], N. Akdeniz[1] and N. Canoruç[2]
[1]Department of Obstetrics and Gynecology, [2]Department of Clinical Biochemistry, Dicle University School of Medicine, Diyarbakir, Turkey. E-mail: drakale@dicle.edu.tr

Objective: To investigate amniotic fluid changes of calcium, phosphorus, iron, copper and magnesium levels in pregnancies between 16 and 19 weeks of gestation.

Materials and methods: We studied 160 normal consecutive singleton pregnancies presenting between January 2004 and June 2005, all of whom underwent amniocentesis for fetal karyotype from 16 to 19 weeks of gestation (mean 17.4 weeks). The data were divided into four gestational age groups: (1) 40 at 16 weeks, (2) 40 at 17 weeks, (3) 40 at 18 weeks, and (4) 40 at 19 weeks of gestation). Serum Copper (Cu) levels were determined by atomic absorption spectrophotometer. Serum calcium (Ca), phosphorus, magnesium (Mg), iron levels were measured using clinical chemistry kits. Statistical analyses were performed by using One-Way ANOVA (*Post hoc* Bonferroni) test. A value of $P < 0.05$ was considered statistically significant.

Results: Amniotic fluid calcium, phosphorus, iron, copper increased progressively from 16 to 19 weeks of gestation

($P < 0.001$). Amniotic fluid magnesium levels decreased progressively from 16 weeks to 19 weeks of gestation ($P < 0.001$).
Conclusion: Calcium, phosphorus, iron, copper levels in amniotic fluid increased progressively with advancing gestational age which suggest fetus needs more elements as gestation week increased. Concentrations of magnesium levels decreased progressively with advancing gestational age, which suggest the need of magnesium is more in early fetal life.

PP-840
The correlation of thyroid hormone levels and gestational weeks in amniotic fluid

N. Canoruç[1], E. Kale[1], A. Kale[2], N. Akdeniz[2] and A. Yalınkaya[2]
[1]Department of Clinical Biochemistry, [2]Dept Obstetrics and Gynecology, Dicle University School of Medicine, Diyarbakir, Turkey. E-mail: drakale@dicle.edu.tr

Objective: The purpose of this study was to determine thyroid hormone levels of amniotic fluid and correlate with gestational ages.
Method: One hundred and twenty five pregnant women underwent amniocentesis procedure for prenatal diagnosis were included in study between May 2004 and May 2005. Thyroid hormone levels were analyzed with using Roche E170 Modular analytics (Hitachi, Japan) system. Statistical analyses were performed by using One-Way ANOVA test. A value of $P < 0.05$ was considered statistically significant.
Results: The mean age of patients was 34.5 ± 5.6. The mean gestational age of patients who underwent amniocentesis was 17.88 ± 1.58. Karyotype analysis of all patients was normal. Amniotic fluid levels of total and free T4 increased progressively with gestational age ($P < 0.001$). Although total T3, free T3 and TSH levels did not increase with gestational age ($P > 0.05$).
Conclusion: The levels of thyroxine (T4) hormone in amniotic fluid was higher than T3 and TSH hormones. The need of thyroxine (T4) hormone increased with gestational age.

PP-841
Molecular and clinical study of familial FSGS - hematuria in Cyprus and Greece

C. Voskarides[1], V. Neocleous[2], I. Zouvani[3], L. Damianou[4], S. Christodoulidou[4], V. Hadjiconstantinou[4], K. Kyriakou[5], K. Ioannou[6], C. Patsias[7], A. Pierides[6] and C. Constantinou Deltas[1]
[1]Biological Sciences, University of Cyprus, Lefkosia, Cyprus, [2]Molecular Genetics C, The Cyprus Institute of Neurology and Genetics, Lefkosia, Cyprus, [3]Department of Histopathology, Lefkosia General Hospital, Lefkosia, Cyprus, [4]Department of Nephrology, Evangelismos Hospital, Athens, Greece, [5]Department of El.Microscopy, The Cyprus Institute of Neurology and Genetics, Lefkosia, Cyprus, [6]Department of Nephrology, Lefkosia General Hospital, Lefkosia, Cyprus, [7]Department of Nephrology, Larnaca General Hospital, Lefkosia, Cyprus. E-mail: kvoskar@cing.ac.cy

Focal Segmental Glomerulosclerosis (FSGS) is a histopathologic phenotype, showing destruction of podocytes in the glomerulus. We located 10 multigeneration families in Cyprus and one in Greece, all with autosomal dominant inheritance. Mainly, the symptoms of the patients are FSGS, hematuria and in some cases proteinuria. We performed linkage analysis at chromosome regions published to be responsible for primary or secondary forms of FSGS: 19q13 (ACTN4 gene), 11q22 (TRPC6 gene), 6p12 (CD2AP gene) are responsible for dominant FSGS and 2q36 (COL4A3, COL4A4 genes) responsible for a secondary form of FSGS. Our data provide adequate evidence that at least eight families link to 2q36 and 3 to 19q13. Two of them show weak positive linkage to both regions. We estimate that the maximum total lod score will exceed 3.3 thus suggesting COL4A3 and COL4A4, as the putative mutated genes. Mutations in these genes are associated with autosomal recessive and autosomal dominant Alport syndrome and dominant benign hematuria. In the three families with suggestive linkage to 19q13 we sequenced the cDNA of ACTN4 gene (plus 173 bp of the promoter), from affected and healthy members. We did not identify any mutations. We also did not identify any mutations in the exons of NEPH3 and WTIP genes, important podocyte genes in the 19q13 region. It is probable that our approach missed the mutations, or that the responsible gene is a different one in the region. The likelihood of false linkage cannot be excluded.

PP-842
Elevated amniotic fluid amino acid levels in fetuses with gastroschisis

A. Kaplan[2], **A. Kale**[1], E. Kale[2], N. Akdeniz[1] and N. Canoruc[2]
[1]Department of Obstetrics and Gynecology, [2]Department of Clinical Biochemistry, Dicle University School of Medicine, Diyarbakir, Turkey. E-mail: drakale@dicle.edu.tr

Objective: To measure maternal plasma and amniotic fluid amino acid concentrations in pregnant women diagnosed antenatally as having fetuses with a gastroschisis in the second trimester.
Methods: Twenty-one pregnant women who had fetuses with a gastroschisis detected on ultrasonography (gastroschisis group) in the second trimester and 32 women who had abnormal triple screens indicating an increased risk for Down syndrome but had healthy fetuses (control group) were enrolled in the study. Amniotic fluid was obtained by amniocentesis, and maternal plasma samples were taken simultaneously.
Results: The mean levels of essential amino acids (Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, Valine) and non-essential amino acids of alanine, glycine, proline and tyrosine levels in amniotic fluid were found to be significantly higher in fetuses with gastroschisis than control group ($P < 0.05$). Although a significant positive correlations between maternal plasma and amniotic fluid concentrations of alanine, cysteine, glutamine, glycine, ornithine, proline, tyrosine, and essential amino acids were found only in gastroschisis group ($P < 0.05$).
Conclusion: We found significantly higher amino acid levels in amniotic fluid of fetuses with a gastroschisis defect than healthy fetuses suggesting that there is malabsorption of amino acids or leakage of amino acids into amniotic fluid from the fetus.

PP-843
Metabolizm of proline in haricot beetlefly *Acanthoscelides obtectus* (Say)

A. Aghajanyan, A. Aghajanyan, S. Chubaryan, L. Tumanyan, M. Martirosyan and L. Ananyan
Department of Biochemistry, Yerevan State University, Yerevan, Armenia. E-mail: anush@freenet.am

Low activity of proline biosynthesis enzymes with cofactor NADF+ in sediment, received during fractionation of beeties homogenate by 30% salted ammonium sulfate is discovered. Intensification of these enzymes activity in supernatant is observed more than four times. It is explained by presence all enzymes of penthoso-phosphate cycle, which is realizing synthesis of reduced nucleotides as NADFH as cofactor for pyrroline-5-carboxylate reductase (P5CR). It has been discovered that the

activity of proline catabolism enzymes (PO, P5CD) considerably reduces by influence of citrate and ATF in homogenates of larvas and beetles of haricot beetlefly. This fact is original exception, because in other objects (pea seeds, organs of common carp), where activity of enzymes of proline catabolism is not so high, citrate and ATF increase the activity of PO and P5CD 5-10 times. Impression is created that indicated compounds balance metabolism of proline, when degradation of proline intensively occurs in organism. High stimulation of the activity of proline degradation enzymes by arginine and citruline has been observed with both cofactor NAD+ and NADF+. This fact will be able to use for activating PO and P5CD for their preparative reception from insects.

PP-844
Cells secreting anti-CEA antibody molecules: 8B6 and 8E1

E. Derebay, F. Senturk Akfirat, T. Yildirim and I. Y. Sezen
Gebze Institute of Technology, Muallimkoy Campus, Faculty of Science, Department of Biology, Kocaeli TÜRKİYE. E-mail: ederebay@yahoo.com, senturk@gyte.edu.tr, yildirimt@gyte.edu.tr, iysezen@gyte.edu.tr

Carcinoembryonic antigen (CEA) is a widely used tumor marker for colorectal cancers. As in colorectal cancers, serum CEA levels may also be elevated in carcinoma of the breast, lung, stomach, pancreas, bladder, thyroid, liver and some benign diseases. Currently, anti-CEA monoclonal antibodies, alone or in combination with other tumor markers, are effectively being used in the diagnosis of colorectal cancers. In this study, antibody levels in the sera of BALB/c mice immunized with CEA were detected at certain time intervals. The mouse giving the highest antibody activity was used for the fusion process. After the fusion, hybrid cells producing specific antibodies were determined by indirect ELISA. The clones showing positive reactions were subcloned by using limiting dilution procedure. The monospecificity of the monoclonal antibodies obtained by this means was determined by performing cross ELISA activity test using different proteins. Hybrids cells (8B6 and 8E1) producing high specific monoclonal antibody against CEA were obtained. It was then determined that immunoglobulin isotypes of these hybrids cells were IgM. Supernatants of hybrid cells produced *in vitro* in large quantities in medium with or without serum were purified by dialysing against distilled water.

PP-845
Patterns of induction of transcription factors C-Fos and ZENK in avian brain in different behaviors

K. Tokarev, A. Tiunova and K. Anokhin
Department of Systemogenesis, P.K.Anokhin Research Institute of Normal Physiology Mokhovaya 11/4, 125009 Moscow, Russia. E-mail: kirill@bioeng.ru

It is known that learning induces a cascade of intracellular events which results in immediate-early gene (IEG) expression. C-Fos and product of another IEG, ZENK, are essential factors on the initial phase of memory consolidation being transcription factors for many later genes, products of which function in morphological, in particular, synaptic, remodeling of neurons on later phases of memory formation. Due to the different DNA-binding domains of ZENK and c-Fos, they can perform different functions. We used these transcription factors as molecular markers pointing to the cells involved in long-term plasticity in young chickens and adult zebra finches during regular daily activity, food discrimination learning and vocalization (only in latter). These transcription factors were sufficiently induced in the studied structures by two latter behaviors. Our data show that (1) patterns of expression of ZENK are different from those of c-Fos during both food discrimination learning and production/perception of the conspecific song; (2) level and distribution of induced expression of each of these genes are different in food discrimination learning and production/perception of the conspecific song, i.e. depend on the type of behavior; (3) neuronal plasticity in the brain regions involved in two different behaviors may occur either in different neuron populations or in the same cells but by means of different molecular mechanisms, for instance, using different transcription factors.

PP-846
Regulation of androgen receptor translocation in the ovary and fallopian tubes

R. Shao, K. Ljungstrom, B. Weijdegard and H. Billig
Section of Endocrinology, Department of Physiology and Pharmacology, The Sahlgrenska Academy, Göteborg University, SE-40530 Göteborg, Sweden. E-mail: ruijin.shao@fysiologi.gu.se

Androgen receptor (AR) plays an important role in a variety of biological processes including reproductive functions. To date, the hormonal regulatory profile and sub-localization of the AR protein in mouse ovary and fallopian tubes, as well as human fallopian tubes during the menstrual cycle have not been closely elucidated. In the present study, we demonstrated that eCG treatment significantly increased in time-dependent AR expression in mouse ovarian granulosa cells and fallopian tubes, whereas an additional hCG treatment caused a significant decrease in both tissues. In mouse ovarian granulosa cells, AR was expressed in both cytoplasm and nucleus regardless eCG treatment. However, in the epithelial cells of fallopian tubes, expression of AR protein was increased in cell nuclei in parallel with decreases in cytosol after eCG treatment. We also demonstrated that expression of AR protein was high in the follicular phase and remained at low levels in the luteal phase in human fallopian tubes. Moreover, immunostaining of AR was rarely detected in the epithelial cell nuclei of human fallopian tubes. Next, treatment with Flutamide increased AR expression in both ovarian granulosa cells and fallopian tubes. Interestingly, Flutamide had no reversible effect of hCG on AR protein expression but reduced ovulation in mice. Our findings indicate (1) translocation of AR protein may be tissue and species-specific; (2) AR plays important roles during follicular development and ovulation.

PP-847
A novel 2D electrophoresis technique for the identification of intrinsically unstructured proteins

V. Csizmók[1], E. Szőllősi[1], É. Klement[2], K. Medzihradszky[2], P. Friedrich[1] and P. Tompa[1]
[1]*Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest, Hungary,* [2]*Mass Spectrometry Facility at the Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary. E-mail: csv@enzim.hu*

Intrinsically unstructured proteins (IUPs) and protein domains exist in a highly flexible conformational state, thus they defy the dogma that protein function depends on a well-defined, three-dimensional structure. Although these proteins are rather frequent in the proteome, only a few of them have so far been identified. We developed a novel 2D electrophoresis technique

that can separate IUPs from globular proteins by running heat-treated extracts on native and denaturing gels in the first and second dimensions, respectively. The underlying idea is that IUPs resist both high temperature and urea-denaturation and run into the diagonal of the second gel, whereas globular proteins either precipitate upon heat-treatment or get denatured in the second gel and run off-diagonal. Our results demonstrate that this conceived set-up can lead to the identification of novel IUPs. First, we demonstrated the feasibility of the concept by running 10 IUPs and 4 globular proteins, which resulted in the separation of the two classes on the gel as expected. Next, we run *E. coli* and *S. cerevisiae* extracts and spots at or near the diagonal were excised for identification by mass spectrometry. Several IUPs have been identified, some already known, but several not yet described as such. For these, the lack of an ordered structure has been verified by web-based disorder predictors (PONDR), by the hydrophobicity-charge plot (Uversky plot) and by physico-chemical characterisation.

PP-848
Inhibition of diphenolase activity of saffron (*Crocus sativus* L.) polyphenol oxidase by kojic acid

Sh. Saeidian[1], E. Keyhani[1,2] and J. Keyhani[2]
[1]*Institute of Biochemistry and Biophysics, University of Tehran, 13145 Tehran, Iran,* [2]*Laboratory for Life Sciences, 19979 Tehran, Iran. E-mail: saeedyan@ibb.ut.ac.ir*

Polyphenol oxidase (EC 1.14.18.1)(PPO), a copper-containing enzyme, can be found throughout the phylogenetic tree. PPO is the rate-limiting enzyme in the melanin biosynthesis pathway. This enzyme hydroxylates monophenols and oxidizes diphenols to o-quinone. Kojic acid inhibits the activity of PPO when L-DOPA is oxidized by the enzyme diphenolase activity. PPO activity was determined, using L-DOPA as substrate, in extracts prepared from saffron corm cultivated in liquid medium for 0, 3, 6, 10, 20 and 30 days. With increasing concentrations of kojic acid, the residual PPO activity in extracts decreased rapidly. The IC50, determined for the various cultivation periods (0, 3, 6, 10, 20 and 30 days) showed a progressive decrease as a function of increased cultivation period. IC50 was 0.7, 0.66, 0.6, 0.2, 0.15 and 0.1 mM for extracts from 0, 3, 6, 10, 20 and 30 days, respectively. Moreover, kojic acid was a non-competitive inhibitor of saffron PPO, throughout the development. The equilibrium constants for inhibitor binding in extracts from different cultivation periods (0, 3, 6, 10, 20 and 30 days), Ki were 0.11, 0.32, 0.24, 0.13, 0.14 and 0.05 mM, respectively for 0.16 mM kojic acid. Non-denaturing gel electrophoresis of extracts, followed by activity staining for PPO showed at least two isoenzymes. Overall data suggest that various isoenzymes were differentially expressed during development in *Crocus sativus* L. corm.

PP-849
Inhibition of choline oxidase by malachite green and phenoxazine dyes

O. Tacal and I. Ozer
Department of Biochemistry, School of Pharmacy, Hacettepe University, 06100 Ankara, Turkey. E-mail: tacal@hacettepe.edu.tr

Choline oxidase (CHO; E.C. 1.1.3.17) is an FAD-containing enzyme that catalyzes the oxidation of choline to glycine-betaine, with betaine-aldehyde as intermediate and molecular oxygen as primary electron acceptor. The development of biosensors for detection of choline and choline esters such as acetylcholine in serological samples and foods renders this enzyme of clinical and industrial interest. In this study, cationic triarylmethane and phenoxazine dyes (malachite green (MG), meldola blue (MB) and nile blue (NB)) were tested as potential ligand sand inhibitors of CHO. The enzymatic reaction was studied at 25 °C in 50 mM MOPS buffer, pH 7, using 0.1–0.4 mM choline as substrate, 0–40 μM dye and horse radish peroxidase as the reporter enzyme. The progress of choline (o-dianisidine) oxidation was monitored spectrophotometrically at 500 nm. All three dyes were found to act as reversible inhibitors of CHO. The Ki values for MG, MB and NB, based on a simplified rapid equilibrium inhibitory model, were 23 ± 0.4, 29 ± 0.5, and 23 ± 3 μM, respectively. Due to their cationic nature, MG, MB and NB most likely act by competing with choline for the active site. However, diagnostic plots of the data based on the rapid equilibrium model yielded patterns bordering between competitive and noncompetitive inhibition. This and further properties of the system indicate that the dyes may be effective at multiple points along the steady-state catalytic scheme for CHO.

PP-850
Microalbuminuria in children with renal scar as a consequence of vesicoureteral reflux

J. Basic[1], E. Golubovic[2], P. Miljkovic[2], G. Bjelakovic[1] and V. Milosevic[3]
[1]*Institute of Biochemistry, Faculty of Medicine, Nis, Serbia and Montenegro,* [2]*Clinic for Pediatrics, Clinical Centre, Nis,* [3]*Faculty of Medicine, Nis, Serbia and Montenegro.*
E-mail: jelenabasic@yahoo.com

Dysfunction of ureterovesical junction causes vesicoureteral reflux (VUR) of infected urine from the bladder to ureters and pyelocalices that may cause scarring in susceptible kidneys, with the potential for compromise of renal function. The aim of the study was to evaluate the eventual influence of VUR and consecutive renal scar on glomerular damage using microalbuminuria as a parameter. Children with VUR grade I-V detected by voiding cystourethrography were investigated. Patients were separated into two groups. The first group included children with VUR. The second group was consisted of children with VUR and consecutive renal scar detected by DMSA scitigraphy. Control group was consisted of healthy children. Microalbuminuria was examined in samples of morning urine specimens using Microalbumin/Creatinine reagent kit. Serum urea, creatinine levels and creatinine clearance (CCR) were measured as markers of renal function. The mean value of microalbuminuria in children with renal scar showed increase vs. control and decrease vs. VUR, but not statistically significant. CCR in the first and second group was significantly decreased in comparison to a group of healthy children. We discussed increased microalbuminuria and decreased CCR in children with renal scar compared to VUR without scar, but not statistically significant, as a possible consequence of faster developing glomerulosclerosis and consecutive hyper filtration in the presence of scar that will lead to reflux nephropathy soon.

PP-851
A comparative study of the expression of a novel human RNase in prokaryotic and eukaryotic systems

A. M. Economopoulou, E. G. Fragoulis and D. C. Sideris
Department of Biochemistry and Molecular Biology, University of Athens, Athens, Greece. E-mail: meconom@biol.uoa.gr

A novel specific RNase has been previously isolated from the insect C. capitata [1]. This enzyme displays no homology with other proteins, whereas a large group of sequences, with

unknown biological function, display high homology to Cc RNase. The human homologue consists of 98 aa and is found to be expressed in a variety of normal and cancer tissues. Efforts to express the human RNase into a series of prokaryotic vectors, resulted either in lack of transformants or severe toxic effects following IPTG induction. Successful expression was achieved only after transformation of the recombinant pSCREEN vector into BL21 (DE3) plysE host cells. Although the use of this system resulted in large amounts of insoluble fusion protein, the mature recombinant RNase exhibited very low enzymatic activity. On the other hand the human RNase was expressed by the yeast P.pastoris expression system. The cDNA was subcloned into pPICZa A vector and was integrated into the KM71 host strain genome. The obtained transformants were selected on the basis of high antibiotic resistance and the integration of the human RNase cDNA was verified by PCR. Expression of the RNase was achieved after methanol induction and the enzyme was secreted in the culture medium. The use of this system yields relatively low amounts of highly active enzyme. This finding proved to be an important tool for the subsequent detailed characterization of this novel RNase.

Reference:

1. Rampias TN et al (2003) NAR. 31: 3092–3100.

PP-852

The respiratory Complex I of *Zymomonas* as a model to study mitochondrial based diseases

E. Vandera[1], G. Rendesi[1], A. Varsaki[1], G. Stoian[2], **C. Drainas**[1] and A. Perisynakis[1]

[1]*Chemistry Department, University of Ioannina, Ioannina, Greece,* [2]*Biology Department, University of Bucharest, Bucharest, Romania. E-mail: elpiniki_vandera@yahoo.gr*

The inner membrane of mitochondria and the cytoplasmic membrane of bacteria, contain a proton-pumping NAD(P)H oxidase (NADH:ubiquinone oxidoreductase), also called respiratory complex I. Complex I is a multisubunit complex catalyzing the electron transfer from NADH to quinone. Many human mitochondrial diseases involve structural and functional defects at the level of this enzyme complex, such as Leber's hereditary optic neuropathy (LHON) and the drug-induced Parkinsonism in rodents and humans. The Gram-negative α-proteobacterium Zymomonas mobilis can be used as an ideal model for the study of complex I, because of its similarities with mitochondria. This work focused on the detection of NAD(P)H-oxidase activity in *Z. mobilis* and cloning of the corresponding genes. The NADH-oxidase showed no differences under any conditions, whereas the NADPH-oxidase appeared more active in cells growing anaerobically. The genome of Z. mobilis apparently contains only six putative genes, as compared to the 14 components of complex I in other bacteria. Two of them encoding products with NAD(P)H-oxidase activity were amplified, cloned and expressed in *E. coli*. It appeared that the recombinant NADH-oxidase, encoded by the rnfE homologue, is more active as a single component as compared with the NADPH-oxidase. On the other hand the product of the rnfG homologue appears to solubilize after treatment with a chaotropic reagent, indicating its tighter association with membranes.

PP-853

Prenatal diagnosis of common aneuploidies by QF-PCR analysis in the Republic of Macedonia

S. Talaganova[1], P. Dimcev[2], E. Sukarova-Stefanovska[1], G. Efremov[1] and D. Plaseska-Karanfilska[1]

[1]*Research Center for Genetic Engineering and Biotechnology, Macedonian Academy of Sciences and Arts, Skopje, R. Macedonia,* [2]*General Hospital "Re-Medika", Skopje, R. Macedonia. E-mail: sonjatal@freemail.com.mk*

Prenatal diagnosis is offered to pregnant women with an increased risk for fetal chromosomal abnormalities. The rapid prenatal diagnosis of common aneuploidies of chromosomes 13, 18, 21, X and Y has been successfully achieved by quantitative fluorescent (QF) PCR amplification of selected small tandem repeat (STR) markers. The prenatal diagnosis was performed on genomic DNA isolated from fetal cells collected by amniocentesis and chorionic villus from 480 pregnant women at risk of bearing a child with chromosomal aneuploidy. All samples were analyzed by three multiplex PCR assays, amplifying four STR markers on chromosome 21 (D21S1435, D21S1446, D21S1411 and D21S1414), four markers on chromosome 18 (D18S535, D18S1367, D18S978 and D18S386), three STRs on chromosome 13 (D13S631, D13S258 and D13S1817) and two markers specific for chromosome X (DXS6803 and XHPRT) together with the amelogenin locus (AMXY). When needed, additional markers on chromosomes 21 (D21S11, D21S1412, D21S1441 and D21S1256), 18 (D18S51), 13 (D13S634, D13S317 and D13S762) and X (DXS6809, X-22 and CAG repeat in androgen receptor gene) were used. Using this approach, we have detected ten fetuses with trisomy 21, six with trisomy 18, four with trisomy 13 and two fetus with Turner's syndrome. In conclusion, QF-PCR is an accurate and reliable technique for rapid prenatal diagnosis of the most common chromosomal aneuploidies.

PP-854

The expression of AMP-deaminase genes in pathological human tissues (liver, thymus and muscles)

M. Szydowska[1], A. Roszkowska[2], M. apiński[3] and K. Kaletha[1]

[1]*Department of Biochemistry, Medical University, D*ębinki 1, 80-211 Gdansk, Poland [2]*Department of Pharmaceutical Biochemistry, Medical University, D*ębinki 1, 80-211 Gdansk, Poland [3]*Department of Thoracic Surgery, Medical University, Dębinki 7, 80-211 Gdansk, Poland. E-mail: mgazda@amg.gda.pl*

Background: AMP-deaminase (EC 3.5.4.6) is an enzyme of nucleotide breakdown involved in regulation of energetic metabolism in mammalian cells. The enzyme is coded by a family of three independent genes (AMPD1, AMPD2 and AMPD3), synthesizing three different isozymes. In mammalian tissues reaction catalysed by AMP-deaminase constitutes a rate–limiting step in adenine nucleotide catabolism. In mammalian neoplastic tissues, adenine nucleotide catabolism is a subject of many modifications influencing also expression of genes synthesizing important regulatory enzymes of the pathway. Aims: The experimental studies presented here illustrate expression level of AMPD genes in human liver neoplasm tumor (Hepatocellular carcinoma - HCC), human thymus neoplasm (Thymoma) as well as in muscles from patients with Myasthenia gravis.

Methods: RT-PCR and Western blotting methods were used for determining of the goal mentioned above.

Results and conclusion: Expression level of AMPD genes in tumorous fragment (HCC and Thymoma) of liver and thymus do not differ substantially from that found in the normal,

not-affected fragments of these tissues. In each case expression of AMPD2 was prevailing. In skeletal muscle of patients with Myasthenia gravis, expression of AMPD1 gene was significantly prevailing. The experimental results presented here seem to exclude the increased expression of AMPD genes as the cause of augmented activity of AMP-deaminase in the tumor.

PP-855
Cystinuria in south east European countries: mutations in SLC3A1 and SLC7A9 genes

K. Popovska-Jankovic[1], **V. Tasic**[2], R. Bogdanovic[3], P. Miljkovic[4], A. Soylu[5], I. Akil[6], E. Baskin[7], R. Topaloglu[8], G. Efremov[1] and D. Plaseska-Karanfilska[1]

[1]Research Center for Genetic Engineering and Biotechnology, Macedonian Academy of Sciences and Arts, Skopje, Republic of Macedonia, [2]Faculty of Medicine, Pediatric Clinic, Skopje, Republic of Macedonia, [3]Institute for Mother and Child, Belgrade, Serbia and Montenegro, [4]Faculty of Medicine, Pediatric Clinic, Nis, Serbia and Montenegro, [5]Department of Pediatrics, Medical Faculty, Dokuz Eylul University, Izmir, Turkey, [6]Department of Pediatric Nephrology, Celal Bayar University, Manisa, Turkey, [7]Faculty of Medicine, Baskent University, Ankara, Turkey, [8]Faculty of Medicine, Hacettepe University, Ankara, Turkey.
E-mail: katepop@freemail.com.mk

Cystinuria is an autosomal recessive disease caused by defective transport of cystine and dibasic amino acids in the proximal renal tubule and small intestine. Mutations in SLC3A1 and SLC7A9, which encoded amino acid transporter rBAT/b0, +AT, are responsible for cystinuria type I and non-type I respectively. The aim of this study was to determine the molecular basis of cystinuria among patients from South Eastern Europe. A total of 36 cystinuria families from several countries were included in this study (R. Macedonia $n = 11$; Serbia and Montenegro $n = 12$; Turkey $n = 9$; Croatia $n = 2$; Bulgaria $n = 1$; and Slovenia $n = 1$). The methodology included single stranded conformational polymorphism (SSCP), restriction fragment length polymorphism (RFLP), and direct sequencing. Of the 71 studied chromosomes, 65 (91.6%) have been characterized. Ten different mutations in SLC3A1 gene (T216M, C242R, R365L, 1136+2T->C, G398R, R456C, M457T, M457K, S647W, and L573X) and four in SLC7A9 gene (G105R, A331V, 1233-1236delACTC and G73R) were found. Four mutations are new: C242R and L573X mutations in SLC3A1 gene, and G73R and 1233-1236delACTC mutations in SLC7A9 gene. The most common mutation is T216M (21.0%), M467T (16.9%), R365L (12.7%) in SLC3A1 gene and G105R (18.3%) in SLC7A9 gene. T216M was found mainly in Gypsies and Albanians; R365L was predominant in Serbs, while M467T and G105R were found in patients from different ethnic origin.

PP-856
Effects of hyper- and hypothyroidism on enzyme activities of rat brain regions

H. Carageorgiou[1], C. Pantos[1], **A. Zarros**[2], V. Stolakis[3], I. Mourouzis[1], D. Cokkinos[4] and S. Tsakiris[2]

[1]Department of Experimental Pharmacology, Medical School, University of Athens, Athens, Greece, [2]Department of Experimental Physiology, Medical School, University of Athens, Athens, Greece, [3]Department of Biological Applications & Technology, Science and Technology School, University of Ioannina, Ioannina, Greece, [4]Onassis Cardiac Surgery Center, First Cardiology Department, Athens, Greece. E-mail: azarros@med.uoa.gr

The aim of this work was to investigate how the activities of acetylcholinesterase (AChE), (Na^+, K^+)- and Mg^{2+}-ATPase could be affected in brain regions of hyper- and hypothyroid rats. Hyperthyroidism was induced by subcutaneous administration of thyroxine (25 μg/100g body weight) once daily for 14 days, while hypothyroidism was induced by oral administration of propylthiouracil (0.05%) for 21 days. In hyperthyroidism, AChE activity was found significantly increased in hippocampus (+22%) and decreased in cerebellum (-23%). (Na^+, K^+)-ATPase was significantly inhibited in the hyperthyroid rat hippocampus (-47%) and cerebellum (-26%). In hypothyroidism, AChE activity was found significantly increased in hippocampus (+21%), decreased in frontal cortex (-23%) and cerebellum (-17%), while it remained unchanged in hypothalamus. (Na^+, K^+)-ATPase was significantly inhibited in the hypothyroid rat frontal cortex (-35%), hippocampus (-43%) and cerebellum (-27%). Mg^{2+}-ATPase was found unaltered in all regions of both hyper- and hypothyroid rat brains. Our data revealed that thyroid hormones affect the examined adult rat brain parameters, in a region- and state-specific way. The inhibited (by the thyroid hormones) Na^+, K^+-ATPase may increase the synaptic acetylcholine release and thus, could modulate AChE activity. Moreover, the above thyroid hormone-induced changes may affect the monoamine neurotransmitter systems in (at least some of) the examined brain regions.

PP-857
A novel transgenic mouse strain for studying peroxisome related functions at the organismal level

A. Jasim, M. Demester, N. Yousaf and B. Magoulas

Neuroscience Centre, Institute of Cell and Molecular Science, Barts and The London School of Medicine and Dentistry, Queen Mary, University of London, UK.
E-mail: C.B.Magoulas@qmul.ac.uk

We have generated transgenic mouse lines carrying the neuronal Thy cassette expressing a mutant form of IκBα. Homozygous mice of one of these lines exhibit a severe phenotype, characterized by lesions in the genital area, atonia and weight loss, while heterozygous mice appear normal. Here we investigated whether this autosomal recessive manner of inheritance of the phenotype is due to a chromosomal positioning of the transgene. We have found that the transgene has been inserted in a region of the Mpv17-like (M-LP) locus which encodes two previously identified proteins, M-LPl and M-LPs. The functions of these proteins are largely unknown, however, they belong to the Mpv17/PMP22 family and have been associated with peroxisome related processes. By performing a detailed sequence analysis of the M-LP locus, we discovered that the transgene is integrated in the first intron of a novel exon which potentially yields a new protein variant, which we termed M-LPs2. Taken together, these results suggest that the phenotype of this novel transgenic mouse strain is due to misplacing of the M-LPs2 variant, which in turn may cause disruption of peroxisomal related functions. We are currently investigating the mechanisms underlying the phenotype of this novel mouse strain, not only in view to elucidate the functions of the M-LP proteins at the organismal and cellular level, but also to establish their involvement in any clinically known peroxisomal disorders.

PP-858
MSSCP as a novel method for detection of minute changes in avian influenza genome fragments

B. Gromadzka[1], B. Szewczyk[1], K. Smietanka[2] and Z. Minta[2]
[1]*Department of Molecular Virology, Intercollegiate Faculty of Biotechnology, University of Gdańsk, Gdańsk, Poland*
[2]*The National Veterinary Research Institute, 24-100 Pulawy, Poland. E-mail: g.beata@biotech.univ.gda.pl*

Classical single-str and conformational polymorphism (SSCP) analysis is based on the observation that single-stranded DNA fragments attain a number of conformational forms which may be separated by electrophoresis in native polyacrylamide gels giving a characteristic pattern of electrophoretic bands. Even minute sequence changes (e.g. point mutations) may have significant effect on electrophoretic pattern of single-stranded DNA. Changes of gel temperature during electrophoresis increase the sensitivity of mutation detection in PCR products; this technique was named MSSCP (where M stands for 'multitemperature'). We have applied this method modified in our laboratories for characterization of influenza A cDNA fragments. A series of primers were synthesized after the comparison of the hemagglutinin gene sequences of different origin. PCR reactions were run using these primers and the products were denatured. Single-stranded DNA fragments were subjected to MSSCP electrophoresis where, after silver staining, they gave ssDNA band patterns characteristic for each genotype. This technique can be potentially applied to detect avian influenza in faeces of migratory birds as well as from bird carcasses collected in the field,. Minor differences within a serotype can be easily detected without need for sequencing the PCR products which makes the characterization of influenza variants much faster.

PP-859
The effects of proteasome manipulation in ageing, survival and longevity

N. Chondrogianni and E. S. Gonos
Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, Athens, Greece.
E-mail: nikichon@eie.gr

The proteasome is the major cellular proteolytic machinery responsible for the maintenance of cellular homeostasis. Alterations of proteasome function have been recorded in various biological phenomena including cellular response to oxidative stress and ageing, while recently it has been implicated to longevity. Here we report that proteasome can be manipulated, either up-regulated or down-regulated, resulting in different effects on ageing, survival and longevity of the cells. Once proteasome is activated through stable overexpression of beta 5 catalytic subunit, proteasome activities are stimulated, due to elevated amount of assembled proteasome. These increased levels of assembled proteasome result to enhanced cellular capacity to cope better with oxidative stress, mainly through an up-regulated rate of proteolysis. Furthermore, stable transfectants exhibit an extended lifespan and a delay of senescence. In contrast, upon replicative senescence the proteasome is down-regulated resulting to lower amount of assembled and thus, functional proteasome. Additionally, when proteasome is partially inhibited in young primary human fibroblasts, the cells exhibit an irreversible senescence-like phenotype. We have identified p53 pathway to be responsible for this accelerated appearance of senescence, since cells with inactivated p53 pathway can escape this phenotype. In conclusion, these data demonstrate the central role of the proteasome during senescence, survival and longevity.

PP-860
Genotypes of recurrent tuberculosis causing mycobacteria

M. Bauskenieks[1], A. Nodieva[3], G. Skenders[2], I. Jansone[1] and V. Baumanis[1]
[1]*University of Latvia Biomedical Research and Study Centre,*
[2]*State Agency of Tuberculosis and Lung Diseases of Latvia,*
[3]*Riga Stradins University. Latvia. E-mail: Ramases_3@yahoo.com*

Latvia is a country with medium tuberculosis (TB) incidence rate. In 2004 there were 69.7 cases per 100 000 residents. Among them 15% are recurrent cases. All the recurrent cases are subdivided in two groups: reactivations (after not fully cured disease) and reinfections (new infection cases). Reactivations and reinfections can be identified using molecular genotyping of TB causing agent Mycobacterium tuberculosis. Identical mycobacterial DNA isolates in both TB cases indicate the reactivation whereas different ones indicate reinfection. The aim of this research were analysis of Latvian reactivation and reinfection cases and studying main genotypes involved in TB resurrection in Latvia. The research includes 66 Latvian recurrent TB patients. Mycobacterial DNA samples have been analysed from both cases of all these patients. Samples were isolated from mycobacterial cultures. IS6110 RFLP genotyping method was applied to analyse these samples. Finally the IS6110 RFLP patterns of these samples were compared with computer program GelCompar 4.2. IS6110 RFLP patterns for 43 recurrent cases showed that 16% of cases were reactivations and 84% were reinfections. The dominant genotype groups were C (42%) and Beijing - 30%. There were a big difference between Beijing genotype proportion in primary cases (20%) and recurrent cases (40%). These results show that *M. tuberculosis* Beijing strains possibly have advantages to infect previously cured host.

PP-861
Locally generated elastin peptides increase invasive potential of melanoma cells

P. Pócza[1], Zs. Darvas[1], K. Pócza[2] and A. Falus[1]
[1]*Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary,* [2]*Department of Pathology, Uzsoki Hospital, Budapest, Hungary. E-mail: pocpet@gmail.com*

Melanoma is a highly malignant tumor type and elastin plays a key role in the progression of melanoma. The VGVAPG and VAPG peptide-sequences are repeating several times in the human elastin and they are most likely to be the breakdown products after the degradation of elastin. We demonstrate the elastin protein and the VGVAPG sequence with histochemical and immunohistochemical methods. We present evidence that both VGVAPG and VAPG elastin peptides bind to three identical receptors: galectin-3, integrin $\alpha v\beta 3$ and elastin-binding protein. We investigated the effects of VGVAPG and VAPG elastin peptides on several metastatic markers of human melanoma cell lines with different invasive potential. Immunocytochemistry, flow cytometry and quantitative real-time RT-PCR were applied to evaluate the changes of the expressions. In conclusion: interaction between phylogenetically conserved elastin sequences (VGVAPG, VAPG) and melanoma cells appears to be a significant point of tumor progression: (i) elastin and its fragments are potential substrates of MMP-2 and MMP-3; (ii) they have chemotactic effect on the melanoma cells; (iii) elastin peptides increase the expression of CXCR-4 and CXCL-12; (iv) the cleaved peptide fragments have the ability to increase the expression of MMP-2 and MMP-3; (v) they could reduce the expression of adhesion molecules on the cell surface, but increase the mRNA level of these adhesion molecules and (vi) increase the expression of VEGF-C.

PP-862
Carboxypeptidase T with the reconstructed primary specificity pocket of carboxypeptidase B

A. Grishin, V. Akparov and G. Chestukhina
VM Stepanov Laboratory of Protein Chemistry, Institute of Genetics and Selection of Industrial Microorganisms, Moscow, Russian Federation. E-mail: grishin@genetika.ru

Regulatory and digestive metallocarboxypeptidases (MCP's), forming one enzymatic family, play an important role in many physiological processes. Prokaryotic metallocarboxypeptidase T (CPT) is a promising model object of the enzymes of the whole family of MCP's, because they are highly homologous. Structural studies revealed that a primary substrate specificity of these enzymes is determined by interaction of a substrate C-terminal amino acid side-chain with the side chains of amino acid residues forming an enzyme primary specificity pocket, namely the residues 203, 207, 243, 250, 253, 255 and 268. In this study we verified this generally accepted hypothesis in the case of CPT. We replaced all the five residues in the CPT specificity pocket, differing in carboxypeptidase B (CPB) and CPT. It was expected, that mutant CPT would possess the CPB substrate specificity. However the kinetic characteristics of mutant CPT, particularly the Km value, virtually didn't change as compared to the wild-type enzyme. These data indicate, that the influence of the above-mentioned five residues on the substrate specificity is not so pronounced for CPT. On the other h and we can suggest that the spatial arrangement of the mutant CPT primary specificity pocket doesn't entirely reproduce that of CPB. We founded, that the surface loop, forming a part of the specificity pocket in CPT, differs from that of CPB. Moreover the enzyme structure may be altered upon mutations.

PP-863
Novel mutations in the porphobilinogen deaminase gene in Czech acute intermittent porphyria patients

D. Ulbrichova, M. Hrdinka, E. Flachsova, J. Prochazkova, J. Zeman and P. Martasek
Department of Pediatrics, Charles University, 1st School of Medicine, Prague, Czech Republic. E-mail: dana.u@email.cz

Acute intermittent porphyria is an autosomal dominant inborn disorder. It is manifested by life-threatening acute attacks of abdominal pain, gastrointestinal dysfunction, neurological disturbances, and accumulation of porphyrin precursors in the urin. AIP results from an error in heme biosynthetic pathway due to the half-normal activity of porphobilinogen deaminase (PBGD, EC 4.3.1.8). To date, over 250 various mutations in PBGD gene have been identified. We were analyzed DNA of Czech and Slovak AIP patients from seven unrelated families. Mutation screening was performed by PCR, denaturing gradient gel electrophoresis (DGGE) and DNA sequencing. Total of 36 individuals were analyzed to detect asymptomatic carriers. Eight mutations were identified, including three novel mutations (610 C > A, 675 delA, 966 insA), and five previously reported mutations (76 C > T, 77 G > A, 518 G > A, 771 + 1 G > T, 973 insG). Of particular interest, one patient had two mutations, 518 G > A and 610 C > A, both located in the same allele of exon 10. To establish the effect of 518 G > A and 610 C > A mutations we have prepared mutant constructs and the enzymatic studies are under current investigation.

Conclusions: Three novel mutations were identified in seven unrelated AIP families. These studies further emphasize the molecular heterogeneity of AIP, and provide accurate detection of asymptomatic carriers.

Acknowledgment: (Supported by grant GAUK 10/2004/C and in part by GACR 303/03/H065 and MSMT 0021620806).

PP-864
Puzzles in formation of trimeric dUTPases

E. Takács, A. Békési and B. Vértessy
Genome Metabolism and Repair Group, Institute of Enzymology, Budapest, Hungary. E-mail: teniko@enzim.hu

dUTPase is an enzyme involved in nucleotide metabolism. On one hand it produces dUMP, a precursor of thymidine nucleotides, on the other hand dUTPase decreases the intracellular dUTP level. Lack of the enzyme leads to erroneous uracil incorporation into DNA. High uracil content in DNA induces hyperactivity of base-excision repair that results in chromosome fragmentation and thymine-less apoptotic cell death. dUTPase inhibition is therefore a promising anticancer strategy. In most dUTPases, a homotrimer has to be formed for correct function. The homotrimer has three structurally identical active sites located at the clefts between two neighboring subunits. The third subunit donates its C-terminal arm that bends over the active site. Interestingly, a highly conserved proline occurs at the hinge region of this arm that is supposed to rigidify the main chain and therefore promote proper oligomerization. By site-directed mutagenesis we conclude that this residue has significant, but not indispensable role in trimer formation. To date, extremely little is known about the interacting protein partners of dUTPases *in vivo*. In our laboratory we managed to fish out and identify some promising proteins that may bind to the dUTPase. One of these proteins is the heat shock protein Hsc70 that may mediate crucial cellular functions under non-stress conditions beside assisting protein folding *in vivo*.

PP-865
Mutations in genes coding for ATP7A and ATP7B (causing Menkes and Wilson Diseases) in Czech Republic

L. Pospisilova[1], L. Kralik[1], E. Flachsova[1], Z. Marecek[4], H. Hansikova[1], R. Bruha[2], P. Fruhauf[3], J. Zeman[1] and P. Martasek[1]
[1]Department of Paediatrics and Center of Applied Genomics, 1st School of Medicine, Charles University, Prague, Czech Republic, [2]Department of Internal Medicine 4, 1st School of Medicine, Charles University, Prague, Czech Republic, [3]Department of Paediatrics, Faculty General Hospital Prague, Czech Republic, [4]Department of Gastroenterology and Hepatology, 1st School of medicine, Charles University, Prague, Czech Republic. E-mail: l.pospisilova@seznam.cz

Background: Copper plays an essential role in biology as a cofactor for many enzymes. There are two intracellular copper binding P-ATPases in human: ATP7A and ATP7B. Deficit in ATP7A cause X linked Menkes disease (MD). ATP7B defect cause autosomally recessive inherited Wilson disease (WD). We report here clinical, biochemical and molecular investigation in three patients with occurrence of Menkes disease. We also report a group of 74 WD-patients from the Czech Republic screened for the H1069Q mutation (prevalent in Central Europe) in the WD gene.

Methods: Genomic DNA was used to amplify exon 9 of ATP7A gene and exon 14 of ATP7B gene. PCR products were examined by RFLP.

Results: Molecular analysis in the patients with occurrence of Menkes disease revealed three mutations in ATP7A gene, two of them previously not published (Q724X and E1249X). In 74 WD-patients, 17 patients were homoallelic, 27 heteroallelic, and in 30 the H1069Q mutation was not detected. The frequency of mutated allele is 41%. Sequence analysis in heteroallelic patients revealed other mutations in ATP7B gene (3402delC, W779X, 2299insC).

Conclusions: Molecular analysis of ATP7A gene allows genetic counselling and prenatal diagnosis in families affected by Menkes disease. Screening of prevalent mutation in ATP7B gene shows, that the frequency of H1069Q mutation - 41% of analyzed alleles- is in accordance with its occurrence in Central Europe.

Acknowledgment: Supported by Grants MŠMT 1M6837805002 and 0021620806.

PP-866
Structural and functional characterization of Lamin B Receptor (LBR) Tudor domain

S. Liokatis[1], C. Edlich[2], M. Sattler[2], S. D. Georgatos[3] and A. S. Politou[1]

[1]Laboratory of Biological Chemistry, Medical School, University of Ioannina, Ioannina, Greece, [2]Structural and Computational Biology Unit, EMBL, Heidelberg, Germany, [3]Laboratory of Biology, Medical School, University of Ioannina, Ioannina, Greece. E-mail: me00537@cc.uoi.gr

Lamin B Receptor (LBR) is a ubiquitous protein of inner nuclear membrane. It contains a hydrophilic N-terminal domain, which projects into the nucleoplasm and interacts with macromolecular structures, like chromatin and nuclear lamina. This domain is consisting of two globular sub-domains, which are separated from a highly basic region, rich in serine/arginine (SR) motifs. Employing biocomputing tools we have found that the first globular domain belongs to the family of Tudor domains. The aim of the study is to investigate LBR's Tudor domain role, using both structural and functional approaches. In this direction, we used NMR spectroscopy to solve the atomic structure of the domain. Bacterially expressed and purified Tudor domain was prepared and a set of 2-D and 3-D NMR spectra were recorded. In addition, different kinds of nuclear extracts were tested in GST pull-down assays for Tudor domain binding. The solution structure of Tudor domain shows that the protein adopts a beta-barrel like structure. Biochemical data obtained so far indicate that LBR's binding to chromatin is mediated by a region encompassing the Tudor domain and the S/R rich part.

PP-867
The NAT motif defines integrity, specificity and affinity of the purine pathway of YgfO transporter

P. Karatza, E. Georgopoulou, P. Panos and S. Frillingos

Biological Chemistry, University of Ioannina Medical School, Ioannina 45110, Greece. E-mail: efriligo@cc.uoi.gr

We have analyzed the role of the nucleobase-ascorbate transporter (NAT) signature motif, a conserved sequence motif of the ubiquitous NAT/NCS2 family, by Cys-scanning mutagenesis of sequence 315-GSIPITTFAQNNGVIQMTGVASRYVG-340 in the *E. coli* purine-specific homologue YgfO. Inactive or marginal-activity single-Cys mutants include P318C, Q324C, N325C, T332C and G340C. These positions, and the remaining two ones of conserved motif residues (Gly327, Gly333) were subjected to extensive site-directed mutagenesis and mutants analyzed for kinetics of xanthine uptake and for inhibition by a series of purines and purine-related drugs or analogues. We find that Pro318 and Gly340 are essential for expression in the membrane, Gly333 is an important determinant of the lig and specificity profile, Gln324 is critical for high affinity uptake, Asn325 is fully irreplaceable with respect to active transport. Site-directed alkylation shows that single-Cys 323-329 are highly sensitive to inhibition by N-ethylmaleimide (NEM), 315-322 are insensitive and 330–340 follow a pattern indicative of an alpha-helical conformation. Strong inhibition, observed with single-Cys mutants at eight positions, is attributable to severe blocking effects of the maleimidyl adduct. No protection from NEM was evident in the presence of xanthine substrate. Most plausible interpretation is that these eight residues line the purine translocation pathway and are critically involved in the conformational changes during turnover.

PP-868
A comparison of the cytokine responses of two serpins, alpha-1 antichymotrypsin and angiotensinogen

C. Baker, L. Morgan, K. Morgan and N. Kalsheker

Institute of Genetics, University of Nottingham, Nottingham, UK. E-mail: mrxcab1@nottingham.ac.uk

Serine protease inhibitors (serpins) are a diverse superfamily of proteins that have arisen by gene duplication. Alpha-1- antichymotrypsin (ACT, SERPIN A3) is an abundant serpin in human plasma and binds cognate proteases and the Alzheimer's peptide beta amyloid (1–42). In contrast, angiotensinogen (AGT, SERPIN A8) acts as a hormone precursor for the vasoactive peptide angiotensin. The expression of both serpins are modulated by cytokines. ACT has 2 STAT binding sites within its promoter, and 2 NFκB sites within a 5' enhancer. AGT also has multiple STAT sites within its promoter but no reported NFκB sites. We used quantitative PCR to compare dose and time curves of cytokine effects on the two serpins in HepG2 cells. Interleukin 6 (IL6) increased ACT expression in a time and dose dependent manner with the maximum 100-fold effect observed after 24 h, whereas AGT expression is biphasic with an initial 2-fold peak observed at 16 h and a second 6-fold peak seen at 64 h. Peak responses to oncostatin M (OSM) in ACT (200-fold) and AGT (4.5-fold) occurred at 16 h. Interleukin 1 (IL1) caused a 7-fold increase in ACT at 24 hours, but decreased AGT expression 2-fold. Typical of many acute phase proteins ACT demonstrates up-regulation in response to IL1, IL6 and OSM. AGT retains blunted responsiveness to IL6 and OSM, but is down-regulated by IL1. These comparative studies are aimed at understanding the diversity of mechanisms for regulating gene expression within a superfamily.

PP-869
Functional characterization of a new human endogenous calcineurin inhibitor

M. C. Mulero Roig, A. Aubareda Rodriguez and M. Pérez-Riba

Cell Signaling Laboratory, Centre de Genètica Mèdica i Molecular, IDIBELL, Institut de Recerca Oncològica (IRO), Hospitalet de Llobregat, Catalunya, Spain. E-mail: mmulero@iro.es

Calcineurin (Cn), a calcium and calmodulin dependent Ser-Thr protein phosphatase plays a pivotal role in modulating many cellular processes, including T cell activation. Nowadays, the Cn inhibitor drugs, cyclosporin A and FK506, are the cornerstone of the immunosuppressive therapy but its continuous administration bears severe side effects. Several endogenous Cn inhibitors have been reported, among them the Calcipressin family members. In humans, this family includes calcipressin 1 (CALP1), calcipressin 2

(CALP2) and calcipressin 3 (CALP3). Recently, our group has characterized the immunosuppressive role of CALP1 in human T cells. The high identity of CALP3 and CALP1 has lead us to analyse CALP3 in T cells. The human *DSCR1L2* gene, which codifies for CALP3 protein, is present in Jurkat and Hut78 cells at the basal level. Moreover, the *DSCR1L2* gene expression is not regulated by Cn as its mRNA levels are not affected either by an increase on the intracellular calcium concentration or by the use of Cn inhibitor drugs. However, the main finding here is that the CALP3 C-terminal region inhibits NFAT nuclear translocation when Cn is active suggesting a modulatory role of CALP3 forward NFAT-dependent gene expression. Further analysis is required to ascertain the putative therapeutic use of CALP3 in allograft rejection therapy and autoimmune diseases treatment.

PP-870
Genetically-modified osmoprotectant accumulation alters thylakoid surface charge under cold stress

V. Doltchinkova[1], P. Angelova[1], D. Parvanova[2] and E. Ivanova[3]
[1]*Department of Biophysics and Radiobiology, Sofia University "St. Kliment Ohridski", 1164 Sofia, Bulgaria,* [2]*AgroBioInstitute, Sofia, Bulgaria,* [3]*Department of Gene Regulations, Institute of Molecular Biology - Bulgarian Academy of Sciences, Sofia, Bulgaria.*
E-mail: virjird@biofac.uni-sofia.bg

Electrokinetic and light scattering properties of freezing-tolerant tobacco plants transformed to accumulate osmoprotectants (proline: AtP5Cs, VacP5Cs; fructan: SacB; glycine betaine: codA) were studied. Tobacco plants of wild type (WT) and transformed variants were cultivated at 2 °C (cold acclimated) and -2 °C (cold stressed). We measured the electrophoretic mobility (EPM) of thylakoids as a sensitive marker for the surface charge density (σ) dynamics for assessment of osmotically-induced changes of the membrane surface by the method of particle microelectrophoresis. Cold stress on VacP5Cs, AtP5Cs and codA plants induced a significant increase in σ of 'low-salt' thylakoids without light treatment. 'Low-salt' thylakoids of WT exhibited a decrease in EPM after cold acclimation. WT and AtP5Cs thylakoids (22 °C) in 'low-salt' media expressed substantial increase in σ upon illumination. We observed that SacB thylakoid membranes in both ionic strengths increased the net negative electrical charge on their membrane surfaces during the process of acclimation. We evaluated the LS properties of thylakoids in order to specify the level of aggregation during photoenergization of their membranes. Cold acclimation and cold stress on WT and transformed plants resulted in a decrease in aggregation of thylakoids at both ionic strengths. The physico-chemical aspect of genetically modified osmolyte protection under low temperature stress in the thylakoid surface was discussed.

PP-871
Detection of tritiated, endomorphin-related products in rat brain extracts after 3HTyr-Pro treatment

E. Szemenyei[2], A. Rónai[1], E. Kátó[1], M. Al-khrasani[1] and G. Tóth[2]
[1]*Semmelweis University, Faculty of Medicine, Department of Pharmacology and Pharmacotherapy, Budapest, Hungary,* [2]*Biological Research Center of the Hungarian Academy of Sciences, Biochemistry Institute, Szeged, Hungary.*
E-mail: eszemenyei@yahoo.co.uk

In spite of focused efforts, the biosynthetic route of μ-opioid receptor agonist brain tetrapeptide endomorphins (endomorphin-1, Tyr-Pro-Trp-Phe-NH_2 and endomorphin-2, Tyr-Pro-Phe-Phe-NH_2), discovered in 1997, is still obscure. We report presently that 30 minutes after intracerebroventricular (icv.) injection of 20 or 200 μCi [^{3} H]Tyr-Pro (49.9 Ci/mmol) the incorporated radioactivity was found in endomorphin-related tetra- and tripeptides in rat brain extracts. As detected by the RP-HPLC (Reverse Phase High Performance Liquid Chromatography) with radiodetection, a peak corresponding to endomorphin-2-OH could be identified in two of four extracts of '20 μCi' series. Radioactive peaks in position of Tyr, Tyr-Pro, Tyr-Pro-Phe or Tyr-Pro-Trp appeared regularly in both series and also in the 'tetrapepide cluster' constituted by endomorphins and their free carboxylic forms. In one of the four extracts in the '200 μCi' series a robust active peak in the position of endomorphin-2 could be detected. The identification of labelled peptide peaks could be declared with safety if their retention time was identical with the actually co-injected standard. Icv. injected 100 nmol cold Tyr-Pro (devoid of opioid activity *in vitro*) caused a naloxone-reversible prolongation of tail-flick latency in rats, peaking between 15–30 min. We suggest that Tyr-Pro may serve as a biosynthetic precursor to endomorphin synthesis.

PP-872
Evidence for the ribonucleoprotein entity of nonhistone protein LMG160 as a RNP component of the RNP-containing nuclear matrix

M. Shahhoseini and A. Rabbani
Department of Biochemistry, Institute of Biochemistry and Biophysics, University of Tehran, Tehran-Iran.
E-mail: hoseini@ibb.ut.ac.ir

Nuclear matrix is a framework scaffolding of the nucleus which consists of the two separatable fractions of RNP-depleted and RNP-containing nuclear matrix. The latter fraction is primarily composed of nuclear proteins and ribonucleoproteins with important roles on nuclear metabolism. In this study a fraction of nonhistone nuclear proteins named LMG160, has been isolated from rat liver nuclei and identified as a ribonucleprotein. Ribonuclease treatment experiment revealed that this molecule is very sensitive to RNase and phenol/chloroform extraction analysis showed a RNA moiety of about 300 b in its structure. In order to identify the nuclear position of this molecule, different fractions of the nuclear matrix were extracted from the hepatocyte cell nuclei, using 2 M NaCl and 8 M urea. Western blot analysis with anti-LMG160 rabbit antisera, on different fractions of the nuclear matrix extract revealed that LMG160 is detectable in the RNP-containing fraction of the nuclear matrix. Concerning the ribonucleoprotein entity of this molecule, this result indicates, for the first time, the nuclear position of LMG160, which might be an important finding for better understanding of the biological role of this ribonucleoprotein particle in rat liver nuclei.

PP-873
Enhancement of protein and RNA synthesis in alveolar macrophages stimulated with concanavalin A

M. Motevalibashi, A. Rabbani and **S. Abdossamadi**
Department of Biochemistry, Institute of Biochemistry & Biophysics, University of Tehran, Tehran, Iran.
E-mail: abdossamadi@ibb.ut.ac.ir

Difference between gene in the eukaryotic cells, is accompanied by the regulatory proteins and change in chromatin structure. In

the present study, alveolar macrophages were prepared from rat lung and stimulated by different concentrations of Concanavalin A in the absence and presence of radiolabeled leucine for various incubation times. The chromatin proteins were then isolated and compared. The results show that although there were no changes in the pattern of histones in the stimulated and the control cells, the content of nonhistone proteins HMG_B, HMG_N and Ubiquitin were increased considerably. Also a new band was appeared between H_{2A} and H_4 with unknown function. Analysis of total RNA extracted from Concanavalin A treated and the control cells showed a remarkable increase in the level of RNA synthesis in the stimulated cells compared to the control. In conclusion it is suggested that HMG proteins may contribute a role in the activation of the cells proceeding alteration of chromatin condensation and gene expression.

PP-874
The activity of 5′-nucleotidase and adenosine deaminase in Fas ligand induced mice kidney damage

T. Jevtovic, G. Kocic, T. Cvetkovic and A. Topalovic
Institute of Biochemistry, School of Medicine, University of Nis, Nis, Serbia and Montenegro. E-mail: tjevtovic@yahoo.com

Adenosine, a cyclic nucleoside consisting of the purine adenine coupled to a ribose sugar is an important, biologically active molecule that has a wide range of physiological actions. When adenosine binds to membrane receptors on a variety of cell types in the kidney, it stimulates functional responses that span the entire spectrum of renal physiology (alterations in hemodynamics, hormone and neurotransmitter release and tubular reabsorption). The FasL/Fas system regulates renal cell apoptosis, but at the same time it is responsible for renal cell injury. Having in mind many different roles of adenosine in kidney, as well as the main mechanism of pro-apoptotic action of FasL the aim of this study was to investigate the influence of anti-Fas antibody on adenosine metabolising enzymes in mice kidney. We have used Balb/c mice. Tha animals were divided in two groups: I group was control treated with 0.85 NaCl intraperitoneally; II group were mice treated with anti-Fas antibody (2×0.8 microgl/kg B.W). The activity of 5'-nucleotidase and adenosine deaminase was measured. The results showed statistically significant increase of 5'-NT activity ($P < 0.05$) and decrease of adenosine deaminase activity in group treated with anti-Fas antibody versus control. In conclusion, the evidence reported in this review indicates that increased production of extracellular adenosine, in Fas-induced renal cell apoptosis culd be result of tissue adaptation in order to protect itself from ischemic injury.

PP-875
Development of a microtiter plate fluorescent assay for inhibition profiling of retroviral proteases

G. Miklóssy, P. Boross, J. Kádas, A. Fehér, T. Sperka, J. Tözsér and P. Bagossi
Department of Biochemistry and Molecular Biology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary. E-mail: miklgabi@indi.biochem.dote.hu

The HIV-1 protease (PR) has proved to be an important target for antiretroviral therapy of AIDS, and various PR inhibitors are now in clinical use, however, resistance usually rapidly develops against these compounds as a consequence of PR mutations. The amino acid residues appearing in drug resistant HIV-1 PRs can frequently be found in structurally equivalent positions of other retroviral PRs, therefore, their comparative studies are expected to help the design of improved PR inhibitors, efficient against several different proteases including highly drug resistant HIV-1 PR forms. We have developed a microtiter plate fluorescent assay for various retroviral proteases to perform their inhibition profiling. This method measures the protease activity utilizing synthetic peptides with a fluorescent donor and a quenching molecule. Fluorescence signal developed after the proteolytic cleavage was corrected by a new method for elimination the inner filter effect. We have determined the inhibition profiles of wild-type and mutant HIV-1 PR, human T-cell leukemia virus type-1 PR, bovine leukemia virus PR and murine leukemia virus PR. Based on our results, single drug-resistant mutations caused only moderate (typically less then ten-fold) decrease in binding affinity of clinically used PR inhibitors. On the other hand most of these compounds were unable to inhibit the other wild-type PRs.

Acknowledgment: (Research supported in part by OTKA T43482 and OTKA F034479 grants).

PP-876
A retroviral dUTPase: modulatory effect of flexible segments on protein structure and function

V. Nemeth[1], O. Barabas[1], M. Fuxreiter[1], D. Svergun[2], M. Petoukhov[2] and B. G. Vertessy[1]
[1]Hungarian Academy of Sciences, Institute of Enzymology, Budapest, Hungary, [2]European Molecular Biology Laboratory, Hamburg Outstation, Germany and Russian Academy of Sciences, Institute of Crystallography, Moscow, Russia.
E-mail: pongracz@enzim.hu

The fusion protein nucleocapsid-dUTPase is present in virions of Mason-Pfizer monkey betaretrovirus and in virus-infected cells where it potentially contributes to RNA/DNA folding and reverse transcription. Effects of N- and C-terminal segments on protein folding and function was analyzed. High resolution 3D structures of wild type and C-terminally truncated dUTPases reveal that conformation within the flexible C-terminus is dramatically altered as compared to other dUTPases possibly due to fusing of nucleocapsid protein onto dUTPase that perturbs orienting role of a critical beta-strand. Dynamic modeling suggests that this segment is capable of double backing upon the active site of its own monomer. The molecular shape of the full-length native protein was characterized by small-angle X-ray scattering. Oligonucleotide binding has a major compacting effect in ordering the nucleocapsid domains around the trimeric dUTPase core. Interacting protein partners of the enzyme were identified *in vitro*. Some viral proteins (integrase, capsid) were found to be capable of physical interaction with NC-dUTPase. Experimental results argue for direct structural and possible physiological consequences of fusing nucleocapsid and dUTPase proteins, an organisation unique to betareroviruses.

PP-877
Extracting the cholesterol from chip-immobilised vesicles with methyl-β-cyclodextrin

M. Besenicar, A. Bavdek and G. Anderluh
Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia.
E-mail: mojca.besenicar@bf.uni-lj.si

Methyl-β-cyclodextrin (MBCD) is a cyclic oligomer of seven glucose residues with the capacity of sequestering lipophilic

molecules in its hydrophobic core. It is water soluble and known to enhance the solubility of cholesterol in aqueous solutions by forming soluble inclusion complexes with it. Cyclodextrins have been in use in pharmacological research for years as carriers of lipophilic drugs and only in last two decades their use in membrane studies has been appreciated. It has been shown that β-cyclodextrins are able to selectively remove cholesterol from various types of cultured cells by neither binding nor inserting into the plasma membrane. In our experiments, performed on Biacore X$\reg$, we have tried to evaluate the process of extraction of cholesterol and its derivatives from membranes of various compositions. How the formation of shingomyelin-cholesterol membrane domains influences the process of extraction of cholesterol from the membranes and its kinetics. With the combination of surface plasmon resonance (SPR) and fluorimetric measurements we monitored the effect MBCD has on the integrity of the vesicles. We have shown that in the presence of sphingomyelin the depletion of cholesterol is slower, less effective and has different kinetics than in the membranes composed solely from DOPC (di-oleyl glycero-3-phosphocholine) and cholesterol.

PP-878
Physical localization of the 14-3-3 protein C-terminal stretch and its possible function

J. Silhan[1], V. Obsilova[2], J. Vecer[3], P. Herman[3], M. Sulc[4], J. Teisinger[2] and T. Obsil[1]
[1]*Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, 128 43 Prague, Czech Republic,* [2]*Institute of Physiology, Academy of Sciences of the Czech Republic, 142 20 Prague, Czech Republic,* [3]*Institute of Physics, Faculty of Mathematics and Physics, Charles University, 121 16 Prague, Czech Republic,* [4]*Institute of Microbiology, Academy of Sciences of the Czech Republic, 142 20 Prague, Czech Republic.*
E-mail: jansilhan@biomed.cas.cz

14-3-3 proteins are involved in regulation of numerous cellular processes. They bind to phosphorylated protein ligands and regulate their functions. The C-terminal part of 14-3-3 protein is involved in the regulation of 14-3-3 binding properties, but its structure is unknown. Phosphorylation of Thr232 located within the 14-3-3 C-terminus seems to have inhibitory effect on ligand binding. It has also been suggested that C-terminal stretch can be located within the 14-3-3's ligand binding groove. We investigated the physical location of 14-3-3 C-terminal stretch and its changes upon the ligand binding using Förster resonance energy transfer (FRET) measurements and molecular dynamics (MD) simulation. FRET measurements between Trp242 located at the end of the C-terminal stretch and a dansyl group attached at either Cys25 or Cys189 indicate that C-terminal stretch occupies ligand binding groove of the 14-3-3 protein. Our data also show that phosphopeptide binding displaces the C-terminal stretch from the ligand binding groove. Intramolecular distances calculated from FRET measurements fit well with distances obtained from MD simulations of full length 14-3-3 protein. Recent MD simulations of 14-3-3 protein phosphorylated at Thr232 show possible interactions responsible for ligand binding inhibition.

Acknowledgment: Support: Grant 204/06/0565 of the Grant Agency of the Czech Republic, Grant B/CH/331/2005 of the Grant Agency of the Charles University, and Centre of Neurosciences LC554.

PP-879
Implication of clusterin/apolipoprotein J in resistance acquisition of cancer cells to chemotherapy

M. Lourda, I. P. Trougakos and E. S. Gonos
Institute of Biological Research & Biotechnology, National Hellenic Research Foundation, Athens, Greece.
E-mail: mlourda@eie.gr

Clusterin/Apolipoprotein J (CLU) is a heterodimeric glycoprotein that has been implicated in numerous physiological disturbance states including in vivo cancer progression. However, due to the intriguingly distinct and usually opposing functions attributed to this molecule, its precise role in different cell types and biological processes is still largely unknown. To study the involvement of CLU in the acquisition and maintenance of resistance of human cancer cells to chemotherapeutic drugs, we developed multi-drug resistant osteosarcoma (OS) cell lines by continuous exposure to gradually increasing, clinically relevant, concentrations of the drug doxorubicin (DXR). The created DXR-resistant (DXR-R) OS cells appeared to be cross-resistant to various unrelated cytotoxic agents. Moreover, the DXR-R OS cells were found to exhibit elevated CLU mRNA and protein amounts. Our subsequent functional analysis revealed that the increased CLU levels in the DXR-R OS cells were related to the acquisition and maintenance of OS cells multi-drug resistance. Therefore, we suggest that CLU may represent a marker for cancer response prediction and effective cancer therapy.

PP-880
Cloning and expression of the coat protein gene of PLRV in *Saccharomyces cerevisiae* and *E. coli*

E. Suluja[1], A. Palucha[1], A. Kazaks[2] and A. Zeltins[2]
[1]*Department of Protein Biosynthesis, Institute of Biochemistry and Biophysics PAS, Pawińskiego 5a, Warsaw, Poland* [2]*Biomedical Research and Study Centre, University of Latvia, Ratsupites 1, LV-1067, Riga, Latvia. E-mail: suluja@ibb.waw.pl*

Potato leafroll virus (PLRV) is the member of the polerovirus genus. The virus posses a small, 23 nm in diameter, isomeric virion, encapsidating ssRNA genome. Capsids of PLRV are composed of two structural proteins: the 23 KDa coat protein (CP) and a 65 KDa read-through protein, which is translated by occasional suppression of the CP termination codon. The CP can be divided into two acidic domains: an N-terminal arginine-rich domain (R) and shell domain (S). The CP was found to self-assemble into virus-like particles (VLPs) which can be used as carry/delivery system. In our preliminary study, the CP of PLRV tagged with six histidine residues at the N-terminus was found to self-assemble into VLPs in *S. cerevisiae* strains AH22 and DC5. Electron microscopy (EM) analysis revealed a mixture of capsid-like particles. The diameter of the formed particles was between 10 and 30 nm in comparison to 23 nm of native virions. In *E. coli*, the expression of a CP protein with truncated 36 N-terminal amino acids resulted in the formation of T = 1 VLPs. EM showed formation of uniform particles of approximate 10 nm diameter. Similar VLPs were observed when 64 amino acids covering the entire R domain were removed from the CP protein. Our preliminary results showed that *S. cerevisiae* and *E. coli* cells are suitable for expression and characterization of VLP formation of PLRV and that deletion of N-terminal residues of the CP affected the formation of T = 3 particles.

PP-881
New type of inhibitors of hepatitis C virus (HCV) helicase activity

A. Stankiewicz-Drogoń[1], V. Kostina[2], L. Palchykovska[2], M. Krawczyk[1], A. Alexeeva[2], A. D. Shved[2] and A. M. Boguszewska-Chachulska[1]
[1]*Institute of Biochemistry and Biophysics PAS, Warsaw, Poland*
[2]*Institute of Molecular Biology and Genetics Ukr NAS, Kiev, Ukraine. E-mail: annas@ibb.waw.pl*

The non-structural protein 3 (NS3) of hepatitis C virus (HCV) is a very promising target for anti-HCV therapy because of its multiple enzymatic activities, such as RNA-stimulated NTP-ase, RNA helicase and serine protease, as well as the fact that it is indispensable for virus replication. We developed a novel direct fluorometric test of helicase activity based on a DNA substrate that allows for direct and fast measurements of the enzyme activity of multiple samples in the microplate format. This assay was applied to test a new class of small-molecule compounds (named N1 - N25) as potential inhibitors of the HCV helicase activity. For further studies three compounds were selected that inhibited 50% of helicase activity at concentration below 100 μM (IC_{50} < 100 μM). The values obtained for N20, N21 and N22 were: 9 μM, 6 μM and 20 μM, respectively. To elucidate the mechanism of inhibition we verified if these compounds inhibited strand displacement activity of the helicase by interacting with the enzyme, by competing with DNA or ATP for the enzyme or by affecting the helicase ability to hydrolyze ATP. The results obtained indicate that N20, N21 and N22 do not interact with the enzyme. Neither do they compete with ATP nor affect ATP-ase activity of the HCV helicase. They interact however with the DNA substrate, though not as intercalators. Thus we estimate N20, N21 and N22 to be competitive inhibitors of the HCV helicase activity with respect to DNA.

PP-882
Kinetic evaluation of phenoxazine and benzimidazole derivatives as cholinesterase ligands

T. Tuylu Kucukkilinc and I. Ozer
Department of Biochemistry, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey. E-mail: ttuylu@hacettepe.edu.tr

The effects of 2 phenoxazine dyes medulla's blue (MB), nile blue (NB)) and of 2-(aminomethyl) benzimidazole (AMBI) on human plasma cholinesterase (ChE) activity were studied at 25 °C, in 100 mM MOPS buffer, pH 8. The assay mixture contained 0.125 mM 5-5'-dithio-bis(2-nitrobenzoic acid), 0.1–0.4 mM butrylthiocholine (BTC) and 0–1 mM effectors. AMBI was found to be a moderate competitive inhibitor of enzyme activity, with Ki = 0.1604 μM ± 0.043. The phenoxazine dyes were much better ligands. The inhibitory patterns observed with MB and NB were complex. The kinetic data could be analyzed either in terms of competitive inhibition or according to a multisite inhibition model. Analysis of the inhibition at different substrate concentrations using the Hill Equation (log[vi/(vo-vi)] = -nlog [MB] + logK' yielded n and K' values which varied with [BTC]. Limiting values for n and K' at [BTC] = 0 for MB and NB were 1.65 ± 0.23, 1.57 ± 0.27 and 0.7299 ± 0.1139 μM, 0.2888 ± 0.0114 μM respectively. The complex patterns of inhibition by MB and NB were compatible with earlier reports on the multiplicity of ligand binding sites on plasma cholinesterase. The result of this study should be useful for research on inhibitor design for therapeutic use.

PP-883
Development of a cellular system for the study of Cr (VI)-induced lung carcinogenesis

A. N. Costa[1], V. Moreno[2], A. M. Urbano[1] and M. C. Alpoim[1]
[1]*Department of Biochemistry, University of Coimbra, Coimbra, Portugal,* [2]*Department of Inorganic Chemistry, University of Barcelona, Barcelona, Spain. E-mail: andrenunocosta@gmail.com*

Some Cr (VI) compounds are recognized as occupational human lung carcinogens through chronic inhalation. Within the cell Cr (VI) undergoes reduction originating a variety of chromium and carbon based reactive species. Several studies using cellular systems have demonstrated the mutagenic potential of these species as well as other molecular and cellular events possibly related with carcinogenesis. However, as the cell types utilized were not the major targets of Cr (VI) toxicity (i.e., lung epithelial cells) and the exposure regimens were not representatives of carcinogenic conditions (i.e., chronic exposure to low doses of chromium), the results obtained must be interpreted with caution. Here we describe a study on which Cr (VI)-induced carcinogenesis was mimicked by chronically exposing normal human lung epithelial cells to sub-lethal Cr (VI) insults. When cells were submitted to 24, 48 and 72 h exposures, we were able to identify, in order of increasing doses, loss of contact inhibition (an essential step of the tumorigenic process), growth arrest and apoptosis (two active mechanisms against the emergence of transformed phenotypes) and finally necrosis. Interestingly, when cells with delayed cell cycle were maintained in culture for longer periods of time in the presence of Cr (VI) they acquired growth and morphologic characteristics typical of cancer cells. Thus, we anticipate that this in vitro system will be invaluable in future studies of Cr (VI)-induced carcinogenesis.

PP-884
Modified gold particles for oriented immobilization of immunoglobulins for subsequent immunoassay of some antigens

N. S. Khachatryan[1], H. E. Yeritsyan[1] and **V. K. Gasparyan**[2]
[1]*Department of Biochemistry, Yerevan State University, Yerevan, Armenia,* [2]*Institute of Biochemistry, National Academy of Sciences, Yerevan, Armenia. E-mail: armishka2004@mail.ru*

Immunoassays based on agglutination of colloidal gold are considered as very promising for use in detection of various antigen/antibodies. In contrary to traditional EIA analysis here the process is one-step and therefore any interference is minimized. But colloidal gold particles have tendency to spontaneous agglutination upon sensitization by some antibodies and it creates some problems. Moreover, here takes place random immobilization of antibodies that decreases effectiveness of antibody-antigen interaction. We have modified surface of gold particles by mercaptoethylamine to overcome these problems. Colloidal gold has high affinity to SH groups and therefore modified surface has exposed functional NH2 groups. On the other h and oxidation of immunoglobulin by periodate brings to modification of Fc fragment and consequent binding of these antibodies to NH2 groups on gold surface results to oriented immobilization of antibodies with exposed Fab fragments. It permits them to effective binding with appropriate antigens. This procedure was applied for estimation of effectiveness assays for some human antigens (ferritin, CRP, PSA). Results are compared with assays with non-modified gold particles. Effects of pH, ionic strength, stabilizators were studied.

PP-885
Analysis of the G. *mellonella* juvenile hormone binding protein core promoter

A. Niewiadomska-Cimicka and A. Sok
Department of Biochemistry, Wroclaw University of Technology, Wroclaw, Poland. E-mail: anna.niewiadomska@pwr.wroc.pl

There is considerable variability in the DNA elements that constitute core promoters, like TATA box, BRE, DPE and Inr. Sequence analysis in the vicinity of the transcription start point (tsp) of *jhbp* from *G. mellonella* revealed the presence of the following putative elements: -29TATAAA-24, +14TCAGTA+19, +38AGGTG+42 (TATA box, Inr, DPE, respectively). In contrast to any known DPE from insects, the distance of this element in *jhbp* relative to tsp is +38 bp instead of +28 bp and Inr does not encompass tsp. These differences and correct localization of TATA box suggest that *jhbp* contains TATA dependent promoter. It should be noted that up to now no functional analyses concerning *jhbp* regulation have been made. In this report we present studies concerning identification of regulatory elements in *G. mellonella jhbp* core promoter. Nuclear extracts (NE) from larval fat body (place of JHBP synthesis) were examined for binding to the radiolabeled probes containing putative core regulatory elements. EMSA showed the presence of transcription factors in NE, which bind to above-mentioned core elements. Competition experiments performed with specific and non-specific probes confirmed specificity of those interactions. The concentration of received complexes was as follows: TATA box > Inr > DPE. EMSA studies support the thesis, that *jhbp* is a TATA-Inr dependent promoter.

Acknowledgement: This work was supported by Ministry of Education and Science.

PP-886
The N-terminal regions of A and B1 ecdysteroid receptors are not fully unstructured *in vitro*

M. Nocula-Lugowska, G. Rymarczyk and A. Ozyhar
Department of Biochemistry, Wroclaw University of Technology, Wroclaw, Poland. E-mail: malgorzata.nocula@pwr.wroc.pl

The insect ecdysone receptor is a heterodimer of the two nuclear receptors, EcR (ecdysteroid receptor) and Usp (Ultraspiracle). It is believed to become useful in human gene therapy as a molecular switch regulated by an exogenous ecdysteroid stimulus. The EcR from *Drosophila melanogaster* exists in three tissue specific isoforms, EcRA, EcRB1 and EcRB2. They differ exclusively in their N terminal A/B regions, which indicates that the A/B domain itself is responsible for the isoform-specificity of the receptor. Our previous gel filtration and circular dichroism spectroscopy analyses have shown that A/B domains of both EcRA and EcRB1 adopt an extended conformation in solution. To understand the structural determinants for the EcR isoform specificity and to verify existence of putative structured regions, we carried out limited proteolysis experiments on the N-terminal region of both isoforms using trypsin and bromelain. Although most of the sequence of each isoform is cleaved, proteolytic patterns obtained by both specific and non-specific cleavages are discrete and partially similar, even at high enzyme concentrations. This may indicate that both EcRA-A/B and EcRB1-A/B are mainly unstructured *in vitro*, but still contain short structured regions, as judged by their resistance to protease digestion. Residual structure within the intrinsically unstructured proteins (IUP) is important, as it has been suggested to play role during their ligand induced folding.

PP-887
New nanosphere system for treatment of full-thickness burn on rabbit

A. D. Sezer[1], F. Hatipoğlu[2], Z. Oğurtan[3], A. L. Baş[4], E. Cevher[5] and J. Akbuğa[1]
[1]Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Marmara University, 34668, Haydarpaşa, Istanbul, Turkey, [2]Department of Pathology, Faculty of Veterinary, Selçuk University, 42075, Kampüs, Konya, Turkey, [3]Department of Surgery, Faculty of Veterinary, Selçuk University, 42075, Kampüs, Konya, Turkey, [4]Department of Pharmacology, Faculty of Veterinary, Selçuk University, 42075, Kampüs, Konya, Turkey, [5]Department of Pharmaceutical Technology, Faculty of Pharmacy, Istanbul University, 34116, Beyazıt, Istanbul, Turkey. E-mail: adsezer@marmara.edu.tr

The aim of this study was to prepare a new nanosphere system based on crosslinking of fucoidan with chitosan (FC) and evaluate its treatment efficiency on dermal burns. The nanospheres were prepared by crosslinking method. The particle size, charge and bioadhesion properties of the nanospheres were investigated. The optimum formulation was applied on seven male New Zeal and white rabbits (mean weight, 3.8 ± 0.6 kg) that formed full-thickness burn wounds. Each rabbit had four wounds; A-was treated with FC-nanospheres, B-was treated with fucoidan solution, C-was treated with chitosan nanospheres and D-as control. Biopsy samples were taken at 7, 14 and 21st days and each wound site was evaluated macroscopically and histopathologically. The nanospheres between the size ranges of 0.37 and 1.02 μm were obtained. The bioadhesion values of the particles, with the charges 6.1–26.3 mV, changed between 0.081 and 0.191 mJ/cm^2. Macroscopically observation, wounds epithel elongation and thickness values were showed that the fastest closure of the wounds were obtained in group A after 21 days treatment ($P < 0.05$). The burns nuclear organize regions (NORs)(5.50) and dermal papillary formation (6.83) values were higher treatment with FC-nanospheres than the other groups at the end of 14th days. FC-nanosphere was evaluated *in vitro* and *in vivo* and it can be concluded that this system might be suitable for the treatment of dermal burns on rabbits.

PP-888
Genetic polymorphism in the HLA class III region in CIN and cervical cancer patients from Russia

B. Bidzhieva, I. Tsiganova and N. Mazurenko
Institute of Carcinogenesis, Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Moscow, Russia. E-mail: bbidzhieva@y and ex.ru

The allele variants of the polymorphic microsatellites D6S273 and TNFa located within HLA class III region are associated with some autoimmune and infectious diseases. It is of interest to study the polymorphism of these markers in patients with cervical neoplasia (CIN) and cervical cancer (CC) compared with controls in order to reveal the possible association with HPV-induced carcinogenesis. We investigated 126 cases of cervical cancer and CIN I-III. As a control 139 females of the same age group and nationality (Russian) without any cancer autoimmune and infectious diseases were studied. We have not revealed the significant difference in distribution of the D6S273 alleles between examined groups. However it has been noted the significant decrease of TNF a7 frequency in CC patients versus the CIN patients ($P = 0.039$). The tumor necrosis factor-α gene (TNFα)-308(G→A) polymorphism is associated with the level of TNFα production. The high-secretor allele A association with the

CC risk was shown in some populations. We have analyzed the TNFα–308 polymorphism in 117 cases of CC and CIN. The decrease of the low-secretor genotype (GG) frequency in CC patients ($P = 0.16$) and in CIN patients ($P = 0.049$) was observed. The significant increase of allele A frequency in CIN, prevalently CIN I-II patients ($P = 0.021$) was revealed. We suggest the increase of allele A frequency in CIN group may be associated with the increase of TNFα activity that causes apoptosis.

PP-889
Serum vitamin B12, folate and plasma homocysteine levels in elderly

S. Ozdem[1] and **M. Gultekin**[2]
[1]*Akdeniz University, Medical Faculty, Departments of Central Lab. Clinical Biochemistry Unit, Antalya, Turkey,* [2]*Akdeniz University, Medical Faculty, Departments of Central Lab. Clinical Microbiology Unit, Antalya, Turkey.*
E-mail: ozdem@akdeniz.edu.tr

In the present study, it was aimed to determine the serum vitamin B12, folate and plasma homocysteine levels in elderly and to investigate the relations between these parameters. Serum vitamin B12, folate and plasma homocysteine levels were measured in 226 elderly (71.0 ± 7 years) and 260 non-elderly (46.4 ± 11 years) subjects. Vitamin B12 levels < 150 pmol/l and between 150–200 pmol/l were considered as high risk for deficiency and borderline deficiency, respectively. Deficiency limit for folate and high concentration for homocysteine were accepted as < 11 nmol/l and > 15 μmol/l, respectively. Age correlated negatively ($r = -0.26$, $P < 0.0001$) with vitamin B12 and positively ($r = 0.32$, $P < 0.01$) with homocysteine levels. Vitamin B12 and folate levels were significantly higher and that of homocysteine was significantly lower in elderly compared to non-elderly. In elderly group, 22.6% of subjects had high risk for vitamin B12 deficiency, 30.6% had borderline deficiency, 10% had folate deficiency and 48% had high homocysteine levels. In 64% of elderly with high homocysteine levels, the level of vitamin B12 was < 200 mmol/l, and in 4.6% of the same group, folate level was < 11 nmol/l. There was a significant relation ($r = -0.32$, $P < 0.0001$) between vitamin B12 and homocysteine levels. It was concluded that regular measurements of vitamin B12, folate and homocysteine levels in elderly and vitamin B12 and folate supplement in cases where needed may be helpful in prevention of some diseases frequently observed in this age group.

PP-890
Catalytic properties of o-dianisidine peroxidases in *Satureja hortensis* L. leaf extract

E. Paknia[1], E. Keyhani[1,2], **J. Keyhani**[2] and S. M. Ghaffari[1]
[1]*Institute of Biochemistry and Biophysics, University of Tehran, 13145 Tehran, Iran,* [2]*Laboratory for Life Sciences, 19979 Tehran, Iran. E-mail: epaknia@ibb.ut.ac.ir*

Peroxidases are hemoproteins catalyzing the H_2O_2-mediated oxidation of numerous substrates; plant peroxidases are also involved in a number of physiological processes. *S. hortensis* L. (summer savory), an aromatic and medicinal plant, is renown for its antispasmodic, antioxidant, sedative, and antimicrobial properties. Peroxidase activity was assayed in S. hortensis L. leaf extract prepared by homogenization in phosphate buffer 0.01 M, pH 7, and centrifugation at 3 000 g for 10 min and at 35 000 g for 30 min. The activity was measured by following the H_2O_2-mediated o-dianisidine oxidation at 460 nm. The pH activity profile showed a peak at 5.5 and a shoulder at 6.5. Kinetics parameters were determined at pH optima with Vmax and catalytic efficiency expressed per mg extract protein. With o-dianisidine as the varied substrate, Km, Vmax and catalytic efficiency were, respectively, 0.29 mM, 142 nmol/min and 0.163/min at pH 5.5 and 0.6 mM, 206 nmol/min and 0.115/min at pH 6.5. With H_2O_2 as the varied substrate, Km, Vmax and catalytic efficiency were, respectively, 2 mM, 334 nmol/min and 0.053/min at pH 5.5 and 0.32 mM, 173 nmol/min and 0.181/min at pH 6.5. IC50 for KCN was 0.7 μM at pH 5.5 and 1.5 μM at pH 6.5. No loss of activity was recorded after 5 min in up to 50 °C at pH 5.5 and up to 40 °C at pH 6.5, but 5 min at 75 °C led to complete loss of activity at both pHs. Data suggested the presence of at least two peroxidase ioenzymes in *S. hortensis* L. leaf extract.

PP-891
Cloning and characterization of WAX2 gene in wild barley, *Hordeum spontaneum*

A. Altinkut[1], A. Altinkut[2], A. Distelfeld[2], S. Weining[2], T. Fahima[2] and E. Nevo[2]
[1]*Plant Biotechnology, Research Institute for Genetic Engineering and Biotechnology, TUBITAK, Gebze-Kocaeli, Turkey,* [2]*Institute of Evolution, University of Haifa, Mount Carmel, Haifa, Israel.*
E-mail: ahu@gmbae.tubitak.gov.tr

The deposition of waxes into and on the cuticle layer is an adaptive mechanism that limits transpiration to conserve water in plants. This mechanism underlies plant survival and especially important in water-limited environments such as desert, alpine, and coastal ecosystems. The WAX2 gene which is involved in both cuticle membrane metabolism and wax synthesis was partially sequenced in genotypes of *Hordeum spontaneum*. The sequences of the genotypes from a northern mesic Mediterranean area, semi-xeric steppes, and a southern xeric desert in Israel showed single nucleotide polymorphisms in the coding region of WAX2. Screening of barley BAC library filter set using the WAX2 gene probe and fingerprinting of the positive clones revealed three different band patterns. Up regulation of the WAX2 gene under drought stress in leaves has been found by RT-PCR and different expression profiles were observed between mesic and xeric wild barley genotypes. The development of an integrated strategy for the complete cloning and characterization of the WAX2 gene would contribute to elucidate its role in the molecular mechanism of the unique tolerance of xeric ecotypes to water stress. This knowledge could then be helpful for future plant breeders to develop varieties more tolerant to drought stress.

PP-892
Antimicrobial activity of peptides from *Robinia pseudoacacia* seed

T. Talas-Ogras[1], Z. Ipekci-Altas[1], **K. Bajrovic**[2] and N. Gozukirmizi[3]
[1]*Plant Biotechnology, TÜBİTAK-Research Institute for Genetic Engineering and Biotechnology, 41470 Gebze-Kocaeli, Turkey,* [2]*Institute for Genetic Engineering and Biotechnology Sarajevo, 71000 Bosnia- Herzegovina,* [3]*Department of Molecular Biology and Genetics, Istanbul University, Faculty of Science, 34459 Vezneciler-Istanbul, Turkey. E-mail: tijen@gmbae.tubitak.gov.tr*

A low molecular weight of basic peptide with antimicrobial activity was isolated from *Robinia pseudoacacia* L. seed. The crude extract of seed was precipitated, dialysed, heat treated and small

proteins were obtained via filtration. The heat stable small peptides were fractionated through anion-exchange choromatography and the fractions were tested for antimicrobial activity *in vitro*. Antimicrobial activity of the fractions were tested by paper disk diffusion and turbidity measurement assays against six bacteria (*Bacillus subtilis, Corynebacterium michiganense, Erwinia carotovora subsp. carotovora, Escherichia coli, Pseudomonas syringae pv syringae, and Staphylococcus aereus*). Inhibition concentration IC_{50}values of a small cationic seed peptide against the bacteria changed between 20–120 μg/ml protein and *S. aereus* was found the most sensitive one, however *E. coli* script was not affected much when compared to others. The antibacterial activity of the peptides was negatively affected by addition of $CaCl_2$ into the assay medium.

PP-893
Inhibition kinetics of yeast glutathione reductase by zinc ion

B. Tandogan and N. Ulusu
Department of Biochemistry, Faculty of Medicine, University of Hacettepe, 06100 Ankara, Turkey.
E-mail: tandogan@hacettepe.edu.tr

Glutathione reductase (GR, type IV, from Baker s yeast, EC 1.6.4.2) is a crucial enzyme which catalyzes the reduction of oxidized glutathione (GSSG) to glutathione (GSH). Glutathione reductase is essential for the glutathione redox cycle that maintains adequate levels of reduced cellular GSH. Glutathione is a ubiquitous intracellular thiol present in all tissues and it serves as an antioxidant, reacting with free radicals and organic peroxides, in amino acid transport. and GSH is a substrate for the glutathione peroxidases and glutathione-S-transferases in the detoxification of organic peroxides and metabolism of xenobiotics. Because of this reason reduced form of GSH is very important for the cell functions and survival and may be involved in the development of diseases such as cancer and human immune deficiency. In this study, we have found that glutathione reductase is inhibited up to 2 mM concentration of Zn^{2+} and activated above this concentration. Kinetic characterization of the inhibition effects of Zn^{2+} on yeast glutathione reductase was investigated. We have studied the effect of Zn^{2+} (between 0.05 and 1 mM) on GR activity is consistent with non-competitive inhibition pattern, when the varied substrate is the oxidized glutathione and the NADPH, respectively.

PP-894
Diagnostic value of serum ghrelin levels in prostate cancer

A. Mungan[1], **G. Mungan**[2], S. Eminferzane[1], C. Yesilli[1], I. Seckiner[1], M. Can[2] and B. Akduman[1]
[1]Department of Urology, Zonguldak Karaelmas University, Zonguldak, Turkey, [2]Department of Biochemistry, Zonguldak, Turkey. E-mail: anmungan@yahoo.com

Objective: Expression of recently identified growth hormone releasing peptide, ghrelin, and its receptor has been demonstrated in prostate cancer cell lines. It was also demonstrated that ghrelin has increased cell proliferation *in vitro* when added to prostate cancer cell lines. The aim of this study was to evaluate diagnostic value of serum ghrelin levels in prostate cancer patients.
Material and method: Thirty patients with prostate cancer (PCA) and 50 patients with benign prostate hyperplasia (BPH) were enrolled in the study. The serum ghrelin levels of PCA and BPH patients were compared. Moreover, the correlations between ghrelin and age groups, body mass index, total PSA levels, free/total PSA ratio, Gleason score, prostate volume were also studied.
Results: There were no statistically significant differences between two groups and parameters mentioned above in terms of serum ghrelin levels ($P > 0.05$).
Conclusion: Although, ghrelin has been shown to induce prostate cancer cell proliferation and in-vitro studies suggests ghrelin may have a role in the control of neoplastic prostate cancer cell, its role in the diagnosis of prostate cancer was not demonstrated in our clinical study. The possible explanations for controversy of the results of *in-vitro* studies could be insufficient secretion of ghrelin into serum, insufficient sensitivity of the ELISA kit or the affect of other sources of ghrelin to serum ghrelin levels.

PP-895
Identification of elicitor-induced plant cytochrome P450s using differential display of mRNA

N. Turgut-Kara and S. Ari
Department of Molecular Biology and Genetics, Faculty of Science, İstanbul University, 34118, Vezneciler, Istanbul, Turkey.
E-mail: neslihantk@istanbul.edu.tr

Secondary metabolites are compounds with a restricted occurrence in taxonomic groups, that are not necessary for a organism to live, but play a role in the interaction of the organism with its environment, ensuring the survival of the organism in its ecosystem. The classification of plant secondary metabolites based on biosynthetic origin has as major examples the phenylpropanoids, terpenoids and polyketides. The phenylpropanoid pathway is one of the most important metabolic pathways in plants. They play important roles in protection against biotic and abiotic stressors. Plant cytochrome P450 monooxygenases (P450s) are membrane-bound heme proteins that function as the terminal component of a short electron transport chain. Plant P450s form a superfamily catalyzing a variety of reactions in plant secondary metabolism as well as in the metabolism of xenobiotics. The majority of the plant P450s are functional on phenylpropanoid pathway and we know that phenylpropanoid pathway can be upregulate by inductive conditions such as elicitor treatments. Elicitors which are derived from yeast induce expression of the phenylpropanoid pathway genes and other secondary metabolites in numerous plant species. In this study, we aimed to do P450-specific differential display of mRNA-reverse transcription-PCR (DD-RT-PCR) for the searching of cDNAs corresponding to P450s that are differentially regulated in response to yeast extract on the phenylpropanoid pathway.

PP-896
Binding properties of Nile blue and fisetin to plasma alpha-1-acid glycoprotein

F. Ulutas and N. Sumer
Department of Biochemistry, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey. E-mail: fulutas@hacettepe.edu.tr

Alpha-1-acid glycoprotein (AAG) was purified from human plasma obtained from Hacettepe University Hospitals Blood Bank by Cibacron blue F3GA affinity chromatography (PAAG). Purity was approved by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Binding properties of Nile blue (NB) as a phenoxazine dye and fisetin as a herbal flavonoid to PAAG and commercially available AAG were determined by flourometric titration assays. In the fluorometric titration assay of commercially available AAG with NB; Kd (dissociation

constant) and binding stochiometry (n) were calculated as Kd = 0.58 ± 0.04 μM and n = 0.28 ± 0.03 respectively. Similar findings were obtained in the assay of PAAG (Kd = 0.52 ± 0.04 μM, n = 0.32 ± 0.02). From the titration assays of commercially available AAG with fisetin before and after dialysis binding stochiometries were determined as 0.13 ± 0.01 and 0.22 ± 0.02 respectively, and dissociation constants were not changed (Kd = 0.37 ± 0.04). From the titration assays of commercially available AAG with NB before and after dialysis binding stochiometries were determined as 0.28 ± 0.03 and 0.47 ± 0.05 respectively, and lower value of dissociation constant (0.25 ± 0.01) was obtained after dialysis. These findings suggested that, low stochiometric values which were determined in NB and Fisetin arrays arise from heterogenity of preparation rather than the effect of endogenous ligand.

PP-897
Searching of nuclear import and export signals within ecdysteroid receptor

T. Gwóźdź[1], J. Dutko-Gwóźdź[1], C. Nieva[2], J. Dobrucki[3], M. Spindler-Barth[2], K. D. Spindler[2] and A. Ożyhar[1]
[1]Department of Biochemistry, Faculty of Chemistry, Wroclaw University of Technology, Wrocaw, Poland [2]Department of General Zoology and Endocrinology, University of Ulm, Germany, [3]Laboratory of Confocal Microscopy and Image Analysis, Department of Biophysics, Faculty of Biotechnology, Jagiellonian University, Kraków, Poland . E-mail: tomasz.gwozdz@pwr.wroc.pl

Nuclear receptors are ligand -regulated transcription factors that control key metabolic and developmental pathways in metazoan. The fruitfly *Drosophila melanogaster* has only 18 nuclear-receptor genes which represent all six subfamilies of vertebrate receptors. This establish the fly as an ideal system for studying the regulation and function of nuclear receptors during development. In insects there is only one known steroid hormone, 20-hydroxyecdysone which exerts its effect through functional ecdysteroid receptor – heterodimer of nuclear receptors EcR and Usp. From our previous investigation it is known that EcR can be localized in the nucleus as well as in the cytoplasm or mainly in the cytoplasm. To find out whether EcR contains nuclear import or export signals, series of deletion mutants tagged to yellow fluorescent protein were prepared and examined in mammalian cells. This analysis revealed that nuclear export signal is localized within ligand binding domain and suggested that N-terminal region (A/B) has the significant impact for distribution of EcR. The importance of A/B region was additionally supported by observations of difference in distribution of full-length isoforms of EcR which vary only in their N-terminal regions.

Acknowledgment: This study was supported by grant (2P04C04829) from the Polish Ministry of Education and Science.

PP-898
Determination of interaction between components of functional ecdysteroid receptor - Usp and EcR

J. Dutko-Gwóźdź[1], T. Gwóźdź[1], J. Dobrucki[2] and A. Ożyhar[1]
[1]Department of Biochemistry, Faculty of Chemistry, Wroclaw University of Technology, Wrocaw, Poland [2]Laboratory of Confocal Microscopy and Image Analysis, Department of Biophysics, Faculty of Biotechnology, Jagiellonian University, Krakow, Poland. E-mail: joanna.dutko-gwozdz@pwr.wroc.pl

Nuclear receptors are vital transducers of hormone signaling in regulation of development and homeostasis of higher eukaryotes. EcR and Usp are nuclear receptors which form functional ecdysteroid receptor in *Drosophila melanogaster*, and like all members of the superfamily contain highly conserved DNA and ligand binding domains (DBD and LBD, respectively). From our previous investigation it is known that Usp promotes nuclear localization of EcR in living cell. To investigate which regions of EcR play the most important role in Usp-dependent presence of EcR in the nucleus, analyses of living cells coexpressing Usp fused to CFP with all regions of EcR tagged with YFP were carried out. Only in case of EcRLBD there was significant change of localization. To prove that nuclear colocalization of full-length EcR and Usp can be caused by interaction of both proteins in LBD we used bimolecular fluorescence complementation method (BiFC). LBDs of both proteins were attached to non-fluorescence fragments of YFP and coexpressed in mammalian cells. Fluorescence which was observed predominantly in the cytoplasm indicates recombining of fluorescent protein caused by LBDs interaction. Here we show for the first time usefulness of BiFC to investigation of nuclear receptors interaction in living cells.

Acknowledgment: This study was supported by grant (2P04C01529) from the Polish Ministry of Education and Science.

PP-899
Brain natriuretic peptide and P wave durations in dialysis patients

C. Taskapan[1], S. Senel[2], O. Ulutas[2], Y. Aksoy[3] and H. Taskapan[2]
[1]Department of Malatya, Medical Faculty Biochemistry, Inonu University, Turkey, [2]Department of Malatya, Medical Faculty Internal Medicine, Inonu University, Turkey, [3]Department of Malatya, Medical Faculty Cardiology, Inonu University, Turkey. E-mail: taskapanmc@yahoo.com

Objectives: P wave duration and P dispersion [Pd = the maximum (Pmax)-minimum P (Pmin) durations] are considered to be most important non-invasive ECG markers to assess the atrial arrhythmia risk. Plasma Brain Natriuretic Peptide (BNP) level was reported as an independent predictor of atrial fibrillation. The aims of this study were to compare the effects of chronic hemodialysis (HD) and peritoneal dialysis (PD) on P wave duration, Pd and BNP and to examine the relationship between BNP levels, P wave duration, and Pd.

Design and methods: Age matched 22 HD (mean age, 52.3 ± 14.0 years) and 19 PD patients (mean age, 46.7 ± 10.9 years) were studied. Results: BNP levels were greater in HD patients before HD seans (459.0 ± 465.1 pg/ml) than in PD patients (139.0 ± 170.1 pg/ml). The P wave durations, and Pd rates were similar in both groups ($P > 0.05$). BNP levels were negatively related with Pmin duration (r = -0.518, P = 0.019) and BNP levels were positively correlated systolic and diastolic blood pressures(r = 0.672, P = 0.001; r = 0.497, P = 0.022 respectively) in HD patients.

Conclusions: Whereas BNP levels are higher in HD patients when they are on peak volume status, just before HD, P wave durations and Pd were similar both groups. Expansion of extra-cellular volume causing myocardial stretching may be the main cause of increased BNP in HD patients. The functional significance of BNP on P wave has not been defined. Additional studies are needed to evaluate the influence of BNP on P wave.

PP-900
Functional assessment of human alcohol dehydrogenase family in ethanol metabolism

S.-J. Yin[1], C.-T. Yao[2] and S.-L. Lee[3]
[1]*Department of Biochemistry, National Defense Medical Center, Taipei, Taiwan,* [2]*Department of Emergency Medicine, Cathay General Hospital, Taipei, Taiwan,* [3]*Division of Biotechnology, Animal Technology Institute Taiwan, Chunan, Taiwan.*
E-mail: yinsj@ndmc.idv.tw

This study was undertaken to quantitatively assess relative contributions of human alcohol dehydrogenase (ADH) isozymes and allozymes in ethanol metabolism in the context of the entire family. Kinetic parameters for ethanol oxidation for recombinant human class I ADH1A, ADH1B1, ADH1B2, ADH1B3, ADH1C1 and ADH1C2, class II ADH2, class III ADH3, and class IV ADH4 were determined. The composite numerical formulations for organ steady-state ethanol clearance were established by summing up the kinetic equations of constituent isozymes/allozymes with the assessed contents in livers and gastric mucosa with different genotypes. In *ADH1B*1* individuals, ADH1B1 and ADH1C allozymes were found the major contributors to hepatic alcohol clearance; ADH2 made significant contribution only at high ethanol levels (> 20 mM). ADH1B2 was the major hepatic contributor in *ADH1B*2* individuals. ADH1C allozymes were the major contributor at low ethanol (< 2 mM) whereas ADH1B3 the major form at higher levels (> 10 mM) in *ADH1B*3* individuals. For gastric mucosal alcohol clearance, the relative contributions of ADH1C allozymes and ADH4 were converse as ethanol concentration increased. The quantitative assessments support that the hepatic alcohol clearance of *ADH1B*2* individuals is higher than that of the *ADH1B*1*, and those of the *ADH1B*3* versus the *ADH1B*1* vary depending on sinusoidal ethanol levels.

PP-901
Transcription factor Sp1 and CpG methylation regulate the expression of human podocalyxin gene

N. Butta, S. Larrucea, S. Alonso, R. Rodriguez, E. Arias-Salgado, M. S. Ayuso and R. Parrilla
Centro de Investigaciones Biologicas (CSIC). Madrid. Spain.
E-mail: nbutta@cib.csic.es

Podocalyxin is a heavily glycosylated transmembrane protein expressed on the apical membrane of rat podocytes, endothelial and hematopoietic cells, human embryonal carcinomas and invasive breast cancer. The promoter region of the human podocalyxin gene has been cloned and its structure and function were analyzed. The basic promoter activity seems to rely entirely on Sp1 transcription factor: the minimal transcriptional activity (from 66 to -111 nts) increases as the number of Sp1 sites availability augmented. The role of the Sp1 sites was further studied in Sp1-lacking insect cells, and by directed mutagenesis of these sites. We also analyzed whether methylation of the CpG dinucleotides present in the first ˜210 nts of the promoter region of podocalyxin could explain the variable rates of expression in different types of cells. Inactivation of methyltransferases by 5'-aza-2'deoxicitidine showed a dose dependent increase of podocalyxin content in cells. *In vitro* methylation of some podocalyxin promoter constructs reduced their activity when transfected in HEK293 cells. Moreover, a correlation was found between the degree of methylation of the CpG promoter dinucleotides and the rate of podocalyxin expression. Our results indicate that transcriptional regulation of podocalyxin gene promoter is supported primarily by Sp1 site(s) and that DNA-methylation of the CpG promoter islands may also regulate the rate of podocalyxin expression.

PP-902
Bioelectrochemical sensing of phenolic compounds in organic solutions using modified horseradish peroxidase

H. Ghourchian
Biophysics, IBB (Tehran University), Tehran, Iran.
E-mail: molla@ibb.ut.ac.ir

Phenolic compounds, generally soluble and environmentally mobile class of compounds, can be introduced through a variety of industrial activities. It has been also reported that oxidoreductases are being explored for the removal of phenols from industrial wastewaters. Among them, horseradish peroxidase (HRP) is a promising c and idate for industrial application due to broad substrate specificity and its ability to operate at wide ranges of pH and temperature. In this study we tried to model modified HRP-catalyzed phenol coupling in the presence of hydroxylated aromatic compounds and H2O2 as HRP substrates. First, horseradish peroxidase was cleaved into the heme and apoenzyme. Apoenzyme was recombined with porphyrins to form a reconstituted holoenzyme. Then, modified HRP was immobilized on the surface of glassy carbon electrodes using an electron-donating mediator. Electron transfer kinetics of modified HRP was analyzed through comparing electrochemical properties of native molecule with those of artificially designed molecules. The results imply that the re-organization energy due to the structural change at the redox center during electron transfer reaction plays an important role in the electron transfer kinetics. In addition, the results show that electrochemical techniques as valuable tools for detection of phenolic compounds. Among the several compounds used as substrates for modified HRP, the detection limit of aniline was measured to be lower than the others.

PP-903
Detection of anti-CA II antibodies in patients with rheumatoid artritis and endometriosis

A. Menteşe[1], E. E. Keha[1], A. Alver[1], H. Çakırbay[2], M. Serdaroğlu[2], S. Cengiz[1] and F. Balaban[1]
[1]*Biochemistry, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey,* [2]*Physical Medicine and rehabilitation, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey. E-mail: amentese@ktu.edu.tr*

Autoimmune diseases arise from the immune responses of the body to its own antigens. In 1991, the presence of anti-CA II antibody has been first reported by Inagaki and et al. in the serums of patients with systemic lupus erythematosus and Sjögren's syndrome. After this year, anti-CA II antibodies were shown to exist in several autoimmune diseases by ELISA and Western blotting techniques. Here, it has been intended to establish an ELISA method in our laboratories in order to be able to determine the anti-CA II antibodies for some autoimmune diseases. For this purpose, by using the serums of the patients with endometriosis in which the presence of anti-CA II antibody had been formerly detected by Western blotting, performance evaluation for the ELISA method has been made. In addition, anti-CA II antibody levels have been detected in the serums of eight patients with endometriosis and 20 patients with rheumatoid arthritis. The presence of anti-CA II antibody in the serums of three of eight patients with endometriosis (37.5%) and 10 of 20 patients with rheumatoid arthritis (50%) have been shown. In conclusion,

it has been seen that the presence of anti-CA II antibodies for autoimmune diseases could be detected by this ELISA technique.

PP-904
Anti-carbonic anhydrase antibodies in autoimmune thyroid diseases

A. Alver[1], A. Menteşe[1], S. C. Karahan[1], C. Erem[2], E. E. Keha[1], K. Arıkan[1], O. Değer[1] and M. Çolak[1]
[1]Departments of Biochemistry, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey, [2]Internal Medicine, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey. E-mail: amentese@ktu.edu.tr

Carbonic anhydrase II (CA II) has an important role in thyroid hormone synthesis via regulating iodide (I-) transport across thyroidal cell membranes and the existence of autoantibodies against CA I and /or CA II have been showed in sera from patients with various autoimmune diseases such as Sjögren's syndrome, systemic lupus erythmatosus, type 1 diabetes, primary biliary cirrhosis and ulcerative colitis. The aim of this study was to investigate the presence of anti-CA I and CA II antibodies in autoimmune thyroid disease and the relationships between these autoantibodies and others clinical parameters. We studied 40 autoimmune thyroid patients (20 Hashimoto's thyroiditis, HT and 20 Grave's disease, GD) and 22 healthy control subjects. Serum anti-CA I and CA II antibodies were quantified by ELISA method. Positive results of anti-CA II (25 %) antibody were significantly higher in GD patients as compared to HT patients and control subjects ($P < 0.05$). There was no significant difference in the positive results of anti-CA I antibody. In addition, a significant correlation between serum anti-CA antibody titers and other studied clinical parameters were not found. The results suggest that anti-CA II antibodies may be involved in the pathogenesis of GD.

PP-905
Thermal stabilization of lipocalin-type prostagl and in D synthase induced by hydrophobic small ligand

T. Inui[1], S. Nishimura[1], M. Mochizuki[1], A. Tanaka[2], Y. Urade[3] and T. Iida[4]
[1]Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Sakai, Japan, [2]Faculty of Bioresources, Mie University, Tsu, Japan, [3]Department of Molecular Behavioral Biology, Osaka Bioscience Institute, Suita, Japan, [4]Department of Food and Nutrition, Tsu City College, Tsu, Japan. E-mail: inuit@bioinfo.osakafu-u.ac.jp

Lipocalin-type prostagl and in D synthase (L-PGDS) is a dual functional protein, acting as a PGD2-synthesizing enzyme and as an extracellular transporter protein for lipophilic ligands. In the previous study, we showed that two equilibrium states were formed during the unfolding process of L-PGDS by denaturants, an activity-enhanced state and an inactive intermediate. In this study, we investigated the thermal unfolding mechanism of L-PGDS and the effect of a hydrophobic small ligand biliverdin (BV), on the stability of L-PGDS. The recombinant mouse L-PGDS was dialyzed against 20 mM acetate buffer (pH 4.0). The protein solutions contained L-PGDS at 0.1–1 mg/ml, 20 mM acetate buffer (pH 4.0), and the appropriate amount of BV for circular dichroism (CD) and differential scanning calorimetry (DSC) analyses. The thermal unfolding of L-PGDS monitored by the CD measurements was shown to be approximately a two-state equilibrium folding. By the thermodynamic analyses, however, DSC curve was fitted the results given by a two-sequential transition model which considers the presence of an intermediate state between the native state and the unfolding one. In addition, the peak temperature of DSC curve of L-PGDS/BV complex was shifted to higher temperature with increasing concentration of BV. These results, taken together, demonstrated that L-PGDS possessed an equilibrium intermediate in a reversible thermal unfolding and was stabilized by the binding of hydrophobic small ligand .

PP-906
MMP-9 and TIMP-1 levels in the sputum of patients with chronic obstructive pulmonary disease and asthma

A. Ünlü[1], M. Çalıkoğlu[2], D. Gün[3], L. Ayaz[3] and **L. Tamer**[3]
[1]Department of Biochemistry, Meram Medical School, Selçuk University, Konya, Turkey, [2]Department of Chest Disease, Mersin University, Medical School, Mersin, Turkey, [3]Department of Biochemistry, Mersin University, Medical School, Mersin, Turkey. E-mail: aunlu@selcuk.edu.tr

Chronic obstructive pulmonary disease (COPD) and asthma are associated with destruction of lungand metalloproteinases are thought to play a role in this destruction. The aim of this study is to measure the levels of metalloproteinases in the sputum of COPD and asthma patients. We measured matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) levels in the sputum of 19 asthma and 17 COPD patients. MMP-9 and TIMP-1 levels were measured by using ELISA kits. There were no significant difference between COPD patients and asthma patients in MMP-9 levels (P : 0.196). On the other hand TIMP-1 levels were significantly higher in COPD patients than in asthma patients (88.3 ± 57 vs. 55.1 ± 52 μg/mg protein, respectively, P : 0.01). Atopic asthma patients TIMP-1 levels were also higher than non-atopic asthma patients. Atopic asthma patients TIMP-1 levels were nearly similar of COPD patients. There was no significant correlation between FEV1 and MMP-9 or TIMP-1 levels. COPD patients display higher TIMP-1 levels than asthma patients. Increased TIMP-1 levels and the imbalance between MMP-9/TIMP-1 may be associated with bronchial morphological changes.

PP-907
Impact of angiotensin-converting-enzyme polymorphism on hemorheological parameters

M. Bor Kucukatay, S. Turgut, G. Emmungil, G. Turgut and V. Kucukatay
Department of Physiology, Faculty of Medicine, Pamukkale University, Denizli, Turkey. E-mail: mbor@pau.edu.tr

An insertion/deletion (I/D) polymorphism within the angiotensin-converting enzyme (ACE) gene is known to increase the risk of cardiovascular events. Hemorheological disturbances have also been associated with several cardiovascular pathologies. This study aimed at investigating the possible relationship between ACE gene I/D polymorphism and hemorheological parameters in healthy, young volunteers (n = 28; 13 female, 15 male, mean age 24 ± 2). ACE I/D polymorphism was identified by polymerase chain reaction (PCR). Red blood cell (RBC) deformability and aggregation were measured using an ektacytometer (LORCA). Plasma and whole blood viscosities were determined with a Wells-Brookfield cone-plate rotational viscometer. The prevalence of the I and D alleles was 30.4% and 69.6%, respectively. The I/I genotype (II) was found in 21.4%, I/D genotype (ID) in 17.9% and D/D genotype (DD) in 60.7% of the subjects tested. No significant relationship between ACE I/D polymorphism and RBC aggregation or whole blood and plasma viscosity was observed.

In contrast, RBC deformability was significantly increased in the subjects with the DD genotype compared with the II ($P < 0.05$) and ID ($P < 0.01$) genotype or with the D allele with respect to the I allele ($P < 0.01$). We suggest that, RBC deformability of individuals with the ACE D allele who have higher risk for cardiovascular pathologies may have been increased probably by a compensatory mechanism.

PP-908
Acute effects of orexin A on plasma cortisol levels of rats

S. Turgut[1], B. Kaptanoğlu[2], **G. Turgut**[1], H. Erken[1], Y. Enli[2] and O. Genç[1]
[1]Department of Physiology, Faculty of Medicine, Pamukkale University, Denizli, Turkey, [2]Department of Biochemistry, Faculty of Medicine, Pamukkale University, Denizli, Turkey.
E-mail: gturgut@pau.edu.tr

Orexin A is hypothalamic peptides that regulate feeding behavior, energy metabolism, and sleep-wake cycle. Peripheral effects of this substance have become important due to the presence of orexin receptors in various peripheral tissues and the determination of orexin A in blood. Aim of our study was to determine acute effects of orexin A administered in different doses intraperitoneally on plasma levels of cortisol produced by adrenal glands. 10 Wistar albino adult male rats were used in this study and divided into two as first and second groups randomly. 10 and 15 μgr/kg doses of orexin A were intraperitoneally administered to first and second groups respectively. Blood samples were collected from the animals 2 h later and plasma levels of cortisol were analysed. The results were evaluated with Mann–Whitney U and Pearson Correlation tests. According to our results, a positive correlation was found between orexin A and plasma cortisol levels ($P = 0.007$ $r = 0.783$). The cortisol levels between the two groups increased with the doses ($P = 0.016$). This study shows that orexin A has an acute effect on plasma cortisol levels in a dose-dependent manner.

PP-909
Comparative analysis of the Bgs proteins family from *Schizosaccharomyces pombe*

I. M. Martins, J. C. Cortés, J. Muñoz, B. Moreno, A. Durán and J. C. Ribas
Instituto de Microbiología Bioquímica CSIC / University of Salamanca, Spain. E-mail: ivonemarisa@gmail.com

The cell wall is a vital structure that confers mechanical strength and support to the fungal cell and represents a specific target for the development of new antifungal agents. *S. pombe* contains four Bgs (beta glucan synthesis) proteins that share a high identity and are putative catalytic subunits of the (1,3)β-D-glucan synthase (GS) activity. The *bgs1*$^+$ mutants *cps1-12*, *cps1-N12* and *cps1-191* present septation and polarity defects. *bgs4*$^+$ mutants *cwg1-1* and *cwg1-2* display a thermosensitive lethal phenotype and a dramatic GS decrease, *orb11-59* is defective in cell polarity and *pbr1-1, -2, -3, -6 -8* are resistant to GS-specific antifungal drugs. In order to analyze whether each mutant phenotype is specific of the corresponding protein, we have performed a comparative analysis of Bgs1p, Bgs3p and Bgs4p. We located the mutations responsible for the bgs1 and bgs4 mutant phenotypes and created the corresponding mutation in *bgs1*$^+$, *bgs3*$^+$ and *bgs4*$^+$ sequences. We included Papulacandin or Echinocandin resistance mutations described in the *Saccharomyces cerevisiae* homologous proteins Fks1p and Fks2p. The mutants have been tested for a series of phenotypes and we show that although the analyzed aminoacids are identical or well conserved, each mutation produces different phenotypes, conferring even lethality or no effect depending on the protein.

PP-910
The kringle domain of T-PA inhibits endothelial cell migration by perturbing FAK and ERK1/2 activation

Y. A. Joe, H.-K. Kim, S.-B. Lee, B.-S. Shim and Y.-K. Hong
Cancer Research Institute, The Catholic University of Korea, Seoul 137-701, Korea. E-mail: youngjoe@catholic.ac.kr

We previously showed that the two kringle domain of tissue-type plasminogen activator (TK1-2) inhibits endothelial cell proliferation *in vitro*, and tumor cell growth *in vivo*. Since angiogenic process involves cell migration, we examined the effect of TK1-2 on migration and its mechanisms of action. In a modified Boyden chamber and wound migration assays, Pichia-derived TK1-2 inhibited dose-dependently HUVEC migration induced by VEGF, bFGF, or HGF, whereas it did not inhibit the migration of cancer cells, U87, A549, and HT1080. TK1-2 inhibited the attachment and spreading of endothelial cells to fibronectin or gelatin coated plates. In addition, it caused disassembly of actin stress fiber and focal adhesion induced by bFGF in HUVECs, and blocked bFGF-, VEGF-, or HGF-induced phosphorylation of ERK1/2. TK1-2 alone induced the phosphorylation of FAK, but inhibited bFGF- and HGF-induced phophorylation of FAK in HUVECs. However, TK1-2 did not inhibit the ERK1/2 phosphorylation induced by HGF in U87 cells. When dissecting the effects of TK1-2 on growth factor mediated- or integrin mediated-signaling pathway, TK1-2 inhibited the ERK1/2 activation in bFGF-stimulated suspended cells and fibronectin-stimulated cells. These results suggest that anti-migratory effect of TK1-2 in endothelial cells may be involved in abnormality of cytoskeleton rearrangement by perturbing both growth factor- and integrin-mediated signaling pathways.

PP-911
Erythrocyte membrane proteins changes in heterozygous and homozygous thalassemic cases

S. Yuzbasioglu, E. Yalin, S. Menziletoglu Yildiz and K. Aksoy
Department of Biochemistry, The Faculty of Medicine, Cukurova University, Adana, Turkey. E-mail: syuzbasioglu@cu.edu.tr

Erytrhocyte morphology has damaged in beta thalassemic cases. Globin chains accumulate at the membrane and its skeleton they cause alterations in deformability and stability. Many of these changes seen to follow the early accumulation of alpha globin and its co-localization with band 4.1 and spectrin. The deformation of erythrocytes is influenced by membrane stiffness, cellular viscosity and cell shape. The viscoelastic behavior of the erythrocyte membrane depends on membrane constituents and membrane-cytoplasmic constituents interactions. In this study we investigated changes of erythrocyte membrane proteins in heterozygous and homozygous thalassemic cases. We determined 49% normal, 41% one protein deficiency and 10% combine proteins deficiency of erythrocyte membrane proteins in 69 heterozygous thalassemic cases and also showed 49% one protein deficiency, 45% combine protein deficiency and 6% normal erythrocyte membrane proteins in 55 homozygous thalassemic cases. We think that erythrocyte membrane protein changes irresponsible first reason for shortening red cell survival due to both group also showed protein deficiencies.

PP-912
Membrane deformation in sickle cell anemia

S. Yuzbasioglu[1], S. Yalin[2], E. Yalin[1], S. Seydel[1] and **K. Aksoy**[1]
[1]*Department of Biochemistry, The Faculty of Medicine, Cukurova University, Adana, Turkey,* [2]*Department of Biochemistry, The Faculty of Pharmacy, Mersin University, Mersin, Turkey.*
E-mail: syuzbasioglu@cu.edu.tr

Sickle cell anemia is characterized by a life long hemolytic anemia. The vaso-occlusive manifestations of the sickling disorders probably result from the altered rheologic properties of red cell containing a high concentration of Hb S. It seems likely that physical trapping of erythrocytes in the microcirculation plays a major role in shortening red cell survival. In this study we inspected erythrocyte membrane protein deficiencies in with 39 sickle cell anemia patients. Spektrin, ankryn and band 3 protein levels were found different ($P < 0.05$) from control group ($n = 57$). Moreover we also determined 33% one protein deficiency, 62% combine protein deficiency and 2% normal of erythrocyte membrane proteins levels in patients. The most deficiency was detected spectrin and band 3 proteins. So that we think that membrane protein deficiency is effect of membrane deformation to erythrocyte shape and short cell life span.

PP-913
Thermoresistance elimination of multicellular tumor spheroid by an Hsp70 induction inhibitor

S. Khoei[1], A. Amerizadeh[2], D. Eslimi[2] and B. Goliaei[2]
[1]*Department of Biology, Faculty of Basic Medical Science, Iran University of Medical Science, Tehran, Iran,* [2]*Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran.*
E-mail: khoei@iums.ac.ir

We previously reported that in samples treated at 43°C for 120 min, the multicellular tumor spheroid culture expressed a higher level of Heat shock protein 70 as compared to monolayer culture of DU 145 prostate carcinoma cell line. Therefore, we showed that the acquired thermal resistance of spheroid cultures may be attributed to the higher level of Hsp70 production. In this study, we examined the role of Hsp70 in the thermoresistance of multicellular tumor spheroid model of DU 145 prostate carcinoma cell line using an inhibitor of Hsp70 induction by Western blotting and semiquantitative RT-PCR method. Our result showed that, Hsp70 induction with mild hyperthermia (43°C, 1 h) was completely blocked by quercetin at the concentration of 500 μM. Under similar conditions of quercetin and /or hyperthermia treatment, the heat resistance of spheroid cells was significantly decreased with quercetin as judged by the number of colonies that they formed in suspension cultures. These results support our previous study that the acquired thermal resistance of spheroid cultures attributed to the higher level of Hsp70 production and consequently use of an Hsp70 induction inhibitor decreased this resistance.

PP-914
Apoptotic effect of ABCG1 expression and function

L. Homolya, N. B. Elkind, L. Seres, J. Cserepes and B. Sarkadi
Research Group for Membrane Biology and Immunopathology, Hungarian Academy of Sciences, Budapest, Hungary.
E-mail: homolya@biomembrane.hu

ABCG1, a member of the ATP binding cassette transporter family, is expressed in numerous tissues, and its expression is highly activated by the nuclear receptor LXR in various cell types including macrophages. Several recent studies demonstrated an ABCG1-dependent cholesterol efflux from monocyte-derived macrophages. However, direct transport of cholesterol mediated by ABCG1 has not been shown. We observed that a substantial fraction of monocyte-derived macrophages undergo apoptosis in response to LXR activation in parallel with the increase of ABCG1 expression. To explore this phenomenon, we expressed ABCG1 and its inactive mutant variant, ABCG1KM, in different cell types. In an Sf9 cell, heterologous, transient expression system, the wild type (wt) ABCG1 accelerated cell death, as compared to the death of cells expressing either ABCG1KM or ABCG2. We found that various mammalian cells transfected with wt ABCG1 undergo early apoptotic events, such as phosphatidyl serine translocation and caspase 3 activation. In contrast, the expression of the inactive ABCG1KM variant does not cause apoptosis in these cells. ABCG1-induced apoptosis was prevented by the inhibitors of ABCG1 function, thyroxin and benzamil. These data indicate that the early apoptotic events and the subsequent cell death are connected with the activity of the ABCG1 transporter. We assume a significant contribution of ABCG1 expression and function to the LXR-induced apoptosis in monocyte-derived macrophages.

PP-915
Detection of SOCS-1 (suppressor of cytokine signaling-1) methylation in CML (chronic myeloid leukemia) patients

O. Hatirnaz[1], C. G. Ekmekci[1], C. Ar[2], U. Ure[2], T. Soysal[2], B. Ferhanoglu[2] and U. Ozbek[1]
[1]*Genetics Department, Institute for Experimental Medicine Research, Istanbul University, Istanbul, Turkey,* [2]*Hematology Department of Internal Medicine, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey.*
E-mail: ozdenhatirnaz@yahoo.com

SOCS-1 (Suppressor of Cytokine Signaling-1) gene is one of the members of SOCS protein family which are suppressors of cytokine signaling and inhibition of its transcription could cause severe defects. Like all the members of SOCS family, SOCS-1 has a cytokine specific inhibition. Especially the signaling of IL-2, IL-4, IL-6 and IFN-α are suppressed by SOCS-1 protein. The aim of this study was to detect the methylation of SOCS-1 gene in CML patients and to find out any possible relation. Seventy patients and 16 controls were studied for the methylation of SOCS-1 promoter and exon 2 regions. After the sodium bisulfite treatment, the DNA samples were amplified by two different primer sets (one for methylation status and one for unmethylation status). The methylation of exon 2 was found to be 64.4% and 93.8% in CML patients and controls respectively [$P = 0.020$, OR = 0.121(0.015–0.957) 95% CI]. The promoter region methylation was not detected in any patient samples or controls. Although a previous study revealed a relation between the methylation of SOCS-1 promoter and CML development, the results of this study showed no correlation between the SOCS-1 methylation and development of CML, neither in the exonic region nor in the promoter region. On the other hand our results could be representing a hypomethylation in CML patients for the studied regions and the samples should be studied in this manner, which will be the next step of this study.

PP-916
Production and characterization of an extracellular lipase activity from mucor hiemalis *f. corticolus* IDM11B

A. Özen[1], S. Ülker[2], Ş. Alpay Karaoğlu[2] and A. Çolak[3]
[1]*Department of Chemistry, Rize Faculty of Arts and Sciences, KTU, Rize, Turkey,* [2]*Department of Biology, Rize Faculty of Arts and Sciences, KTU, Rize, Turkey,* [3]*Department of Chemistry, Faculty of Arts and Sciences, KTU, Trabzon, Turkey.*
E-mail: arzuozen61@hotmail.com

In this work, Mucor hiemalis *f. corticolus* IDM11B fungus isolated from soil was chosen as a source for lipase. The level lipase activities and the preliminary kinetic data for its characterization in the crude extracts from Mucor hiemalis *f. corticolus* IDM11B were obtained. The lipase activity was assayed in the presence of substrates as p-nitrophenyl acetate, p-nitrophenyl butyrate, p-nitrophenyl palmitate. Moreover, pH and temperature optimum and stability were determined. The effect of some metals on lipase activities was also tested. The activity was determined using all substrates by measuring absorbance at 405 nm. One unit of lipase activity was defined as the amount of 1 μM p-nitrophenolate released per minute. The greatest activities were observed in the presence of p-nitrophenyl acetate. The maximum velocity (Vmax) was 250 000 U/l and Michaelis-Menten constant (Km) was 175 mM. The optimum activity was seen at pH 8.5 and 30. The enzyme was extremely stable at its optimum pH of 8.5 in presence of p-nitrophenyl acetate as the substrate and it retains 100% of original lipase activity after 24 h of incubation at +4 °C at this pH. The preliminary data obtained in this study have shown that this fungus contains an active lipase having similar biochemical characteristics to other lipases.
Acknowledgment: (This work was supported by TUBITAK).

PP-917
Structural and functional studies of bovine liver catalase upon chemical modification of carboxyl residues

S. Hashemnia, H. Ghourchian and A. A. Moosavi-Movahedi
Institute of Biochemistry and Biophysics, University of Tehran, Tehran. E-mail: Hashemnia@ibb.ut.ac.ir

The chemical modification of carboxyl residues of bovine liver catalase (BLC) at different concentrations of Woodward's Reagent K (WRK) (0.5–15 mM) was studied by enzymatic activity measurements and optical methods such as fluorescence and absorption spectroscopies. Reaction of carboxyl groups of glutamic and aspartic acid residues with WRK in 50 mM phosphate buffer, pH 7, is happened by covalently attached chromophores with an absorption maximum at about 340 nm. This can be used to quantitate the reaction of reagent with carboxyl groups in BLC and calculated modification number (moles of modified residues/mole of the enzyme). The enzymatic activity was found to be changed in the concentration range over 2 mM of WRK. The intrinsic fluorescence and 1-anilino-8-naphtalene sulfonate (ANS) fluorescence of BLC were found to be dependent on the chemical modification of the enzyme with WRK. The modified enzyme exhibits a blue shift in the emission maximum as well as a decrease in the fluorescence intensity. A decrease in ANS fluorescence intensity with increasing modifier concentrations was observed. These observations indicate that during the chemical modification the tertiary structure of catalase was altered and hydrophobic clusters were buried.

PP-918
Production and characterization of an extracellular lipase activity from *Trichoderma harzianum* IDM14D

S. Ülker[1], A. Özen[2], Ş. Alpay Karaoğlu[1] and A. Çolak[3]
[1]*Department of Biology, Rize Faculty of Arts and Sciences, KTU, Rize, Turkey,* [2]*Department of Chemistry, Rize Faculty of Arts and Sciences, KTU, Rize, Turkey,* [3]*Department of Chemistry, Faculty of Arts and Sciences, KTU, Trabzon, Turkey.*
E-mail: s.ulker@ktu.edu.tr

In this work, *Trichoderma harzianum* IDM14D fungus isolated from soil was chosen as a source for lipase. The level lipase activities and the preliminary kinetic data for its characterization in the crude extracts from *Trichoderma harzianum* IDM14D were obtained. The lipase activity was assayed in the presence of substrates as p-nitrophenyl acetate, p-nitrophenyl butyrate, p-nitrophenyl palmitate. Moreover, pH and temperature optimum and stability were determined. The activity was determined using all substrates by measuring absorbance at 405 nm. One unit of lipase activity was defined as the amount of 1 μM p-nitrophenolate released per minute. The greatest activities were observed in the presence of p-nitrophenyl butyrate. The maximum velocity (Vmax) was 4348 U/l and Michaelis-Menten constant (Km) was 67 mM. The optimum activity was seen at pH 8.5 and 40. The enzyme was extremely stable at its optimum pH of 8.5 in presence of p-nitrophenyl butyrate as the substrate and it retains over 90% of original lipase activity after 24 h of incubation at +4 °C at this pH. Thermal stability graphic shows that this enzyme is very heat sensitive. The preliminary data obtained in this study have shown that *Trichoderma harzianum* IDM14D contains an active lipase having similar biochemical characteristics to other lipases.
Acknowledgment: (This work was supported by TUBITAK).

PP-919
Fluorescence and circular dichroism studies for AGEs formation of human serum albumin in turnover incubation of glucose

N. Sattarahmady and **A. A. Moosavi-Movahedi**
Institute of Biochemistry and Biophysics, Tehran University, Tehran, Iran. E-mail: sattar@ibb.ut.ac.ir

Glycation, a deleterious form of post-translational modification of proteins has been linked to disease such as diabetes, cataract, Alzheimer's, dialysis related amyloidosis and Parkinson's as well as physiological aging. The objective of this investigation was to determine the effect of nonenzymatic glycation by glucose on the secondary structure of human serum albumin (HSA) and to also demonstrate the *in-vitro* formation of AGEs (advanced glycation end products) by this sugar under turnover time of HSA in the circulation. The formation of AGEs was monitored by fluorescence spectroscopy and selected excitation / emission wavelengths as follows: 322/431, 342/440, 352/453, 375/460 and 381/460 for detection of AGEs. The changes in the secondary structure were determined by circular dichroism (CD) profiles of the native and glycated proteins. For this purpose, HSA (40 mg/ml) was incubated with glucose in various concentration (50–500 mM), that is suggested as a model for long-term entrapment of plasma proteins in aging tissue, in phosphate buffer in 37 °C at 36 days. It is used these samples for investigation after dialysis against phosphate buffer. The results indicate strong conformational changes in the modified protein by the changes of α-helix structure to β-pleated and random coil conformation of HSA and increase the level of AGEs correspondence to the value of glucose.

PP-920
The effect of cold stress on adrenomedullin levels in some rat tissues

N. Cicikoglu Yildirim and M. Yurekli
Department of Molecular Biology, Inonu University, 44069 Malatya, Turkey. E-mail: nuran@inonu.edu.tr

Exposure to extreme environment is a form of stress to be competed by the organism. Recently animal models are being developed utilizing cold application to study the physiological mechanisms and the effect of stress. AdM is produced as apart of a 185 amino acid pro-hormone, called preproadrenomedullin which also contains on unique 20 amino acid residue in its N-terminus, exerting hypotensive action and named proadrenomedullin N-terminal 20 peptide (PAMP). AdM has been reported to be present in a normal adrenal medulla, heart, lung and kidney as well as in plasma and urine. In this study, the effects of cold stress on adrenomedullin levels were investigated in some rat tissues such as kidney, smooth muscle and striate muscle. 12 Sprague Dawley rats weighing 200–250 g were used. Rats were housed under diurnal lighting conditions (12–12 h) with free access to food and water. For this study rats were divided into two groups, control group ($n = 6$) and group receiving cold stress. AdM levels were measured by high performance liquid chromatography. ANOVA and least significant differences test (LSD) was used in statically analysis and the values were expressed as mean ± SD. AdM levels were lower that controls. The differences were significant ($P < 0.05$). AdM levels were decreased depend on cold stress. The results suggested that AdM may play possible role in adaptation to stress.

PP-921
The role of the complement regulatory protein factor H in the hellp syndrome pathogenesis

Ş. Tekin[1], E. Arı[2], G. Haklar[1], Y. Taga[1] and Ç. Özener[2]
[1]Department of Biochemistry, Faculty of Medicine, Marmara University, Istanbul, Turkey, [2]Internal Medicine, Subdepartment of Nephrology, Faculty of Medicine, Marmara University, Istanbul, Turkey. E-mail: sebnemtekin_71@yahoo.com

The Hellp Syndrome is a manifestation of pre-eclampsia occurring in 0.6% of pregnancies. Its pathologic features are endothelial cell injury and vasospasm with thrombosis in microcirculation. Factor H is a major regulatory protein that limits the auto activation of the alternative complement pathway. The aim of this study was to show the correlation of factor H with the increase of C3 during normal pregnancies in comparison with pregnancies with Hellp syndrome. In this study 22 patients with Hellp syndrome, 21 with pre-eclampsia, 23 healthy pregnant and 24 healthy women, all of the same age, were enrolled. Serum AST, ALT, LDH, total and direct bilurubin, uric acid and creatinine concentrations were measured spectrophotometrically (modular p800, Roche, Germany). Furthermore, we measured Factor H levels with the radial immunometric method and C3 levels nepholometrically (BN ProSpec, Dade Behring, Germany). Hemoglobin and thrombocyte concentrations were also measured (Coulter LH750, Beckman Coulter, USA). In Hellp Syndrome patients AST, ALT, LDH, total and direct bilurubin levels were significantly increased ($P < 0.005$), whereas Hband thrombocyte concentrations were decreased ($P < 0.005$). The uric acid and creatinine concentrations were normal. The Factor H and C3 levels showed an increase in all pregnant patients; therefore they were not shown to be important in the pathogenesis of Hellp syndrome. Intensive research with more patients is necessary to show the possible relation between them.

PP-922
Gene expression profiling with target specific preamplified cDNA using a TaqMan® low density array

K. Lee, F. Chan, R. Samaha, S. Stanley, J. Stevens and N. Tuason
Applied Biosystems, Foster City, CA, USA. E-mail: leeky@appliedbiosystems.com

Quantitative gene expression analysis of multiple gene targets from small amounts of RNA is highly desirable when screening samples with limited material (FFPE, LCM, blood) for biomarkers or for developing gene signatures. A preamplification reagent, TaqMan® PreAmp Master Mix, allows unbiased, uniform amplification of up to 100 gene specific targets from as little as 1 ng total RNA for subsequent analysis with TaqMan® Gene Expression Assays. When a large number of samples need to be analyzed, a Taqman® Low Density Array can be used. The TaqMan Array is a consumable consisting of 384 wells connected by a series of micro fluidic channels and preloaded with a specific set of TaqMan Assays as in a fixed TaqMan® Low Density Array Gene Signature Panel (Human Immune Profiling Panel, Human Endogenous Control Panel, Human Protein Kinase Panel and Human GPCR Panel). In this study we will use a Gene Signature Panel to compare the relative gene expression data generated from preamplified cDNA from a very small amount of RNA to the same non-amplified sample. We will show that TaqMan® Low Density Array can be used with preamplified samples with the same precision and sensitivity as non-amplified samples.

PP-923
Role of asymmetric dimethylarginine and vascular endothelial growth factor in etiopathogenesis of preeclampsia

Z. S. Altun[1], S. Uysal[2], G. Guner[3], O. Yilmaz[4] and C. Posaci[5]
[1]Departmant of Oncology, Dokuz Eylul University The Institute of Health Sciences, Izmir, Turkey, [2]Department of Biochemistry, Dokuz Eylul University School of Medicine, Izmir, Turkey, [3]Department of Biochemistry, Dokuz Eylul University School of Medicine, Izmir, Turkey, [4]Department of Laboratory Animals, Dokuz Eylul University School of Medicine, Izmir, Turkey, [5]Department of Obstetrics and Gynaecology, Dokuz Eylul University School of Medicine, Izmir, Turkey. E-mail: zekiye.altun@deu.edu.tr

Preeclampsia is a multifactorial and multisystemic pregnancy specific disease that its pathophysiology is remaining largely unknown. This study was carried out to elucidate vascular endothelial growth factor (VEGF), asymmetric dimethylarginine (ADMA) and nitric oxide levels, which are hypothesized to lead to endothelial dysfunction; and to study the affect of L-arginine in preeclamptic rats. The preeclamptic rat model was used on stress. VEGF in serum was determined using ELISA and plasma ADMA levels by HPLC. There were no meaningful difference of serum VEGF and urinary nitrate levels among all groups. In the preeclamptic group, plasma levels of ADMA were considerably increased when compared to the pregnant control group. Differences in the levels of ADMA between the preeclamptic and L-arginine treated preeclamptic group were statistically significant. Among all the groups, blood pressure measures have a correlation with ADMA levels ($r = 0.492$, $P = 0.015$) and proteinuria ($r = 0.748$, $P = 0.000$). Our findings concluded that arginine-ADMA-nitric oxide pathway and VEGF are affected in preeclampsia and have a significant role in the etiopathology of

the disease. Returning the diagnostic criteria of preeclampsia to normal levels is showing that taking L-arginine has a significant role in decreasing the preeclampsia risk. However, since blood pressure levels did not decrease to normal pregnant levels, it is suggested that further research in L-arginine prophylaxis should be useful.

PP-924
Malate dehydrogenase activity in seminal plasma may be a new marker for infertility

H. Leventerler[1], S. Taga[1], M. T. Cetin[1], S. Solmaz[3] and **N. Dikmen**[2]
[1]*Cukurova University, Faculty of Medicine Gynecology and Obstetric Department-Center of Assisted Reproduction, Adana, Turkey,* [2]*Department of Biochemistry, Adana, Turkey,* [3]*Department of Histology and Embryology, Adana, Turkey. E-mail: hleventerler@cu.edu.tr staga@cu.edu.tr mtcetin@yahoo.com ssolmaz@cu.edu.tr ndikmen@cu.edu.t*

Energy production in most cells requires the enzymes of glycolytic pathway and Krebs cycle. Malate Dehydrogenase (MDH) catalyzes the oxidative decarboxylation of malate to pyruvate together with the reduction of the NAD^+ or $NADP^+$. MDH has an effective role on energy metabolism of sperm. In this study, semen samples were evaluated by light microscopy. NAD and NADP dependent MDH activities in seminal plasma were evaluated spectrophotometrically in normozoospermic (control $n = 40$) and subnormal group ($n = 13$). Following results (mean ± SD) were obtained for the normozoospermic group: Sperm concentration 53.1 ± 31.6 (xmillion/ml), motility 71.3 ± 6.6 (% motile), morphology 17.8 ± 4.4 (% normal). MDH-NAD activity 93.9 ± 52.1 (mU/ml) and MDH-NADP activity was 155.1 ± 77.7. Subnormal group consists of semen samples which contain lower spermiogram values and the cut-off value for MDH-NAD activity in this group was 20 mU/ml. We observed that the men in the subnormal group were infertile and also half of them were azospermic with the absence of sperm in their semen. We believe that MDH-NAD activity in seminal plasma may be an important marker to evaluate sperm functions related to male infertility.

PP-925
Characterization of the novel CK1-mediated phosphorylation of sulfatide- and cholesterol sulfate-binding proteins

F. Kawakami[1], T. Yamamoto[2], K. Suzuki[1] and K. Ohtsuki[1]
[1]*Laboratory of Signal Biology, Graduate School of Medical Sciences, Kitasato University, Sagamihara, Japan,* [2]*Biological Laboratory, Center for Natural Science, Kitasato University, Sagamihara, Japan. E-mail: kawakami82@hotmail.com*

Purpose: Recently, we reported that sulfatide- and cholesterol sulfate (CS)-binding proteins (SCS-BPs), such as HMG1, C-kinaseη, complement C3a, FGF-binding protein (p37) and myelin basic protein (MBP), are highly phosphorylated by casein kinase 1 (CK1) when incubated with ATP in the presence of either sulfatide or CS *in vitro* [Kawakami *et al.*: *Biol. Pharm. Bull.*, 27, 282, 2004]. However, the novel phosphorylation sites for CK1 on these SCS-BPs and the physiological significance of their phosphorylation by CK1 remain to be elucidated. Therefore, we biochemically characterized the CK1-mediated sulfatide- and CS-dependent phosphorylation of SCS-BPs *in vitro*.
Results and Discussion: The novel phosphorylation sites for CK1 were determined using several fragments obtained from two SCS-BPs (MBP and tau protein) and their synthetic fragments *in vitro*. Some fragments were effectively phosphorylated by CK1 in a sulfatide- or CS-dependent manner *in vitro*. At least two distinct common novel CK1 recognizing motifs [K/RXK/RXXS/T and S/TXXS/TXXXK/R] were found in these phosphorylated fragments. In addition, the fragments of two novel motifs containing K/R has a binding ability with sulfatide or CS *in vitro*. Finally, it is concluded that CK1 may be responsible for the regulation of SCS-BPs, including MBP and tau protein, through their preferential phosphorylation at an increased sufficient level of sulfatide and /or CS in brain.

PP-926
Disruption of *hom* gene of *Streptomyces clavuligerus and* its effects on cephamycin C yields

A. Koca Caydasi[1], **E. Ince Yilmaz**[2] and G. Ozcengiz[1]
[1]*Biology, Middle East Technical University, Ankara, Turkey,* [2]*Biology, Dicle University, Diyarbakir, Turkey. E-mail: e118000@metu.edu.tr*

The members of the genus *Streptomyces* are well-known microorganisms for their capacity to synthesize a vast repertoire of secondary metabolites, including many useful antibiotics and proteins. *Streptomyces clavuligerus*is the producer of the medically important β-lactam antibiotics, including cephamycin C and clavulanic acid. The aspartate pathway of *S. clavuligerus* is an important primary metabolic pathway which provide substrates for the antibiotic biosynthesis. Carbon flow through the lysine-specific branch of aspartate pathway is a rate limiting step for the formation of cephamycin C. The other branch of the pathway which leads to L-methionine, L-isoleucine, and L-threonine biosynthesis starts with the enzyme homoserine dehydrogenase. The aim of this study is the disruption of the homoserine dehydrogenase gene (*hom*) encoding for the first enzymatic step of this branch and determination of its effect on cephamycin C production. In the study, *hom* gene previously cloned in our laboratory was blocked via insertion of a kanamycin resistance cassette. The disrupted *hom* gene was transferred to *S. clavuligerus* cells using the Streptomyces plasmid vector PIJ486. The hom mutants resulting from the homolog recombination between the chromosomal *hom* gene and the disrupted *hom* gene on the plasmid was next selected. The present study describes volumetric and specific antibiotic production patterns of the knock out mutants.

PP-927
Effect of homologous multiple copies of *ask* gene on cephamycin C biosynthesis in *Streptomyces clavuligerus*

B. Taskin[1], S. Tunca[1] and **G. Ozcengiz**[2]
[1]*Biotechnology, Middle East Technical University, Ankara, Turkey,* [2]*Biology, Middle East Technical University, Ankara, Turkey. E-mail: tbilgin@metu.edu.tr*

Streptomyces clavuligerus is a gram-positive filamentous bacterium well known for its ability to produce an array of β-lactam compounds (secondary metabolites) including cephamycin C, clavulanic acid and other structurally related clavams. Carbon flow through the lysine branch of the aspartate biosynthetic pathway is a rate-limiting step in the formation of cephamycin C, a broad spectrum beta-lactam antibiotic produced by *Streptomyces clavuligerus*. The genes which encode the enzymes catalyzing the first two steps of the aspartate pathway, *ask* (aspartokinase) and *asd* (aspartate semialdehyde dehydrogenase) in *S. clavuligerus* NRRL 3585 were cloned, sequenced and heterologously expressed for the first time in our previous studies. Amplification of ask gene

alone in a multi-copy *Streptomyces* plasmid vector, pIJ486, and determination of the effects of multiple copies on cephamycin C biosynthesis were the goals of the present study. For this purpose, *ask* gene was cloned into this vector and *S. clavuligerus* protoplasts were efficiently transformed with the recombinant plasmid named pTB486. After stable recombinants were obtained, the Ask activity, and growth patterns as well as the volumetric and specific cephamycin C yields of the recombinants were determined and compared in both rich and defined media.

PP-928
Cytochrome c based biosensor for H2O2 measurements

A. Altuğ, A. Akkaya, N. Pazarlıoğlu and E. Dinçkaya
Biochemistry, Ege University, Izmir, Turkey.
E-mail: cagri19782000@yahoo.com

The determination of H2O2 is of interest to many fields, such as clinic, food, pharmaceutical and environmental analyses, because H2O2 is released during the oxidation of substrates in the presence of oxygen. Electroanalytical methods are suitable for the determination of H2O2 since they can achieve low detection limits and rapid response time on the basis of direct reduction or oxidation of H2O2. Biosensors have utility in analytical research but also in clinical diagnosis, food and pharmaceutical industry, environmental control and process monitoring. The interest in biosensor development has partly arisen from the need of fast and routine analysis of a large number of samples. *In vivo*, Cyt-c are part of the energy-conserving electron transport system. In living systems no catalytic activity of Cyt-c has been described. However, forty years ago the ability of Cyt-c to induce lipid peroxidation as well as its involvement in hydroperoxide cleavage were reported. Cyt-c presents several advantages for use as a biocatalyst: does not lose its heme catalytic group in organic systems, while peroxidases do; active over a wide range of pH; is able to perform biocatalytic reactions at higher temperatures; is cheap. Cyt-c was immobilized by gelatin and glutaraldehyde onto dissolved oxygen electrode to measure H2O2 concentration in pH 7, 50 mM phosphate buffer. Optimum pH, repeatability of the biosensor and peroxidase activity were investigated.

PP-929
Genetic relationship between Latvians and neighbouring European populations, revealed by mitochondrial DNA analysis

A. Krumina[1], L. Pliss[2], M. Lazdins[2] and V. Baumanis[2]
[1]Riga Stradins University, Riga, Latvia, [2]University of Latvia, Riga, Latvia. E-mail: Astrida.Krumina@rsu.lv

The analysis of mitochondrial DNA (mtDNA) polymorphisms has been shown to be a powerful tool in unraveling the past of human populations. Studies of the diversity of European mtDNA variants so far has been restricted to Western and Central Europe. The aim of the research was to compare the mtDNA sequences of Latvians with those of neighbouring populations. MtDNA data for 299 healthy unrelated Latvians (L.Pliss et al., 2005) were compared with published data for nine neighbouring populations. The principal component analysis performed by POPSTR program showed that all compared populations, except Finns, formed a tight cluster. Calculation of genetic pair wise distances by use of ARLEQUIN package gave controversial results, depending on the type of comparison (haplogroups only, haplotypes only, or combined data), which can be explained by methodological imperfection. Results of the admixture analysis (ADMIX 2.0 program) and lineage sharing analysis were similar: the highest proportions of shared haplotypes of Latvians were with Estonians (56.2%), Lithuanians (39.5%), Germans (38.8%), Russians and Poles (19.4%). The performed comparison of mtDNA data showed that Latvians share haplotypes with all neighbouring populations, in particular Estonians, irrespective of their linguistic affiliation.

PP-930
Erythrocyte arginase activities in inactive lepromatous leprosy patients

S. Temizer Ozan[1], M. Erişir[1], S. Yılmaz[1], G. Ozan[2] and A. Seyran[1]
[1]Department of Biochemistry, Faculty of Veterinary Medicine, Fırat University, Elazığ/ Turkey, [2]Department of Biochemistry, Faculty of Medicine, Fırat University, Elazığ/ Turkey.
E-mail: sematemizerozan@yahoo.com

Leprosy, an infection caused by Mycobacterium leprae, is a chronic infectious disease which attacks superficial tissues, especially the skin, peripheral nerves and mucouse membranes (eyes, respiratory track). Arginase (L-arginine amidinohydrolase; E.C. 3.5.3.1), which catalyses the conversion of arginine to urea and ornithine, is one of the five members of urea cycle enzymes that convert ammonia to urea as the principal product of nitrogen excretion. The purpose of this study was to investigate erythrocyte arginase activities in inactive lepromatous leprosy patients. The subjects for this study were healthy human volunteers ($n = 20$) and inactive lepromatous leprosy patients released from treatment ($n = 34$). The levels of erythrocyte arginase activities in inactive lepromatous leprosy patients released from treatment and in healthy human volunteers were 25.27 ± 1.82 and 20.91 ± 1.57, respectively. Erythrocyte arginase activity was slightly increased but not significantly in inactive lepromatous leprosy patients in comparison to control group. The results suggest that the erythrocyte arginase activities are not significantly influenced in inactive lepromatous leprosy.

PP-931
N-acetyl transferase (NAT1&NAT2) and glutathione-S transferase (GSTM1&GSTT1) polymorphisms in breast cancer

T. Guray[1], A. Atalay[1], Y. S. Su[1], M. Altinbas[2] and M. A. Atalay[3]
[1]Department of Biological Sciences, Middle East Technical University, Ankara/Turkey, [2]Department of Internal Medicine, Faculty of Medicine, Erciyes University, Kayseri/Turkey, [3]Department of Microbiology, Faculty of Medicine, Erciyes University, Kayseri/Turkey. E-mail: aycinatalay@gmail.com

Breast cancer is the most frequent malignancy among women, especially in Western societies. Highly penetrant genes such as BRCA1 and BRCA2, together with the reproductive history can constitute only 30% of the cause, so there should be other common genes, which may play a role in breast carcinogenesis according to one's lifestyle. In our case, the effect of N-acetyl transferases (NAT1, NAT2) and glutathione-S transferases (GSTM1&GSTT1) were investigated, since variations in these genes may alter their enzymatic activity and therefore their capacity to biotransform xenobiotic compounds. To evaluate the potential association between NAT1, NAT2, GSTM1 and GSTT1 genotypes and development of breast cancer, a hospital based case-control study was conducted in a Turkish study population consisting of 52 histologically confirmed incident breast cancer cases and 48 control subjects with no present or previous history of cancer. The only recognizable difference between case and control groups is the percentage

of GSTM1 deletion, 35% and 21% respectively ($P = 0.066$). The frequency of rapid NAT2 acetylator genotype is 24% in cases and 11% in controls. Especially, women with NAT2 rapid acetylator and GSTM1 null genotypes were at the elevated risk. NAT1 rapid acetylator genotype showed no association with breast cancer. These results suggest that GSTM1 null genotype is a susceptibility factor for breast cancer, particularly in the presence of NAT2 rapid acetylator genotype.

PP-932
The investigation of homocysteine and vitamin -B12 folic acid levels in ovary cancer

A. Ç. Özkiliç and M. Cengiz
Department of Medical Biology, Cerrahpaşa Medical Faculty, İstanbul University, İstanbul, TÜRKİYE.
E-mail: aozkilic@istanbul.edu.tr

The primary function of folate is a carrier for single carbon fragments in the conversion of Hcy to methionine and in purine and pyrimidine synthesis. Methylentetrahydrofolatereductase (MTHFR) plays a central role in folate metabolism, DNA synthesis and repair. Hcy plasma level is that associated with the MTHFR 677 genotype is a representative marker of intracellular folate status. It was suggested that decreasing folate levels related to hyperhomocysteinemia due to genetic polymorphism could be associated with carcinogenesis. The level genetic damage in patients with ovarian malignancy has been studied by the comet assay. This technique is a simple, rapid, sensitive and visual technique for measuring and analyzing DNA damage in mammalian cells. In our study Hcy, folic acid, vitB12 levels were studied for 39 ovary cancer patients and 52 healthy controls respectively. Hcy levels were measured by Elisa, B12 folic acid levels were measured by chemiluminescent assay. The Student-*t* test was used for comparisons between groups. The mean levels of Hcy, folic acid, vitB12 in ovary cancer were 8.35 ± 4.32; 6.38 ± 3.64; 257.97 ± 117.65 in control groups were 8.73 ± 3.92; 6.55 ± 2.19; 302.7 ± 96.76. There were no significant differences found in Hcy, folic acid and vitB12 levels between ovary cancer and healthy control groups. Comparison of the results of comet assay in patient and control subject showed a significant difference in the number of damaged cells.

PP-933
The assessment of biochemical parameters, carotid IMT and fibrinogen levels in chronic renal failure patients

T. Tunçkıran Sofu[1], **B. Arslan Şentürk**[1], F. Üstüner[1], S. İnce[2] and M. Cirit[3]
[1]Department of Biochemistry and Clinical Biochemistry, 35360, Atatürk Training Hospital, Izmir, Turkey, [2]Department of Radiology, Atatürk Training Hospital, [3]Department of Nephrology, Atatürk Training Hospital.
E-mail: ttsofu19@mynet.com

This study aimed to determine the relationship between severity of chronic kidney disease and plasma fibrinogen levels and development of atherosclerosis. The patients were categorized according to glomerular filtration rate (GFR) into five groups. The classification was made as GFR < 15, GFR 15–29, GFR 30–59, GFR 60–89, GFR≥ 90 and in each stage there was 10 patients. Subjects whose GFR was ≥ 90 was accepted as control group. All groups serum uric acid, BUN, creatinin, triglyceride, total cholesterol, HDL, LDL, VLDL, ApoA1, ApoB, MCP-1 levels and plasma fibrinogen level were measured. For determination of atherosclerosis ultrasonographic carotid artery IMT measurements were made. When assessed between mean IMT and maximum IMT measurements and GFR, there was statistically significant differences between the four groups and the control group. Also, plasma fibrinogen levels between the four groups and the control group showed statistically significant differences. There were no statistically significant differences between age, sex, hypertension, weight and triglyceride, total cholesterol, HDL, LDL, VLDL, uric acid, Apo B, ApoA1, ApoB/ApoA1, MCP-1 levels of the four groups and the control group. In conclusion, for some biochemical parameters there were no statistically significant differences between the four groups and the control group. The reason for this was the usage of the antihypertensives and antihyperlipidemics in the patient groups.

PP-934
Effects of balneotherapy on serum IL-1, PGE2 and LTB4 levels in fibromyalgia patients

S. Rota[1], F. Ardıç[2], M. Özgen[2], H. Aybek[1], D. Çubukcu[2] and A. Gökgöz[3]
[1]Department of Biochemistry, Faculty of Medicine, Pamukkale University, Denizli, Turkey, [2]Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Pamukkale University, Denizli, Turkey, [3]Department of Geological Engineering, Faculty of Engineering, Pamukkale University, Denizli, Turkey.
E-mail: siminrota@yahoo.co.uk

We investigated the clinical effects of balneotherapy in the treatment of Fibromyalgia Syndrome (FMS) and determined if balneotherapy influences serum IL-1, PGE2 and LTB4 levels. 24 primary fibromyalgia female patients were included to the study. FMS patients were r and omly assigned in two groups as, group 1 ($n = 12$) and group 2 ($n = 12$). Group 1 received 20-min bathing, once in a day for five days per week for 3 weeks (total of 15 sessions) in Denizli. Group 2 did not receive balneotherapy. FMS patients were evaluated by tenderness measurements, Visual Analogue Scale, Beck's Depression Index, Fibromyalgia Impact Questionnaire. Ten healthy women recruited group 3 as controls. PGE2, LTB4 and IL1-α levels were measured in all three groups. The biochemical measurements and clinical assessments were performed before and after balneotherapy. Statistically significant alterations in algometric score, Visual Analogue score, Beck's Depression Index and PGE2 levels ($P < 0.001$), numbers of tender points ($P < 0.01$) and FIQ score ($P < 0.05$) were found after the balneotherapy between group 1 and 2. Mean PGE2 level of FMS patients were higher compared to healthy control group ($P < 0.0001$) and decreased after the treatment period only in group 1 ($P < 0.05$). In group 1, after balneotherapy IL-1 and LTB4 significantly decreased ($P < 0.05$). In conclusion, balneotherapy is an effective choice of treatment in patients with FMS relieving the clinical symptoms, and possibly influencing the inflammatory mediators.

PP-935
Polymerization of actin in migrating and pinocytotic *Amoeba proteus*

P. Pomorski, A. Wasik, P. Krzeminski, K. Wierzbicka, J. Baranska and W. Klopocka
Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, Warsaw, Poland.
E-mail: pp@nencki.gov.pl

No exact information is yet available about localization and mechanism of actin polymerization in migrating *Amoeba proteus*. Therefore, we have attempted to detect intracellular localization of Arp3 in migrating amoebae in order to determine sites of actin

polymerization. The microinjection techniques have made use to *in vivo* studies on the role of endogenous Arp2/3 complex in amoeba migration. Arp2/3 complex was concentrated in five different cell areas accompanied by F-actin: in the middle-anterior region where adhesive structures are developed, in the frontal part of advancing pseudopodia, in the cortical network, uroid, and in perinuclear cytoskeleton. Distribution of the examined protein in pinocytosing amoeba was limited to the pinocytotic pseudopodia and the perinuclear cytoskeleton where it also colocalised with F-actin. Blocking the *A. proteus* endogenous Arp3 by microinjection migrating amoebae with antibody against mouse Arp3 caused an inhibition of frontal-edge progression as well as the uroid retraction by more than 40% when compared with control amoebae. We show here that actin polymerisation occurs in multiple regions, all of them not related to active front progression. Locomotion of amoeba seems to be related mainly to the intensity of microfilaments aggregation caused by contraction of the cortical layer in the middle region of the cell, not polymerization on the cell front.

PP-936
Hemoglobin immobilization on the Clark electrode for development of a hydrogen peroxide biosensor

E. Dinçkaya
Biochemistry, Ege University, İzmir, Türkiye.
E-mail: msezginturk@hotmail.com

The detection of hydrogen peroxide, H2O2, plays an important role in many fields including industry, environmental protection and clinical control. Hydrogen peroxide can be toxic if ingested, inhaled, or by contact with the skin or eyes. Hemoglobin is a molecule with four electroactive iron hemes, which can be used as an ideal model molecule for the study of electron transfer reactions of heme proteins and also for biosensing and electrocatalysis. The present study describes the immobilization of hemoglobin on a Clark electrode surface to develop a novel electrochemical biosensor for the detection of hydrogen peroxide. The principle of the measurements was based on the electrocatalytic activity of the immobilized hemoglobin to the reduction of hydrogen peroxide. Hemoglobin was cross linked with gelatine using glutaraldehyde and fixed on a pretreated Teflon membrane. Firstly, the optimum conditions for the biosensor were established. In the optimization studies of the biosensor, the most suitable hemoglobin and gelatin amounts and glutaraldehyde ratio were determined. Characterization studies of the biosensor such as optimum pH and optimum temperature were carried out. The repeatability experiments were done and the average value (x), standard deviation (SD) and variation coefficient (C.V.) were calculated. After the optimization and characterization studies the proposed biosensor was applied to determination of H2O2 in real samples.

PP-937
K735 is the key residue for the ATP-binding to the TRPV1

L. Simunova[1], Z. Lansky[1], E. Friedlova[1], M. Kubala[2] and J. Teisinger[1]
[1]*Institute of Physiology, Academy of Sciences of Czech Republic, Prague, Czech Republic,* [2]*Department of experimental physics, Faculty of Science, Palacky University, Olomouc, Czech Republic.*
E-mail: teisinger@biomed.cas.cz

TRPV1 is nonselective transmembrane ion channel that interferes peripheral nociception by stimuli like heat, extracellular acidification and vanilloid-like ligand excitation. It is known that intracellular ATP modulates activity of TRPV1 channel by a direct interaction with two Walker type nucleotide-binding sites. We have studied the role of several amino acids within the Walker type nucleotide-binding site by the means of site-directed mutagenesis and fluorescence spectroscopy. The experiments were focused on the TRPV1 carboxyl terminus containing highly conserved Walker A motif. We have picked up single amino acids from this sequence and performed the following point mutations: P732A, D733A, G734A, K735A, D736A, D737A. All constructs were expressed as a fusion protein with the GST tag in *E.coli* bacteria. For each C-tail construct, we have analysed the ability to bind the nucleotide using the titration and competition experiments with the ATP and its fluorescent analog TNP-ATP (2‘, 3‘-O-(2, 4, 6-trinitrophenyl) adenosine 5‘-triphosphate) (1), pointing out the crucial role of the Lys735 residue. Moreover, this hypothesis was strongly supported by the comparison of FITC-labeling and subsequent Anti-Fl quenching experiments performed on both, the K735A construct and a wild-type protein.

Reference:

1. Kubala M et al., Eur. Biophys. J., 2003; 32: 363–369.

PP-938
Actin dynamic in *Amoeba proteus* motility

W. Klopocka, P. Pomorski, P. Krzeminski, A. Wasik and J. Baranska
Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, Warsaw, Poland.
E-mail: aniak@nencki.gov.pl

Migration is performed by reorganisation of actin cytoskeleton. Actin keeps dynamic balance between two forms: monomeric – G-actin, found in the central, solificated zone of the cell and filamentous – F-actin, mainly localised in the areas under the cell membrane, where the three-dimensional network is formed. It is generally assumed that the whole cortical cytoskeleton of *A. proteus* performs isotonic or isometric contraction along periphery of the whole cell, with exception of advancing fronts and that solution of actin network in the uroidal region is an indispensable condition for isotonic character of the contraction. However, no exact information is yet available about changes in the ratio of filamentous (F) actin and total actin in migrating amoebae. Therefore, we visualized here, for the first time, changes in F/T actin in living amoebae cells – along the cell axis, from their leading edge to the uroid and from retracting fronts to the uroid. The F/T-actin ratio depends mainly on the contraction that can be related to the aggregation of microfilaments or let to the degradation of F-actin network. The relative amount of filamentous actin increases from the advancing front to the middle cell region and decreases again toward the uroid but from the top of retracting pseudopodium decreases to cell middle area. Obtained results show, that it is not an actin polymerization what propels progression of the cell front.

PP-939
MKP-1: a novel anti-inflammatory target of glucocorticoids in human endothelial cells

R. Fürst[1], T. Schroeder[2], H. M. Eilken[2], M. F. Bubik[1], A. K. Kiemer[3], S. Zahler[1] and A. M. Vollmar[1]
[1]*Department of Pharmacy, Center of Drug Research, Pharmaceutical Biology, University of Munich, Munich, Germany,* [2]*GSF - National Research Center for Environment and Health, Institute of Stem Cell Research, Neuherberg, Germany,* [3]*Institute of Pharmaceutical Biology, Saarl and University, Saarbrücken, Germany. E-mail: robert.fuerst@cup.uni-muenchen.de*

Glucocorticoids, such as dexamethasone (Dex), are well-established anti-inflammatory drugs. We used Dex in a model of

endothelial inflammation, i.e. human umbilical vein endothelial cells activated by TNF-α. Dex is known to decrease the expression of the TNF-α-induced adhesion molecule E-selectin, which is largely regulated by NF-κB. We found that Dex at low concentrations (1–100 nM) reduces TNF-α-evoked E-selectin expression, but does not influence NF-κB. Aim of the study was to clarify the underlying mechanisms leading to this NF-κB-independent anti-inflammatory action of Dex on endothelial cells. TNF-α-induced E-selectin expression was diminished by a p38 MAPK inhibitor (SB203580). Dex inhibited p38 MAPK activity upon TNF-α treatment. This effect was reversed by a phosphatase inhibitor (vanadate). Therefore, we hypothesized that the effects of Dex could be mediated by a phosphatase. In fact, Dex (1 nM) increased the expression of the MAPK phosphatase MKP-1 mRNA and protein. The attenuation of p38 MAPK activity and E-selectin expression by Dex was strongly impaired in MKP-1-silenced (antisense treatment) endothelial cells and in endothelial MKP-1 knockout cells (obtained by differentiation of mouse embryonic MKP-$1^{-/-}$ stem cells). In summary, our study introduces MKP-1 as a novel, pivotal mediator of the anti-inflammatory effects of glucocorticoids at low concentrations in the human endothelium.

PP-940
Comparison of innogenetics and Roche line assay methods for human papillomavirus typing

I. Sabol, G. Halec, M. Matovina, N. Milutin Gasperov and **M. Grce**

Laboratory of Molecular Virology and Bacteriology, Division of Molecular Medicine, Rudjer Boskovic Institute, Zagreb, Croatia. E-mail: ghalec@gmail.com

Human papillomaviruses (HPVs) belong to the family Papillomaviridae. Until now, 130 HPV types have been identified and fully sequenced. Approximately 40 types infect the anogenital tract and a few types are commonly found in anogenital cancer biopsy specimens. Detection and typing is important for the diagnosis of HPV associated diseases, notably cervical precancerous lesions and cervical cancer. The molecular methods used for HPV testing are based on the method of hybridization, DNA amplification (polymerase chain reaction – PCR) or both. In this study, 86 DNA samples, isolated from cervical scrapes with abnormal cytology (HSIL, high grade intraepithelial lesion) and determined as HPV positive by consensus PCR (MY09/11), were analyzed for the presence of specific HPV types by two commercially available line probe assays: LiPA from Innogenetics and Linear Array (LA) from Roche, that allow the detection of 25 and 37 HPV types, respectively. All 86 (100%) samples were successfully typed with Roche LA, while 79 (92%) samples were typed with LiPA. At least one HPV type was detected with both tests in 45 (52.3%) cases, with LiPA detecting less HPV types as expected. Moreover, in 31 (36.1%) cases, the results were identical between both tests, while in three cases (3.5%) completely discordant. In this study group, we can conclude that Roche LA has an advantage over LiPA for HPV DNA genotyping because it allows the detection more HPV types in a single test with greater sensitivity.

PP-941
Overlap of the gene encoding the novel poly (ADP-ribose) polymerase PARP-10 with the plectin gene

K. Lesniewicz[1], E. Poreba[2], J. Luescher-Firzlaff[3], P. Fuchs[4], G. Walsemann[3], G. Wiche[4] and B. Luescher[3]

[1]Institute of Molecular Biology and Biotechnology, A. Mickiewicz University, Poznan, Poland [2]Department of Molecular Virology, A. Mickiewicz University, Poznań, Poland [3]Abteilung Biochemie und Molekularbiologie, Institut fur Biochemie, Klinikum der RWTH, Aachen, Germany, [4]Institut fur Biochemie und Molekulare Zellbiologie, Universitat Wien, Vienna, Austria. E-mail: lesniew@amu.edu.pl

We have recently identified PARP-10 as a novel functional poly (ADP-ribose) polymerase. The gene encoding PARP-10 is conserved in vertebrates but no orthologs were found in lower organisms. In addition to the poly (ADP-ribose) polymerase domain, PARP-10 possesses several additional sequence motifs, including an RNA recognition motif and two ubiquitin interaction motifs. We characterized the murine genomic locus of the Parp10 gene. We noticed that 3' Parp10 sequences overlapped with the plectin gene in a head-to-tail arrangement. Detailed analyses revealed that the two most 3' Parp10 exons (exons 10 and 11) are also used for plectin. While these two exons code for part of the poly (ADP-ribose) polymerase domain in Parp-10, they are noncoding for plectin due to the lack of appropriate start codons. Furthermore our findings suggest that at least one of the plectin promoters is located within intron 9 of the Parp10 gene.

PP-942
The *Aspergillus nidulans* aspartate/glutamate transporter reveals new mechanisms of amino acid uptake regulation

A. Apostolaki[1], Z. Erpapazoglou[2], C. Scazzocchio[1] and V. Sophianopoulou[2]

[1]Institut de Génétique et Microbiologie, Université Paris-Sud, 91405 Orsay Cedex, France, [2]Institute of Biology, National Center for Scientific Research "Demokritos" (NCSRD), Aghia Paraskevi 15310, Athens, Greece. E-mail: vicky@bio.demokritos.gr

In fungi, amino acid uptake from the growth medium is mediated by integral membrane proteins called amino acid transporters, which belong to three distinct families of the APC (Acid/Polyamine/organoCation) superfamily: ACT (Amino acid/Choline Transporters), LAT (L-type Amino acid Transporters) and YAT (Yeast Amino acid Transporters). In *Aspergillus nidulans*, the study of the *prnB* gene encoding the major proline transporter (YAT) and the *gabA* encoding the γ-aminobutiric acid transporter (ACT), has provided novel insights concerning amino acid transporter gene regulation and structure-function relationships. In the present work, we describe the characterisation of a new YAT member, mediating specifically aspartate and glutamate uptake, the acidic amino acid transporter AgtA. *agtA* gene presents a novel pattern of transcriptional response to the nitrogen source present in the growth medium. Additionally, *agtA* is a common target of several unlinked mutations affecting specifically amino acid uptake at a post-transcriptional level. These mutations have been mapped in different genes and present different phenotypes from those so far described in *Saccharomyces cerevisiae*. Based on sequence similarity data, it is suggested that similar mechanisms to those encountered in *A. nidulans* operate in all other model filamentous ascomycetes to regulate post-transcriptionally amino acid transporter gene expression.

PP-943
Prenatal diagnosis in multiple pregnancies with the hemoglobin disorders in Cukurova plain

A. Tuli[1], E. Yenilmez[1], S. Yuzbasioglu[1], S. Yildiz[1], G. Attila[1], M. A. Curuk[1], C. Evruke[2], F. T. Ozgunen[2] and K. Aksoy[1]
[1]*Department of Biochemistry, Faculty of Medicine, Cukurova University, Adana, Turkey,* [2]*Department of Obstetric and Gynecology, Faculty of Medicine, Cukurova University, Adana, Turkey. E-mail: tuliab@cu.edu.tr*

Objective: To tabulate genetic results in twin pregnancies with the hemoglobin disorders.
Background: Hemoglobinopathies and thalassemias are the inherited disorders of hemoglobin synthesis that are seem to be very frequent in Cukurova plain, southern part of Turkey. Prenatal diagnosis answers the need to detect early in pregnancy a number of genetic diseases. Today prenatal diagnosis is also the unique preventive approach of these diseases.
Methods: Abnormal hemoglobin and thalassemia carrier states of the couples were affirmed at molecular level. Chorionic villus samples (CVS) of twins were obtained at 10–12 weeks of gestation. Amplification refractory mutation system and restriction fragment length polymorphism techniques were applied to determine and confirm the presence of the hemoglobin disorder of the fetuses. Also, the method of variable number of tandem repeats analysis of four different loci [pMCT118, IgJH, ApoB, D4S95] was used to identify each twin and eliminate the maternal contamination of chorionic villus samples.
Results: We performed prenatal diagnoses for hemoglobinopathy and thalassemia in 21 high risk (1:4) couples. CVS did not cause abortion or fetal malformation in any case. Ten prenatal diagnoses revealed an affected fetus; all couples opted for therapeutic abortion. In 21 cases, the fetus was heterozygote, and in 11 cases it was non-carrier of mutated alleles.

PP-944
Ivermectin sensitivity of heteromeric P2X4+P2X1 and P2X4+P2X6 purinergic receptors

I. Jelinkova, M. Jindrichova, V. Vavra and H. Zemkova
Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic. E-mail: jelinkoi@biomed.cas.cz

Purinergic P2X receptors (P2XRs) are ATP-gated ion channels which subunits (termed P2X1-7) are encoded by seven genes. The P2XR subunits become incorporated into homomeric or heteromeric trimeric assemblies. The P2X4 subunit associates with the P2X1 subunit (P2X1 + P2X4 receptor) or P2X6 subunit (P2X6 + P2X4 receptor). We examined the effect of ivermectin (IVM), a selective modulator of P2X4 receptor, on activation and deactivation properties of heteromeric P2X4R channels. Experiments were performed on HEK293 cells co-transfected with cDNAs encoding wild-type rat P2X4 and P2X1 or P2X6 subunits. ATP-induced currents were recorded in a whole-cell configuration. IVM had two effects in homomeric P2X4R: it increased about 2-fold the amplitude of ATP-induced current and greatly prolonged its deactivation time constant. In the presence of IVM, the ATP dose response was leftward shifted, reducing the EC50 values from 7.8 ± 0.8 to 0.6 ± 0.3 μM. In cells expressing heteromeric P2X4 + P2X1 and P2X4 + P2X6 receptors, IVM had similar effects. However, in cells expressing P2X4 + P2X6 receptors, IVM increased the maximum amplitude more than 3-fold. These results indicate that in heteromeric receptors, the P2X4 receptor is dominant with respect to IVM sensitivity and that IVM can be used to differentiate the heteromeric P2X4R channels in native tissues.
Acknowledgment: This work was supported by the Academy of Sciences of the Czech Republic (No. A50114080) and Grant Agency of the Czech Republic (No. 309/04/0496).

PP-945
Attaining basic chemical educational goals in problem based learning: a sample module

N. Yener[1], S. Koçtürk[1], G. Oktay[1], H. İşlekel[1] and M. Sakızlı[2]
[1]*Department of Biochemistry, Dokuz Eylül University, İzmir, Türkiye,* [2]*Department of Molecular Biology, Dokuz Eylül University, İzmir, Türkiye. E-mail: nilgun.yener@deu.edu.tr*

Problem Based Learning (PBL) System was established in Dokuz Eylül University (DEU), Faculty of Medicine in 1997–1998 educational period, and it has been practiced since then. This system is basically structured upon a group of educational modules that the contents of which are defined by a curriculum board founded in our faculty. The scenarios discussed in the PBL sessions that structured upon convenience of specific learning goals are supported by other components of PBL as lectures and practical hours. The duration of a module varies between 1 to 3 weeks upon the number of its integrative elements. The module discussed in our study is the first module withheld for Phase I students which lasts 1 week. It is percepted as an introductory module for the young people sprouting out of the high school education and joining the ''Academia''. Owing to this fact, in contrast to all scenarios classically based on a clinical problem, this one is constructed so that solely chemical educational goals, represented in this case as 'learning the Basics of Acid- Base Concept' are intended. Other goals of the module, specified as 'Organic Functional Groups and Biomolecules' are covered up by accessory lecture and practice hours structured upon molecular modelling and recognition of the basic concepts of chemistry/biochemistry. Processing and conclusions are based upon the feed back from the students and results are expressed in graphical strains.

PP-946
Antidepressant-like effect of endomorphin-1 and endomorphin-2 in mice

J. Fichna[1], M. Piestrzeniewicz[1], J. Costentin[2], J.-C do-Rego[2] and A. Janecka[1]
[1]*Laboratory of Biomolecular Chemistry, Medical University of Lodz, Lodz, Poland* [2]*Laboratoire de Neuropsychopharmacologie Expérimentale, CNRS-FRE 2735, IFRMP 23, Université de Rouen, Rouen, France. E-mail: jfichna@zdn.am.lodz.pl*

Endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2) are two μ-opioid selective peptides with potent antinociceptive activity, involved in a number of physiological processes. However, little is known about the antidepressant effect of endomorphins. In this study we examined the antidepressant activity of endomorphins in the animal behavioral models of depression. Endomorphin-1 and endomorphin-2 were synthesized by a standard solid-phase procedure using techniques for Fmoc-protected amino acids. The antidepressant activity of centrally administered endomorphins was examined in forced-swimming and tail suspension tests in mice, as well as in the locomotor activity assay. In both animal models of depression, endomorphins significantly decreased the duration of immobility, interpreted as an expression of behavioral despair and related to the depression syndrome. The antidepressant-like effect of endomorphins did not result from the stimulation of the motor

activity. The effect of endomorphins was significantly inhibited by naloxone, a μ-opioid receptor antagonist.
Conclusion: Endomorphin-1 and endomorpin-2 produce a potent, μ-opioid receptor-mediated antidepressant-like effect after intracerebroventricular injection in mice. We suggest that the endomorphinergic system might play a key role in the physiopathology of depressive disorders and could serve as a novel target for the development of antidepressant drugs.

PP-947
Kinetic analysis of DNA unwinding by *E. coli* DNA helicase I (TraI) reveals a highly processive monomeric molecular motor

B. Sikora[1], R. L. Eoff[1], **S. W. Matson**[2] and K. D. Raney[1]
[1]Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, USA, [2]Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, USA. E-mail: sikorabartekt@uams.edu

TraI (DNA helicase I) is encoded by *Escherichia coli* F-plasmid and possesses both helicase and transesterase activities. TraI is required for conjugative DNA transfer. Previous work has shown that the 180 kDa TraI protein is a highly processive helicase, catalytically separating ~850 bp under steady-state conditions. In this report, we examine the kinetic mechanism describing DNA unwinding of TraI. The kinetic step-size of TraI was measured under both single-turnover and pre-steady-state conditions. The resulting kinetic step-size estimate was ~4–6 bp step^{-1}. TraI can separate dsDNA at a rate of ~1100 bp/s, similar to the measured unwinding rate of the RecBCD helicase, and appears to dissociate extremely slowly from the 3'-terminus following translocation and strand -separation events. Finally, analysis of pre-steady-state burst amplitudes suggest that TraI can function as a monomer, similar to the bacteriophage T4 helicase, Dda. However, unlike Dda, TraI is a highly processive monomeric helicase, making it unique amongst the helicases characterized at this point in time.

PP-948
An improved method for expression and purification of the hepatitis C virus helicase

D. Sikora and K. D. Raney
*Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, USA.
E-mail: sikoradeniz@uams.edu*

Hepatitis C Virus (HCV) infects over 170 million persons worldwide and is the leading cause of liver disease in the U.S. The viral genome encodes non-structural protein 3, NS3, a bi-functional enzyme containing an N-terminal protease and C-terminal helicase. Enzymological studies of NS3 have been limited by inadequate methods for purification of the protein. We have developed a new method for purifying the enzyme resulting in higher yields of protein with similar specific activity to that of previously reported. A fusion protein was prepared between NS3 and SUMO (small ubiquitin-related modifier) protein which contained an N-terminal histidine tag. After expression in *E.coli*, the chimeric protein was purified from the lysed cells by a metal affinity column. The SUMO tag was cleaved by a very specific and durable protease, Ulp1 (SUMO protease (1). Final purification was performed with metal affinity chromatography. The Ulp1 protease recognizes the tertiary structure of the SUMO tag and does not require a recognition sequence on the N-terminal of the target protein yielding purified protein with a native N-terminus. We found out that the presence of proline residues on the N-terminus of NS3 helicase domain (NS3h) inhibited the action of Ulp 1 although they were not located adjacent to cleavage site of the protease. Therefore, this purification method is very useful but not applicable to all proteins due to sequence limitations.
Acknowledgment: This work was supported by NIH RO1 AI060563.

PP-949
eNOS T-786C gene polymorphism in elite Turkish wrestlers

I. Peker[1] and Z. Kavuncuoglu[2]
*[1]Chemical engineering Biotechnology Laboratory 314,Marmara University, 34722 Goztepe, Istanbul, Turkey, [2]Biology, Institute for Graduates Studies in Pure and Applied Sciences, Marmara University, 343722 Goztepe, Istanbul, Turkey.
E-mail: ipeker1952@gmail.com*

The aim of the present study is to investigate the association between talent of wrestlers and eNOS T-786C polymorphism of the endothelial nitric oxide synthase gene. Association studies of single or multiple gene variants have identified a limited number of genes that appear to influence exercise-related phenotypes. Endothelial nitric oxide synthase enzyme (eNOS) gene is one of these genes. In this study the eNOS gene polymorphism was analyzed in 49 elite Turkish wrestlers (mean age, mean high, mean weight) and 52 normal male populations as control (mean age, mean high, mean weight). The eNOS T-786C polymorphism of the endothelial nitric oxide gene was determine by PCR (Polymerase Chain Reaction) and restriction fragment length polymorphism. There was a significant association between TT and TC genotypes but not CC of the eNOS T-786C gene polymorphism. eNOS TT polymorphism is 28.6% in wrestlers and 59.6% in the control group. eNOS TC polymorphism is 59.2% in wrestlers and 28.9% in the control group ($P = 0.005$). There is no meaningful difference between wrestlers and the sedentary group in the eNOS CC allel. These results showed us a new strategy for determining prospective elite wrestlers even during their childhood.

PP-950
Evaluation of serum hyaluronic acid level and hyaluronidase activity in acute and chronic hepatitis C

M. Kucur[1] and F. K. Isman[2]
*[1]Fikret Biyal Central Research Laboratory, Cerrahpasa Medical Faculty, Istanbul, Turkey, [2]Department of Clinical Biochemistry, Taksim Teaching and Research Laboratory, Istanbul, Turkey.
E-mail: mkucur@hotmail.com*

Background: Viral hepatitis C is one of the leading causes of fibrosis and cirrhosis. Current diagnosis of HCV focuses on the importance of recognizing acute and early disease activity in order to prevent chronic hepatitis C and its complications. Hepatic fibrosis is characterized by the elevation of extracellular matrix component, mainly hyaluronic acid (HA). Hyaluronidase, the enzyme that degrades HA is also elevated due to increased HA deposition, reflecting rapid HA turnover. We assume that both HA and hyaluronidase have predictive value for monitoring early disease activity in cases with acute hepatitis C and may be useful indicators for preventing progression to chronic hepatitis.
Materials and methods: Twenty-six patients with acute, 89 patients with chronic hepatitis C confirmed by liver biopsy, a

total of 115 HCV positive patients and 32 healthy controls were studied. Liver fibrosis was staged using the METAVIR scoring system. According to histopathological findings patients were divided into two groups; F0–F1: no-mild fibrosis and F2–F4: moderate to severe fibrosis. Serum hyaluronidase activity was measured by the determination of released N-acetylglucosamine reducing termini. Serum HA was measured in an enzyme-linked s and wich assay.

Results: Both serum HA levels and hyaluronidase activities were significantly increased in acute hepatitis ($P < 0.001$). HA concentrations were also significantly elevated in chronic hepatitis C ($P < 0.05$). However serum enzyme activities were found significantly lower ($P < 0.05$) in chronic hepatitis. Statistically significant differences in serum HA level and hyaluronidase activity were demonstrated between F0–F1 and F2–F4 stages ($P < 0.001$).

Conclusions: Both hyaluronidase and HA parameters may be useful as early serum indicators of disease activity in acute hepatitis C. We assume that it could be possible to prevent progressive liver damage by monitoring HA levels and hyaluronidase activity.

PP-951
α-isoforms of the Na^+-K^+ atpase (NKA) & VSM function: evidence from transgenic mice

R. Paul[1], D. Bullard[1], M. Tosun[2], R. Lynch[3] and T. Pritchard[3]
[1]Department of Molecular & Cellular Physiology, University of Cincinnati, OH, USA, [2]Department of Pharmacology, Ege University College of Pharmacology, Izmir, Turkey, [3]Department of Physiology, University of Arizona, Tucson, AZ, USA. E-mail: richard.paul@uc.edu

NKA is important in ion homeostasis and regulation of numerous cell processes in vascular smooth muscle (VSM). Differential roles have been proposed for α-isoforms, whose distribution (70% α1, 30% α2) has been reported to change in disease states such as hypertension. To further examine the function of the α-isoforms, we expressed the α-subunits in mice using the α-actin smooth muscle specific promoter. PCR and Southern blot analyses were used to identify mice carrying the transgene for either the α1- or α2-isoform NKA ($\alpha1_{sm+}$, $\alpha2_{sm+}$). Western blot analysis showed that the α1-isoform is increased 1.4 fold in $\alpha1_{sm+}$ mice, and the α2-isoform is increased 2-8 fold in $\alpha2_{sm+}$ mice. In cultured aortic smooth muscle cells from $\alpha2_{sm+}$ mice, we showed that the increased α2-isoform remained reticularly distributed while the α1-isoform maintained its ubiquitous distribution. For KCl stimulation, maximum force in aorta from $\alpha2_{sm+}$mice was 30% greater ($P < 0.01$) and relaxed faster than WT mice (90.9 ± 8.3, $n = 13$ vs. 115.8 ± 9.0 s, $n = 9$; $P = 0.06$). $\alpha1_{sm+}$ male mice had a basal systolic blood pressure (SBP) not different from WT mice. The $\alpha2_{sm+}$ mice have a significantly lower basal SBP than WT mice (109.9 ± 1.6 mmHg, $n = 13$ vs. 121.3 ± 1.4 mmHg, $n = 11$; $P < 0.05$). Our results support the hypothesis that the α2-subunit is important in vascular contractility and blood pressure regulation. Thus our mice provide unique models in analyzing the relative contribution of the α-isoforms to vascular Ca^{2+} homeostasis under pathological conditions.

Acknowledgment: Supported by NIH HL66044 (RJP, RML).

PP-952
Membrane stabilizing effect and antisickling activity of *Senna podocarpa* and *Senna alata*

J. Okpuzor[1], O. Adebesin[2] and V. Okochi[3]
[1]Department of Cell Biology and Genetics, University of Lagos, Akoka-Yaba, Lagos, Nigeria, [2]Department of Cell Biology and Genetics, University of Lagos, Akoka-Yaba, Lagos, Nigeria, [3]Department of Biochemistry, College of Medicine, University of Lagos, Idi araba, Lagos, Nigeria. E-mail: joyokpuzor@yahoo.com

Senna podocarpa and *Senna alata* are used in traditional medicine for the treatment of skin diseases such as eczema, scabies, ringworm and veneral disease. The leaf decoction is given as mild laxative and in large doses, acts as a purgative, it is also employed along with other herbs in the management of sickle cell disease. The quantitative, antisickling and membrane stabilizing effect of aqueous ethanolic extract of *Senna podocarpa*, *Senna alata* and *Cajanus cajan*, on human red blood cells of normal and sickle cell subjects were investigated. Phytochemical analysis of the extracts indicated the presence of alkaloids, glycosides, deoxy ketosugars, flavonoids, anthraquinones, reducing sugars as well as traces of saponin. *Cajanus cajan*, *Senna podocarpa* and *Senna alata* at 3 mg/ml inhibited sickling in the presence of sodium metabisulphite by 55%, 49% and 42% respectively over a thirty minute period. The osmotic fragility used as an indicator of membrane stabilizing effect revealed that *Cajanus cajan*, *Senna podocarpa* and *Senna alata* were more efficient than the controls without supplementation using 50% lysis index. The result suggests that the aqueous ethanolic extract from these plants possess significant antisickling as well as membrane stabilizing activity and may have potential for management of sickle cell disease.

PP-953
ACE I/D, eNOS T-786C and PON1 Q-192R gene polymorphisms and longevity in Turkish nonagenarians

F. Ciloglu[1], **I. Peker**[2], A. Ozyurek[2], B. Tangurek[3], Z. Kavuncuoglu[2] and D. Lukuslu[4]
[1]Genlab Medical Diagnostics and Research Laboratory, Goztepe, Istanbul, Turkey, [2]Chemical Engineering Biotechnology Lab, Marmara University, 314, 34722 Goztepe, Istanbul, Turkey, [3]Siyami Ersek Cardiovascular Hospital, Istanbul, Turkey, [4]Department of Mathematics, Marmara University, 34722 Goztepe, Istanbul, Turkey. E-mail: fciloglu@gmail.com

Although healthy longevity is affected by such factors as nutrition, cigarette and alcohol consumption, physical exercise and stress, it also seems to have a strong genetic component. One of the major risks for mortality and morbidity is cardiovascular disease and long-lived people seem protected from premature vascular death. In relation to cardiovascular disease and mortality, we considered Angiotensin converting enzyme (ACE) which regulates the renin-angiotensin system that plays a major role in maintaining blood pressure homeostasis; endothelial nitric oxide synthase (eNOS) which regulates the bioavailability of nitric oxide which in turn regulates vascular tone and local blood flow, platelet aggregation and adhesion, and leukocyte-endothelial cell interactions; and paraoxonase1 which is a high density lipoprotein associated arlyesterase that hydrolyses lipid peroxidase and protects LDL against oxidative modification. We conducted a study to assess any association between ACE genes I/D polymorphism, eNOS-786 T/C genetic polymorphism, PON1-192 Q/R genetic polymorphism and longevity in 33 healthy nonagenarians and young controls from Turkey. Our results showed that

although there seemed to be a trend between ACE genotypes and longevity, it was not significant. No such trend was found in eNOS or PON1 polymorphisms. Further studies with larger numbers are needed to determine if common variants in genes associated with cardiovascular risk contribute significantly to longevity.

PP-954
A study on the interaction between βlactoglobulin-A and a new antitumor reagent (2,2-bipyridinglycinato Pd (ii) chloride)

A. Divsalar, A. A Saboury and A. A Moosavi-Movahedi
Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran. E-mail: divsalar@ibb.ut.ac.ir

Bovine βlactoglobulin (Blg) is a major whey protein of bovine milk. The physiological function of Blg is considered to be the binding and transportation of small hydrophobic ligands. The interaction between a new Pd (II) complex, as an antitumor component, with βlactoglobulin was studied using fluorescence spectroscopy and Far UV-CD spectropolarimetric techniques. A strong fluorescence quenching reaction of Pd (II) complex with Blg-A was observed and the quenching mechanism was suggested as a static quenching mechanism. The binding constants of Pd (II) complex with Blg-A at different temperatures of 300, 310, 315 and 320 K were calculated as 390, 420, 660 and 630/nM and corresponding the average numbers of binding sites were 4.4, 3.3, 3.2 and 3.5, respectively. Thermodynamic parameters calculated at different temperatures indicate that the hydrophobic forces play a major role in the interaction of this complex to Blg-A. Far-UV-CD studies showed that in the presence of different concentrations of Pd (II) complex, the secondary structure of the protein does not any significant change at different temperatures. From above results, it can be seen that Pd (II) complex can significantly change the tertiary structure of Blg-A, without any effects on the secondary structure of the protein at different temperatures. The new Pd (II) complex can bind to Blg-A to be effectively transported and eliminated in body, which can be a useful guideline for further drug design.

PP-955
A comparative study on the effect of less moisture solvents on solubilized lactase phlorizin hydrolase

T. Valadbeigy[1], M. Habibi-Rezaei[1] and M. N. Sarbolouki[2]
[1]School of Biology, University College of Science, University of Tehran, Tehran, Iran, [2]Institute of biochemistry and biophysics, University of Tehran, Tehran, Iran.
E-mail: tahereh@khayam.ut.ac.ir

The study of enzymes in less moisture solvents is of great importance. Increasing number of reports has been appeared regarding to the pure soluble enzymes under such conditions. Meanwhile there are few reports mirroring the effect of minimally moist solvent systems on membrane bound enzymes. Lactase-phlorizin hydrolase (LPH; EC 3.2.1.23/62) is a membrane bound intestinal hydrolase, with an extracellular domain comprising four homologous regions. It is synthesized as a large polypeptide precursor, pro-LPH that undergoes several intra- and extracellular proteolytic steps to generate the mature membrane bound enzyme which have been emphasized in this study in both in situ and detergent mediated solubilized form. The later type of the enzyme was prepared upon Triton X-114 phase partitioning technique. So, we separated LPH from residual intestinal brush border membrane (IBBM) proteins based on its hydrophobic characteristics which resulted in enriched LPH in detergent poor phase (DPP). In this report the effect of type and percent of water miscible organic solvents in three categories; polar-protic, polar-aprotic and nonpolar-aprotic solvents, are examined on the stability and activity of two mentioned preparations of LPH (IBBM and DPP). Our results indicate acceptable catalytic properties of the LPH in less moisture systems. Furthermore the relative activation of the LPH was observed in nonpolar-aprotic solvents, especially for solubilized LPH.

PP-956
Nickel ions induced substrate inhibition for DAAO

F. Bostanian[1], S. Safarian[2], A. A. Saboury[1], M. Amani[1], H. Heli[1] and A. A. Moosavi-Movahedi[1]
[1]Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran, [2]Department of Biology, Faculty of Science, University of Tehran, Tehran, Iran. E-mail: bostanian@ibb.ut.ac.ir

The effect of Ni ions on the structure and activity of D-amino acid oxidase (DAAO) was investigated at pyrophosphate buffer, 37 Centigrade. The activation and inhibitory concentration ranges of Ni (II) on DAAO were also determined. Ni ions at low concentrations (<2 mM) induced substrate inhibition for DAAO at high concentrations of Alanine as a main substrate accompanying with compaction of the enzyme which was observed by Far-circular dichriosm (CD) experiment.

PP-957
Improving the purification of NAD^+-dependent formate dehydrogenase from *Candida methylica*

E. Bıçakçı[1], R. B. Sessions[2], **A. R. Clarke**[2] and N. Gül Karagüler[1]
[1]Department of Molecular Biology and Genetics, Faculty of Science and Letters, Istanbul Technical University, 34469 Istanbul Turkey, [2]Department of Biochemistry, School of Medical Sciences, University Walk, Bristol BS8 1TD, UK.
E-mail: emelb@yahoo.com

One important use of formate dehydrogenase (FDH) is to regenerate valuable NADH which is required by NAD^+-dependent oxidoreductases (e.g. LDH) in enzymic catalysis. The NAD^+-dependent FDH offers several advantages over any of the other dehydrogenases, and has been extensively studied as a candidate for developing industrial NADH regeneration. The advantages of using FDH are; the availability and low cost, a favourable thermodynamic equilibrium and the inertness of the CO_2 product. On the other hand the low k_{cat}, and high K_M, the lack of extreme thermostability or the solvent tolerance and the limited coenzyme specificity are known disadvantages of FDH. In order to make FDH more thermostable enzyme for its industrial applications, NAD^+-dependent FDH from *Candida methylica* will be engineered by using site directed mutagenesis. To be able to purify each constructed mutant protein more efficiently, *Candida methylica* recombinant wild type FDH gene was cloned into the pQE-2 TAGZyme expression vector and 6his-tagged FDH gene was overexpressed in JM105 cells. By the use of exopeptidases, TAGZyme Purification System allowed the complete removal of the small N-terminal His tag. 1.064 mg/ml cmFDH protein of $>95\%$ purity was obtained after the purification procedure. The

kinetic parameters and thermal inactivation of cmFDH were determined by observing the oxidation of the nicotinamide coenzyme at 340 nm.

PP-958
Molecular characterisation and comparative analysis of the human C/EBP delta promoter to mammalian homologues

F. Kockar[1], H. Yildirim[1] and D. Ramji[2]
[1]*Department of Biology, Faculty of Science and Literature, Balıkesir University Balikesir, Turkey,* [2]*School of Bioscience, University of Cardiff, Museum Avenue, PO Box 911 Cardiff CF10 3US UK. E-mail: fkockar@balikesir.edu.tr*

C/EBPs are the member of the leucine zipper transcription family which consist of six members, C/EBP alpha, beta, gamma, delta, epsilon and zeta. Human C/EBP delta has been mapped to chromosome 8q11 and the highest tissue expression levels of delta are found in the lung, adipose tissue and intestine with only low levels detected in the liver. Although these proteins have significant functions in the control of cell growth, cell differentiation, regeneration of liver and response to inflammations, knowledge about these genes regulation is very limited. We present here the molecular characterisation of the promoter of human C/EBP delta gene and the sequence comparison of delta promoter was performed using bioinformatic analysis with other mammalian C/EBP delta promoters. The putative transcription factor binding domains were identified and compared to the other delta promoters. 1.75 kb of C/EBP delta promoter cloned to the luciferace vector was transfected to the Hep3B cells for the functional analysis of the promoter. PGL2 basic vector and RSV plasmid were used as control and beta-gal for normalizing the results. Initially, transient transfection studies was optimised using different concentrations of plasmid DNA, different incubation times and cells. In addition, the smallest truncated construct of the promoter, 200 bp, was prepared using PCR based strategy in order to determine the basal promoter activity.

PP-959
The effect on plasma NOx levels of eNOS Glu298Asp polymorphism in healthy volunteers from Denizli, Turkey

S. Demir[1], R. Akbay[1], N. Karagenc[2] and S. Rota[1]
[1]*Department of Biochemistry, Pamukkale University, Denizli, Turkey,* [2]*Department of Medical Biology, Pamukkale University, Denizli. E-mail: suleyman@pamukkale.edu.tr*

The aim of the present study was to investigate the relationship between the plasma nitric oxide level and eNOS gene polymorphism in healthy volunteers from Denizli, Turkey (34.23 ± 10.56 (15–61) years old, $n = 120$, M: 69, F: 51) Griess assay and PCR-RFLP analysis were used to measure the plasma nitric oxide metabolites and genotypes, respectively. It was found that Glu/Glu, Glu/Asp and Asp/Asp genotype frequencies of the eNOS were 71.7%, 23.3% and 5.0%, respectively and T allele frequency was 28.3%. Plasma nitrite/nitrate levels no showed a significant difference between the Glu298Asp genotypes. There was also no correlation between plasma nitric oxide levels and the allele frequencies. Our study provides evidence that the Glu298Asp polymorphism does not affect plasma NOx concentrations, which are believed to reflect endogenous production of NO.

PP-960
Plasma amino acid concentrations in healthy pediatric and adult subjects

Z. B. Hizli[1], Y. Ozarda Ilcol[1] and **D. Aslan**[2]
[1]*Uludag University Medical School,* [2]*Pamukkale University Medical School. E-mail: banuh@uludag.edu.tr*

Reference values and intervals serve as the basis of laboratory testing. Amino acid analysis is a procedure extensively used for diagnosis and follow-up of inborn error of metabolism. The objective of this study was to determine reference values for 32 plasma free amino acids from measurements done in 155 healthy children ranging from 0 to 18 years of age and 130 healthy adults ranging from 18 to 45 years of age. Amino acid analysis was performed by gas chromatography. The mean, median, mod, standard deviation, 2.5 and 97.5 percentiles of amino acids were determined. Nonparametric method was used to determine reference values. Statistical comparison of the means of amino acid concentrations from all age groups conducted with one-way ANOVA followed by Tukey's multiple comparison procedure. Glycine, α-aminobutyric acid, methionine, glutamic acid, phenylalanine, cystine, asparagine, aspartic acid, hydroxyproline, hydroxylysine, cystathionine, tyrosine, α-aminopimelic acid, glutamine, ornithine, lysine, histidin and tryptophan demonstrate significant differences ($P < 0.05$) throughout infancy, childhood, adolescence, and adulthood, but not alanine, sarcosine, valine, isoleucine, leucine, β-aminoisobutyric acid, prolin, serine, threonine, α- aminoadipic acid, allo-isoleucine, proline-hidroxyproline, glycine-proline, and thioproline. As far as we know, reference values of amino acids are produced for the first time in all age groups, including adulthood.

PP-961
Effects of vitamin A and E on arginase, ornithine and urea levels in brain tissue of rats received long term alcohol

A. Kundak[1], **H. Erbas**[1], S. Gulen[1], G. Dokmeci[2], H. Celik[2] and T. Ozcan[2]
[1]*Department of Biochemistry, Faculty of Medicine, University of Trakya,* [2]*Department of Internal Medicine, Faculty of Medicine, University of Trakya. E-mail: hakanerbas@hotmail.com*

Arginase is the last enzyme of the urea cycle. It leads to the formation of urea and ornithine from L-arginine using the same substrate, nitric oxide synthase (NOS). In this study, the effect of long-term alcohol intake on brain arginase enzyme activity, ornithine and urea levels and the changes induced by administration of vitamins C and E were investigated. Five study groups of Wistar Albino rats were formed ($n = 15$). All groups were fed with standard laboratory feed. The first (control) group received equivalent amounts of glucose in calorie. Alcohol was mixed the second group's drinking water. The third, fourth and fifth groups received vitamin C, E and C + E, respectively in addition to alcohol. All rats were sacrificed at the end of 20 weeks period. The arginase enzyme activity, ornithine and urea levels were measured in the brain tissue samples. In the treatment groups, arginase enzyme activities, ornithine and urea levels were found to be significantly higher than the alcohol group. These results may indicate that the NOS enzyme, shown to be increased in chronic alcohol intake, may result in a decrease of the arginase enzyme activity via depleting the L-arginine pool. Antioxidant replacement therapy may increase the arginase enzyme activity and therefore this will lead to a decrease in the nitric oxide (NO) production which has been shown to have some negative effects. In the mean time, increased polyamine production may potentate positive effects of those vitamins.

PP-962
Prepartum and postpartum serum paraoxonase activity in dairy cattle and their associations with dystocia

B. Yokus[1], S. Bademkiran[2] and D. U. Cakir[3]
[1]*Department of Biochemistry, Faculty of Veterinary, Dicle University, Diyarbakir, Turkey,* [2]*Department of Reproduction and Clinic for Obstetrics, Veterinary Faculty, University of Dicle, Diyarbakir, Turkey,* [3]*Department of Biochemistry, Public Hospital of Diyarbakır, Turkey. E-mail: beyokus@dicle.edu.tr*

Although the presence of paraoxonase (PON) in serum has been already confirmed in ruminants since 1953, the knowledge about serum PON activity in veterinary medicine is still scarce. The aim of this study was to investigate possible changes of the serum PON activity in prepartum and postpartum stage in Holstein cows and to examine the relation between PON activity and Dystocia. In the beginning of the study total 200 Holstein, aged 3–6 years, were used as the material of the study. The samples were taken both the 5–7 month of pregnancy and within the first 15 minute postpartum. At the end of study the cows were divided two groups according to the sort of partum: (Group 1) difficult labour (n = 18) and (Group 2) normal labour (n = 21). Additionally, Dystocia group was divided three subgroups, according to reason of the Dystocia (1-Absult birth weight, 2- Twin pregnancy, 3-Presentation anomaly). The serum PON activities significantly increased in the prepartum compared to the postpartum in both groups. When the groups were compared according to same period the difference was not observed ($P < 0.05$). However no differences were found in the PON activities depending on the subgroups, it showed that decreased tendency in twin pregnancy. The reason of the decreased PON activities in twin pregnant cows probably is the increased negative energy balance. In conclusions, the increased oxidative stress in postpartum period might be responsible for the decreased PON activity in dairy cattle.

PP-963
Slime degradation by an enzyme produced by *Enterococcus* sp. GMBAE 205b

D. Coskuner Ozturk, A. A. Denizci, D. Kazan and A. Erarslan
The Scientific & Technological Research Council of Turkey (TUBITAK), Research Institute for Genetic Engineering & Biotechnology (RIGEB), Marmara Research Center Campus, P.O. Box 21, 41470 Gebze–Kocaeli, Turkey.
E-mail: dcozturk@gmbae.tubitak.gov.tr

The use of recycled paper has significantly increased to reduce utilization of world's timber resources in the pulp-paper industry. However this usage causes significant problems on the paper machines because of the slime [extra cellular polysaccharides (EPS)] deposition formed by slime producing microorganisms. Using biocides largely prevents slime deposition in paper industry. However the use of EPS degrading enzymes is another alternative for slime removal. In this work, *Klebsiella oxytoca* was isolated as slime former from the slime samples taken from paper machines, and its EPS was used as carbon source for screening of EPS degrading enzyme-producing bacterium. Enzymatic hydrolysis of EPS was detected by measuring the reducing sugar accumulation in reaction medium. *Enterococcus* sp.GMBAE 205b was identified as EPS degrading enzyme producer after screening. This strain was cultivated at 30 °C in a medium consisted from 0.5% *K. oxytoca* EPS and 0.67% yeast nitrogen base dissolved in 50 mM phosphate buffer (PB) pH 7. Culture filtrate was used as the bulk enzyme source. During EPS degradation; 1 ml of culture filtrate was mixed with 5 ml substrate (3% EPS dissolved in 50 mM PB, pH 7) and incubated at 30 °C for 1 h. Highest reducing sugar accumulation was obtained as 4.36% at 6th hour of treatment. The results showed that, the bulk enzyme has an ability to hydrolyse the EPS found in the slimes formed during chipboard production processes.

PP-964
A serine alkaline protease from a newly isolated obligate alkaliphilic *Bacillus* sp. GMBAE 72

M. N. Kerimak Öner[1], A. A. Denizci[2], D. Kazan[2] and A. Erarslan[2]
[1]*Kocaeli University, Hereke O.I. Uzunyol Technical College, 41800 Hereke-Kocaeli/Turkey,* [2]*The Scientific and Technological Research Council of Turkey (TUBITAK), Research Institute for Genetic Engineering and Biotechnology (RIGEB), Marmara Research Center Campus, P.O. Box 21, 41470 Gebze–Kocaeli, Turkey. E-mail: mine@kou.edu.tr*

Bacillus sp. GMBAE 72 was isolated as an obligate alkaliphilic extracellular alkaline protease (AP) producing strain from the compost. AP production was carried out at 37 °C and pH 10.5 by shaking 500 ml medium (1.0% soluble starch, 0.5% yeast extract, 0.1% K_2HPO_4, 0.02% $MgSO_4 \bullet H_2O$) in 2 l flasks at 120 rpm. Flasks were inoculated with the 18-h-old culture of this strain at 1.0% v/v ratio. Highest production was obtained after 54 h cultivation. AP was 5.6-fold purified from culture filtrate by ammonium sulfate precipitation at 55% saturation. Culture filtrate pH was adjusted to 6.0 before salt addition. The precipitate containing 79.5% of total AP activity and 14.2% of total protein available in culture filtrate was dissolved in 50 mM glycine-NaOH buffer at pH 10.5, dialysed against same buffer. The temperature profile of AP was estimated at 30–80 °C interval in the presence and absence of Ca^{2+} ions at pH 10.5. The pH profile of the AP was estimated at 8–13 pH interval and 30 °C. The optimal temperature and pH values of AP were found to be 50 °C and 12.0, respectively. Nevertheless optimal temperature is shifted to 60 °C by 5 mM Ca^{2+} ions. Activation energy of AP for casein hydrolysis was calculated as 15 kcal/mole. Its complete inhibition by 2 mM phenylmethanesulfonylfluoride (PMSF) indicates that the enzyme is a serine AP.

PP-965
ADMA levels in acute and stable chronic obstructive pulmonary disease

S. Erdem[1], S. Abusoglu[1], A. Unlu[1] and F. Kanat[2]
[1]*Department of Biochemistry, Meram Medical School, Selçuk University, Konya, Turkey,* [2]*Department of Chest Disease, Meram Medical School, Selçuk University, Konya, Turkey.*
E-mail: serdem1505@yahoo.com

Chronic Obstructive Pulmonary Disease (COPD) is characterized by the presence of airflow obstruction. Pulmonary hypertension is one of the major complication of COPD and acute attack of the disease is associated with overload of right ventricle. Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NO synthase and associated with high blood pressure. The aim of this study is to determine the levels of ADMA in stable and acute attack of COPD patients. 16 stable COPD and 18 acute attack COPD patients were included in this study. Patients ADMA levels were measured by HPLC with fluorescence detector. Control group is consist of 19 individuals. ADMA levels were significantly higher in the acute attack of the patient (P = 0.026). Although ADMA levels in stable COPD patients were higher than control this was not statistically

significant ($P = 0.052$). In summary, our findings show that elevated levels of ADMA is associated with COPD and may show the presence of pulmonary hypertension.

PP-966
Increasing the substrate specificity of *Bacillus stearothermophillus* lactate dehydrogenase by DNA shuffling

B. Binay and **N. Gül-Karagüler**
Department of Molecular Biology and Genetics, Faculty of Science and Letters, Istanbul Technical University, 34469 Maslak Istanbul, Turkey. E-mail: binayba@itu.edu.tr

L-lactate dehydrogenase (LDH) catalyses the interconversion of an oxo-acid (pyruvate) and hydroxy-acid (lactate) using the NADH / NAD^+ pair as a redox cofactor. The enzyme has a commercial significance as it can be used to produce chiral building blocks for the synthesis of key pharmaceuticals and agrochemicals. Although bs LDH allows the synthesis of chiral hydroxy acids from their corresponding oxo acids with high stereochemical fidelity, this enzyme has the disadvantage of generally not having very broad substrate specificity. DNA shuffling was used to alter the substrate specificity of lactate dehydrogenase (LDH) to mimic that of malate dehydrogenase (MDH). Novel, synthetic mutant LDH (named as BB1) with high activity and commercial potential have been produced by using this DNA shuffling method. Both the wild type and BB1 mutant LDHs have been produced and purified. BB1 has eight amino acid substitutions – asn4gln, ser19thr, gln86arg, thr91ser, ala173val, glu183asp, gln221asn and val267thr changing the substrate specificity of bs LDH from pyruvate / lactate to malate / oxaloacetate. The results of the enzyme assay shows that, these changes led to a new malate dehydrogenase that catalysed the oxidation of malate 1009 times faster than the pyruvate does.

PP-967
Partial purification and characterisation of alkaline protease from *Bacillus clausii* GMBAE 22

H. Bal[1], D. Kazan[2], A. A. Denizci[2] and A. Erarslan[2]
[1]Department of Biology, Faculty of Art and Science, Marmara University, İstanbul, Turkey, [2]The Scientific & Technological Research Council of Turkey (TUBITAK), Research Institute for Genetic Engineering & Biotechnology (RIGEB), Marmara Research Center Campus, P.O. Box 21, 41470 Gebze–Kocaeli, Turkey. E-mail: denizci@gmbae.tubitak.gov.tr

An extracellular alkaline protease producer *Bacillus* strain capable of growing under highly alkaline conditions was isolated from compost. Strain was identified as *Bacillus clausii* according to the investigations on the physiological properties, cellular fatty acid composition and 16SrRNA gene sequences analysis and designated as GMBAE 22. 16S rRNA sequence data of the isolate GMBAE 22 have been submitted to GenBank nucleotide sequence databases under the accession number DQ131908. Alkaline protease produced from *B. clausii* GMBAE 22 was partially purified by DEAE-cellulose anion exchange chromatography followed by ammonium sulfate precipitation. 2.66-fold purification of enzyme was succeeded with 14.69 percent yield. The molecular weight of enzyme was found to be 25.4 kDa by SDS-PAGE analyses. Optimum temperature of enzyme was found to be 60 °C; however it is shifted to 70 °C after addition of calcium ions in 5 mM concentration. The enzyme was stable between 30–40 °C intervals when incubated for 2 hrs at pH 10.5. Only 35% activity loss was observed at 50 °C. Optimal pH of the enzyme was found to be 12. Enzyme was also stable in pH 9.0–11.0 range for 24 h at 30 °C. The strong inhibition of enzyme by phenylmethanesulfonyl-fluoride (PMSF) treatment suggested that enzyme is a serine alkaline protease. K_m and k_{cat} values were found to be 1.32 mg/ml Hammarsten caseine and 469.51 1/min respectively.

PP-968
Serum adenosine deaminase activity in monitoring disease activity and response to therapy in severe psoriasis

Z. Erbağcı[1], A. B. Erbağcı[2], O. Köylüoğlu[2] and H. Sezen[2]
[1]Department of Dermatology, Gaziantep University, Faculty of Medicine, Gaziantep, Turkey, [2]Department of Biochemistry and Clinical Biochemistry, University of Gaziantep, Faculty of Medicine, Gaziantep, Turkey. E-mail: aerbagci@gantep.edu.tr

Adenosine Deaminase (ADA); a main enzyme in purine degradation is considered as a marker for non-specific T cell activation. Serum ADA activity has been investigated in relatively a small number of studies since, yielding conflicting results. İn the present study patients with psoriasis were analysed for serum ADA activity and results were compared with those after therapy as well as healthy controls. Also a relationship between disease activity and ADA was investigated. 38 patients with psoriasis and 24 healthy volunteers were recruited to this study. Psoriasis cases were divided into two groups as mild cases with local and stable lessions (Group I, *n*: 20) and severe cases with extensive involvement (Group II, *n*: 18). Serum ADA activity is determined with modified Guisti procedure in healthy controls and in patients with psoriasis before and after therapy. The mean serum ADA activity of all psoriasis cases was not significantly different from the healthy controls ($P > 0.05$). However it was higher in Group II than in Group I and healthy controls (respectively $P < 0.001$ and $P < 0.05$). A significant decrease was observed after therapy in Group II ($P < 0.05$). Increased serum ADA activity in severe psoriasis is consistent with T cell activation in pathogenesis of the disease. ADA activity might be a useful marker in monitoring disease activity and response to therapy in severe psoriasis.

PP-969
Conversion of DNA methyltransferases to alkyltransferases via cofactor engineering

G. Lukinavicius[1], S. Klimasauskas[1], C. Dalhoff[2] and E. Weinhold[2]
[1]Laboratory of Biological DNA Modification, Institute of Biotechnology, Vilnius, Lithuania, [2]Institute of Organic Chemistry, RWTH Aachen University, Aachen, Germany.
E-mail: grazvyds@ibt.lt

S-Adenosyl-L-methionine (AdoMet) is a biological sulfonium compound known as the major methyl donor in the cell. AdoMet-dependent methyltransferases (MTases) catalyze the transfer of the methyl group from the cofactor S-adenosyl-L-methionine (AdoMet) to defined positions within various substrates like DNA, RNA, proteins and other biomolecules. A series of new AdoMet analogs with extended linear carbon chains replacing the methyl group was obtained by chemical synthesis. Remarkably, we find that extended groups containing a double or triple carbon-carbon bond one unit away from the sulfonium center, as opposed to saturated carbon chains, are readily transferred onto DNA owing to conjugative stabilization of the SN2-like transition state. The MTase-assisted transalkylations of DNA are truly catalytic, efficient and proceed in a sequence-specific

manner yielding corresponding adenine-N6, cytosine-N4 or cytosine-5 derivatives.

PP-970
Importance of the Fecal Elastase in chronic pancreatitis diagnosis

Ş. Gök[1], G. Saydam[1], C. Aydın[1], H. Şimşek[1], T. Himmetoğlu[1], O. Üsküdar[2] and **D. Oğuz**[2]
[1]*Clinical Biochemistry Laboratory, T. Yükses İhtisas Research and Training Hospital, Ankara, Turkey,* [2]*Gastroenterology Clinic, T. Yükses İhtisas Research and Training Hospital, Ankara, Turkey. E-mail: gsaydam@yahoo.com*

Evaluation of exocrine functions of pancreas is important in the diagnosis and treatment of the chronic pancreatitis. Today determination of fecal pancreatic elastase gains importance due to being an non-invasive method. Our aim in this study are to determine the diagnostic importance of Fecal Elastase (FE-1) level on patients diagnosed as chronic pancreatitis in Gastroenterology Clinic, to investigate the effects of the usage of enzyme preparations on the fecal elastase method and to show correlation between FE-1 level and serum lipase level. In our study FE-1 level is measured by micro ELISA method in 26 patients with chronic pancreatitis and 17 healthy subjects. The cut off value was found 240 μg/g. Sensitivity and specificity of the method were found as 85% and 70.6% respectively. FE-1 levels of patient groups when compared with that of healthy subjects, it was found significantly low ($P < 0.001$). As a result we found FE-1 test might only be a supporting parameter as well as Ultrasonography, Computerized Tomography, Endoscopic Retrograde Colangio Pancreotography in the diagnosis of chronic pancreatitis because of the low diagnostic profiency parameters, FE-1 is unaffected by exogenous pancreatic enzyme treatment and there was no correlation between the FE-1 and serum lipase levels.

PP-971
Protective role of artichoke in hepatic and renal cadmium toxicity

E. Gurel[1], E. Taskin[2], N. Yilmazer[3], M. Caner[4], **C. Demirci**[1] and H. Dogruman[2]
[1]*Department of Biology, Faculty of Science, Istanbul University, Istanbul, Turkey,* [2]*Department of Medical Biology, Veterinary Faculty, Istanbul University, Istanbul, Turkey,* [3]*Department of Primary Education, Hasan Ali Yucel Faculty of Education, Istanbul University, Istanbul, Turkey,* [4]*Department of General Surgery, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey. E-mail: ebrugurel@email.com*

Artichoke (Cynara scolymus) is known to be an antioxidant and hepatoprotective plant. Cadmium (Cd), a very toxic heavy metal, has been shown to damage several tissues. This study was performed to investigate protective role of artichoke in tissue toxicity in point of a possible relationship with Nitric oxide (NO), and some biochemical parameters. Male and female Wistar albino rats were divided into four groups: control, Cd (1 mg/100 g CdCl2), Cd plus artichoke and artichoke (3 mg/100 g/bW) only. Kidney and liver tissues were immunohistochemically examined after four weeks exposure. iNOS and eNOS were increased in kidney of both only Cd and artichoke treated animals. However, combined Cd and artichoke treatment resulted in similar iNOS and eNOS immunohistochemistry compared to the controls. iNOS increment was more severe in male liver than the female. iNOS immunohistochemistry in artichoke given female group was same as the control, whereas it was increased in males of artichoke given groups. Cd treatment increased aspartate and alanine aminotransferase (AST and ALT, respectively) levels in both gender, while alkaline phosphatase (AP) was not changed in male, but significantly increasing in female. Combined artichoke and Cd did not decrease those enzymes. In only artichoke given animals AST levels were lowered. However, AP levels were higher than the control and other experimental groups of male. As a result, artichoke extract can protect liver and kidney from Cd toxicity.

PP-972
Diagnostic value of adenosine deaminase in nontuberculous peritoneal effusions

G. Saydam[1], C. Aydın[1], A. Terim Çağır[1], T. Yazıcı[1], M. Balk[1], T. Himmetoğlu[1] and **P. Oğuz**[2]
[1]*Clinical Biochemistry Laboratory, T. Yüksek İhtisas Research and Training Hospital, Ankara, Türkiye,* [2]*Gastroenterology Clinic, T. Yüksek İhtisas Research and Training Hospital, Ankara, Türkiye. E-mail: drcengiz_aydin@yahoo.com*

Adenosine Deaminase (ADA) can aid in the diagnosis of tuberculous peritoneal effusions. The purpose of this study is to assess the ADA levels in non-tuberculous peritoneal effusions of different aetiologies, to investigate false-positive results from peritoneal effusions and to show correlation between ADA levels and white blood count. ADA activity in periton effusions (82 cases) were measured by the Giusti method. Mean ADA activities were 7.47 ± 4.31 U/l in cirrhosis (17 cases), 10 ± 6.47 U/l in chronic liver diseases (23 cases), 26.14 ± 13.37 U/l in peritonitis carcinomatosis (7 cases), 16.1 ± 12.09 U/l in hepatic carcinoma (19 cases), 15.75 ± 13.12 U/l in miscellaneous cases (16 cases). The negative predictive value of ADA for the diagnosis of peritoneal tuberculosis was 96.34%. The peritoneal fluid ADA levels were significantly higher in different types of exudative effusions than transudative effusions ($P < 0.001$) and there was weak correlation between ADA levels and white blood count ($P < 0.001$, $r = 0.648$). As a result, our study shows assessment of ADA in pathologic peritoneal effusions is helpful and it is supporting parameter as well as peritoneal biopsy in the diagnosis of tuberculosis.

PP-973
Polyphenoloxidase potentials of some wild and edible mushroom species harvested from Trabzon

M. Yildirim, E. Sahin, O. Faiz, A. Colak and Y. Kolcuoglu
Department of Chemistry, Faculty of Arts and Sciences, Karadeniz Technical University, Trabzon, Türkiye. E-mail: melikey80@yahoo.com

In this study, Armillaria mellea, Lepista nuda, Handkea excipuliformis and Amanita rubescens were evaluated for their polyphenoloxidase (PPO) potentials. First, the mushroom species were harvested from the Lişer High Plateau-Maçka (Trabzon) and crude extracts were analyzed for both monophenolase and diphenolase activities. Of these mushroom species, A. mellea and L. nuda was determined to possess the greatest PPO activities. Native electrophoresis stained by L-DOPA of the crude extracts of A. mellea showed two bands having Rf values of 0.38 and 0.28 and L. nuda showed one single band having Rf value of 0.43. Each of the crude extract of two mushrooms were able to possess greatest diphenolase activity against 4-methylcatechol. The optimum pH value for each enzyme is determined as pH 7.0. When enzyme extracts were incubated at optimum pH value for 24 hours at 4 °C, A. mellea enzyme activity increased about 11%, but L. nuda enzyme activity decreased about 24%. It was

determined that the optimum temperature of both enzymes were 30 °C. The thermal stability of the two PPO activities rapidly decreased over 40 °C. It was observed that K^+, Cu^{2+} and Cr^{3+} ions inhibited each activity in the crude extracts while Co^{2+} ion activated. However, sodium metabisulphite and ascorbic acid were highly potential inhibitor for each diphenolase activity. Substrat saturation curves obtained for each enzyme indicated that both enzymes followed simple Michaelis-Menten kinetics.

PP-974
The effect of calcium overload on mitochondrial NAD(P)-dependent dehydrogenases

V. Mildaziene[1], J. Grigiene[2], S. Maslauskaite[1] and R. Baniene[2]
[1]*Environmental research center, Vytautas Magnus University, Kaunas, Lithuania,* [2]*Institute for Biomedical Research, Kaunas University of Medicine, Kaunas, Lithuania.*
E-mail: v.mildaziene@gmf.vdu.lt

The response of Krebs cycle dehydrogenases to Ca^{2+} in physiological concentration range is well established, but little is known about Ca^{2+} overload induced changes in the activity of essential mitochondrial dehydrogenases. We examined whether very fast decrease in the amount of mitochondrial NAD(P)H under condition of Ca^{2+} overload may be caused by inhibition of Ca^{2+} sensitive NAD(P)-dependent dehydrogenases. Ca^{2+} overload (increase in extramitochondrial Ca^{2+} concentration from 1 μM to 10 and 30 μM) did not change the activity of mitochondrial pyruvate and 2-oxoglutarate dehydrogenases. Ca^{2+} inhibited NADP-dependent isocitrate dehydrogenase (ICDH) by 12% at 10 μM and 20% at 30 μM Ca^{2+} at subsaturating concentration of substrate. NAD-dependent ICDH was activated (21%) by increase of Ca^{2+} concentration from 1 μM to 10 μM. At saturating substrate concentration Ca^{2+} had not effect on the activity of NADP-dependent ICDH but NAD-dependent ICDH was activated: activity was higher by 43% at 10 μM Ca^{2+} in comparison to that at 5 nM; further increase in Ca^{2+} concentration did not affect the activity. The activity of NADP-ICDH was 100–350-fold higher than NAD-ICDH and increase in Ca^{2+} concentration substantially decreased the ratio of NADP-ICDH vs. NAD-ICDH activity. The fact that supra-physiological Ca^{2+} levels moderately inhibit only NADP-ICDH cannot provide explanation for Ca^{2+} overload induced NAD(P)H depletion and inhibition of respiration in heart mitochondria.

PP-975
Molecular cloning, expression and purification of a thermophilic aldolase gene from *Anoxybacillus gonensis* G2

N. Saglam Ertunga[1], M. Col[1], A. Colak[1], S. Canakci Dulger[2] and A. O. Belduz[2]
[1]*Department of Chemistry, Faculty of Arts and Sciences, Karadeniz Technical University, Trabzon, Turkey,* [2]*Department of Biology, Faculty of Arts and Sciences, Karadeniz Technical University, Trabzon, Turkey. E-mail: nagihanca@yahoo.com*

In living organisms, fructose-1,6-bisphosphate aldolase (FBA) is an ubiquitous enzyme essential for glycolysis, gluconeogenesis, and Calvin cycle. In glycolysis, it catalyses the reversible cleavage of fructose-1,6-bisphosphate into dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. In this study, the FBA gene of a novel thermophilic bacteria, *Anoxybacillus gonensis* G2 strain, was cloned, sequenced and overexpressed in *Escherichia coli* with 6x histidine tag. Whole DNA sequences of the gene were determined by constructing of genomic DNA library and performing inverse PCR. Nucleotide-sequence analysis revealed an open reading frame consist of 861 bp and 286 amino acids. Sequence alignment were done by using BLAST programme. The results showed that amino acid sequences of FBA was similar to amino acid sequences of FBAs belonged to *Bacillus* species in the ratio of 80–90%. The gene were cloned in to pET28a (+) vector and overexpressed under T7 RNA polymerase promoter control with his-tag in *E.coli* BL21(DE3)pLysS strain. It was purified in one step with nickel column. The native gel electrophoresis were done using the purified protein and after the activity staining, the purple band were formed on the paper. SDS-PAGE showed that either the enzyme was composed of one subunit or more than one subunit had the same molecular weight. The calculated subunit weight of the protein with his-tag end and without his-tag were 33.3 and 30.9 kDa respectively.

PP-976
The use of quantitative Anti-HCV, quantitative HCV-RNA and alt tests in the diagnosis of hepatitis C virus infection

M. Balk, G. Saydam, D. Cengiz, A. Türkmen, H. Şimşek and **T. Himmetoğlu**
Clinical Biochemistry Laboratory, T. Yüksek İhtisas Research and Training Hospital, Ankara, Türkiye.
E-mail: mevhibeb@hotmail.com

Hepatitis C Virus (HCV) infection is frequently diagnosed by detection of antibody to the HCV (Anti-HCV). The seropositivity of Anti-HCV could reflect chronic infectious status and/or previous infection. This study was performed in Anti-HCV positive patients to determine the relationships among Anti-HCV, HCV-RNA and Alanine aminotransferase (ALT) levels. Serum samples of 124 patients were tested for Anti-HCV by a MEIA technique, for HCV-RNA by a quantitative PCR and for ALT by IFCC UV test. Sample rate/Cutoff rate (S/CO) values for Anti-HCV tests and ALT levels were directly correlated with the quantitative values of HCV-RNA (respectively, $r = 0.964$, $P < 0.001$ and $r = 0.908$, $P < 0.001$). HCV-RNA values were found to be negative in samples with a S/CO < or = 10. HCV-RNA positivity was found in 33.3% of samples with a S/CO between 11 and 50 and in 100% of samples with a S/CO > 50.All HCV-RNA negative cases had normal serum ALT levels (24.67 ± 8.56 U/l) and relatively low S/CO values (3.81 ± 4.32) for Anti-HCV tests. HCV-RNA positive other cases had greater S/CO values (119.53 ± 37.99) and elevated serum ALT levels (96.36 ± 48.28 U/l). Sensitivity of Anti-HCV cutoff value at 29 S/CO in the diagnosis of HCV viremia was 100% and specificity was 98.4%. The positive predictive value of this test in the diagnosis of hepatitis C viremia was 98.4%. The use of quantitative Anti-HCV assay could facilitate the diagnosis of HCV infection and therefore, quantitative HCV-RNA testing could not be routinely required for all patients.

PP-977
Polyphenoloxidase from a wild and unedible mushroom, *Hypholoma fasciculare*

O. Faiz, E. Sahin, M. Yildirim, A. Colak and M. Col
Department of Chemistry, Faculty of Arts and Science, Karadeniz Technical University, 61080 Trabzon, Turkey.
E-mail: ozlemfaiz61@gmail.com

Polyphenoloxidases (PPO) are a group of copper enzymes catalysing oxidation of polyphenolic compounds in the presence of molecular oxygen. They are widespread in the biosphere from mammals to bacteria. In this work, an unedible mushroom

species, *Hypholoma fasciculare (H. fasciculare)*, was screened for its PPO potential. The mushroom species was harvested from the Lişer High Plateau-Maçka (Trabzon) and crude extract was spectrophotometrically analyzed for either monophenolase or diphenolase activities. Native electrophoresis stained by L-dihydroxyphenylalanine of the crude extract of *H. fasciculare* showed a single band having Rf values of 0.41. It was calculated that *Vmaks* and *Km* values are 0.25 μM/min mg protein and 0.51 mM, respectively from Lineweaver-Burk graphics in the presence of 4-methylcatechol. The highest enzyme activity was achieved at pH 7.0. When enzyme extract was incubated at this pH value for 24 h at 4 °C, H. fasciculare enzyme activity retained about 82% of their original activity. The optimum temperature of *H. fasciculare* is 20 °C. The stimulation of the activity by K^+, Ca^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Al^{3+} and inhibition by Cu^{2+} ve Cr^{3+} at 1 mM concentrations indicate its metal ion dependence. The PPO activity of *H. fasciculare* was inhibited in the presence of benzoic acid, cysteine, sodium metabisulphite, ascorbic acid and sodium azide.

PP-978
Identification of the cellular transcription factors interacting with HPV16 L2 promoter

E. Poreba[1], K. Lesniewicz[2] and A. Gozdzicka-Jozefiak[1]
[1]*Department of Molecular Virology, A. Mickiewicz University, Poznan, Poland,* [2]*Department of Molecular and Cellular Biology, A. Mickiewicz University, Poznan, Poland.*
E-mail: eporeba@amu.edu.pl

Human Papillomavirus type 16 is strongly associated with the development of benign and malignant lesions of the cervix. HPV type 16 infects the basal cells of the epithelium and its transcriptional activity is tightly linked to the differentiation state of the epithelial cells. The late L1 and L2 proteins of HPV16 are only produced in the upper layers of terminally differentiated keratinocytes. 78-bp sequence located in LUR (late upstream region) of HPV16 seems to be critical for the promoter activity of L2 ORF. The aim of our studies was to identify proteins interacting with the L2 promoter of HPV16. The EMSA assays performed with the HPV16 L2 promoter region in the presence of HeLa nuclear extract revealed the presence of four Oct1 binding sites in this promoter located in the positions: 4089–4101, 4103–4121, 4127–4140, and 4177–4190. Southwestern analysis performed with L2 promoter region showed that proteins with molecular weight of approximately 210 kD, 100 kD, 55 kD and 40 kD bind with this DNA fragment. Further studies will be focused on identification of other proteins interacting with HPV16 L2 promoter region. The function of Oct1 protein in L2 expression will be studied by mutational analysis of identified Oct1 binding sites in transfection assays.

PP-979
Significance of heat shock protein 27 expression in patients with renal cell carcinoma

Ö**. Erkizan**[1], G. Kirkali[2], K. Yörükoglu[3] and Z. Kirkali[4]
[1]*Dept. of Biochemistry, Izmir Training and Research Hospital, Izmir, Turkey,* [2]*Dept of Biochemistry, Dokuz Eylul University School of Medicine, Izmir, Turkey,* [3]*Dept of Pathology, Dokuz Eylul University School of Medicine, Izmir, Turkey,* [4]*Dept of Urology Dokuz, Eylul University School of Medicine, Izmir, Turkey. E-mail: guldal.kirkali@deu.edu.tr*

Heat shock proteins (HSPs) were first defined as proteins induced by heat shock and other environmental and pathophysiologic stresses. They are implicated in protein-protein interactions and thought to play an important role in cancer. The expression of heat shock protein-27 (HSP-27) has been shown in some human tumors. In this study we investigated HSP-27 expression in patients with renal cell carcinoma (RCC) and examined its biological significance. Expression of HSP-27 was studied in tumor and normal parenchyma tissue specimens from 76 patients with RCC by immunohistochemistry. Findings were correlated with clinical stage, lymph node metastasis, histologic grade and survival. Of the 76 RCC tissues studied, the presence of HSP-27 was demonstrated in 73 tissues (96%). The expression was low in 10 patients (14%), intermediate in 38 (50%) and high expression was demonstrated in 25 (33%). HSP-27 expression was higher in RCC tissue compared with adjacent non-cancerous renal tissue ($P < 0.001$). There was an inverse relationship between tumor stage and HSP-27 expression ($r = -0.281$, $P = 0.016$). However, there was no difference in progression-free survival with respect to HSP-27 expression. There was no relationship between HSP-27 expression and tumor grade, lymph node metastasis, distant metastasis and cause specific-survival. Our data suggest that HSP-27 expression is not a powerful and significant prognostic indicator for disease free survival of patients with RCC.

PP-980
Effect of ecto-NAD glycohydrolase

L. Turker-Sener, I. Albeniz, O. Coskun and R. Nurten
Department of Biophysics, İstanbul Medicine Faculty, İstanbul University, İstanbul, Turkey. E-mail: leylaturker@hotmail.com

NAD glycohydrolases (NADases), a class of enzymes that catalyze hydrolysis of nicotinamide adenine dinucleotide (NAD) into adenosine diphosphate ribose and nicotinamide, are widely distributed in the nature, ranging from bacteria to mammalian systems. Erythrocytes are particularly rich in ecto-NAD glycohydrolase, a component of erythrocyte membrane, has recently evoked considerable interest as the catalyzer of both synthesis and hydrolysis of cyclic adenosine diphosphoribose (cADPR).In a recent study we found an elevation of erythrocyte NAD glycohydrolase activity in cancer patients in comparison to controls ($P < 0.01$). In order to provide an explanation for this finding, we extended the scope of this study and determined NAD glycohydrolase activity in erythrocytes from patients in a broad variety of different diseases. We indicated that NAD glycohydrolase activity increases significantly in anemia erythrocytes which were both of primer and seconder anemia ($P < 0.01$). There exists a strong correlation between NAD glycohydrolase activity and degrees of anemia, either primary or secondary to some underlying systemic disease. This findings raised the question whether elevation in NAD glycohydrolase activity is due to an increased turnover of red blood cells with appearance of younger erythrocytes in peripherical blood. A correlation of relatively high significance was found between NAD glycohydrolase activity and the degree of anemia and, to some extent, number of reticulocytes in peripheric blood appeared to support this view.

PP-981
Framingham risk prediction models in samples of Turkish adults

N. Tekkesin, E. Aktug, O. Orgen, C. Yegen and K. Keskin
Department of Biochemistry, Memorial Hospital, Istanbul, Turkey. E-mail: nilgun.tekkesin@memorial.com.tr

In Turkey, cardiovascular disease (CHD) is the greatest cause of morbidity and mortality. During the last decade, recommendations

for CHD prevention have emphasized the need of an assessment of coronary risk. We decided to determine 10-year risk for developing CHD using Framingham study as a tool for the estimation of coronary risk in adults aged 30 and older. The study was carried out in Memorial Hospital, Istanbul. Samples from fasting 3169 healthy donors (1800 women (median age 46.8 years) and 1369 men (median age 46.03 years)) were tested and scored according to risk factors (age, total cholesterol, HDL, blood pressure, diabetes, and cigarette smoking) in both genders. High total cholesterol and low HDL-C levels were seen in 20% and 4.64% of men and 32.6% and 1.1% of women, respectively. Both had a low rate of controlled high blood pressure (men 10.57%, women 13.89%) and smoking was especially high (42.5%) in men. The 10-year CHD risk averaged 9.35% in men and 4.59% in women and was found to be lower than age-related low risk for each decade in both genders. About 10.3% of men and 1.17% of women were high-risk (> 20%). Turkish men showed a high risk for CHD, owing to the above factors, especially the high smoking rate. The utilization of Framingham function for the prediction of coronary disease in clinical practice could be useful until we have our own model derived from populations with a baseline risk similar to ours allowing to predict the risk with higher accuracy.

PP-982
Screening survey of beta thalassemia carriers in Diyarbakir province

S. Ekinci, L. Erdinc, **K. Gunes**, S. Batun, A. Kaplan, E. Kale and N. Canoruc

Department of Biochemistry and Clinical Chemistry, Faculty of Medicine, Dicle University, Diyarbakir, Turkey.
E-mail: kemalgunes2002@hotmail.com

Beta (β) thalassemia is characterized by defective β-globin production. Homozygotes and compound heterozygotes of β-thalassemia mutations develop a chronic anemia whereas heterozygotes causes carrier status. Screening survey of carriers is essential in prenatal diagnosis. Turkey is in the thalassemic belt. The incidence of β-thalassemia carriers is 2.1%. Consanguineous marriage within this country is common and this causes an increase in carrier status of β-thalassemia. Therefore prevention of this disease by prenatal diagnosis has been a major objective in Turkey. In this study, β-thalassemia carriers have been detected by screening survey in Diyarbakir province. Blood samples of 10038 individuals of 9–14 ages were collected. Hematological parameters have been analyzed by Cell-dyne 3800 analyzer. Abnormal hemoglobins, HbA2 and HbF have been separated by HPLC. The samples that had ≥3.5% of HbA2 levels were mentioned as β-thalassemia carriers. Among 10038 individuals, 134 samples (1.33%) were evaluated as β-thalassemia carriers. The incidence of β-thalassemia carriers in Diyarbakir region is below that of the Turkey's.

PP-983
Primary brain tumor incidence and association with CYP1A1 and CYP2E1 polymorphisms in a Turkish population

H. Pinarbasi[1], Y. Silig[1], **M. Gurelik**[2], O. Cetinkaya[1] and S. Bakir[1]

Departments of [1]Biochemistry and [2]Neurosurgery, Cumhuriyet Universitesi, Sivas, Turkey. E-mail: hpinar@cumhuriyet.edu.tr

Cytchrome P450 enzymes are essential in the metabolism of chemical carcinogens. Numerous genetic polymorphisms in the cytochrome P450 multigene families have been described in human populations and suggested to contribute to individual cancer susceptibility as genetic modifiers of cancer risk. Primary brain tumors are very poorly understood and heterogeneous group of diseases. It is thought that DNA damage caused by chemical, physical and biologic agents is suspected potential neurocarcinogens. Here we present the preliminary results of a study analyzing the association between genetic polymorphisms of CYP1A1, CYP2E1 and primary brain tumor incidence in 230 Turkish individuals (77 patients with primary brain tumor and 153 controls). Genotypes of CYP1A1 and CYP2E1 were determined by MspI- and PstI-RFLP respectively. The distribution of the CYP1A1 MspI genotype in the patients with primary brain tumor was 88% for m1/m1 genotype and 12% for combination of the m1/m2 and m2/m2 genotypes. The frequency of the polymorphic genotype (m1/m2 and m2/m2) was 11% in controls. Therefore no statistically significant association was found between primary brain tumor incidence and CYP1A1 MspI polymorphism (OR: 1.06, 95% CI: 0.41–2.68, $P = 0.89$). None of the controls had CYP2E1 PstI polymorphism while only one patient was heterozygous. This finding and our previous studies indicate that CYP2E1 PstI polymorphism was extremely rare in Turkish population when compared to other populations.

PP-984
Characterization of purified class II fructose-1,6-bisphosphate aldolase from anoxybacillus gonensis G2

M. Col[1], N. Saglam Ertunga[1], A. Colak[1], S. Canakci Dulger[2] and A. O. Belduz[2]

[1]Department of Chemistry, Faculty of Arts and Sciences, Karadeniz Technical University, 61080 Trabzon, Turkey,
[2]Department of Biology, Faculty of Arts and Sciences, Karadeniz Technical University, 61080 Trabzon, Turkey.
E-mail: melekcol@hotmail.com

Fructose-1,6-bisphosphate (FBF) aldolase (FBA)is a glycolytic enzyme which catalyzes the reversible aldol condensation/cleavage reaction between glyceraldehyde-3-phosphate and dihydroxyacetone-phosphate to generate fructose-1,6-bisphosphate. In this work, thermostable fructose-1,6-bisphosphate aldolase gene from Anoxybacillus gonensis G2 had been cloned and over expressed in *Escherichia coli*. The enzyme was purified and aldolase activity was studied. As a result of native electrophoresis, special to aldolases, activity staining revealed that the protein had been purified. Analyses of the enzyme by SDS-gel electrophoresis revealed a single band. The molecular mass of the purified aldolase estimated by SDS/PAGE was 31.9 kDa. The optimal pH and temperature for FBA enzyme activity were 8.5 and 60 °C, respectively. The activity was decreased at 70 °C and lost at 80 °C. The enzyme almost preserved its activity when kept for 3 h at 30 and 40 °C. The activity was decreased after the incubation of 30 min at 50 °C. At 60 and 70 °C the activity was below 10% for first 30 minutes. In the presence of FBF, Vmax and Km values, were 2.4 μM dak-1/mg protein and 567 μM from Lineweaver-Burk kinetics, respectively. The optimum enzyme concentration was determined as 10 μg/ml for aldolase activity. In the presence of EDTA the enzyme was inhibited completely. Various metal ions were examined and the activity was increased by 3-fold in the presence of Zn^{2+}, especially.

PP-985
Comparative characterization of diphenolases from two mulberry fruits (*Morus alba* L. and *Morus nigra* L.)

Y. Kolcuoglu, A. Colak, O. Faiz, A. Ozen, N. Saglam Ertunga and M. Yildirim
Department of Chemistry, Faculty of Arts and Sciences, Karadeniz Technical University, Trabzon, Turkey.
E-mail: yakupkolcuoglu@yahoo.com

Fruits of two mulberry cultivars (Morus alba L. and Morus nigra L.) were investigated for their polyphenol oxidase potentials. Native electrophoresis of the crude extracts prepared from the cultivars stained with L-DOPA showed similar patterns with Rf values of 0.15 (one major band), 0.42 and 0.54 (two minor bands), respectively. The crude extracts from each mulberry cultivars (Morus alba L. and Morus nigra L.) were highly active against 3-(3,4-dihydroxyphenyl) propionic acid (DHPPA) at acidic and neutral pH values and possessed temperature optima of 40 °C and 20 °C, respectively. pH-stability profiles showed that crude enzymes were extremely stable at both their optimum pH and alkaline pH values. The diphenolase activities from the two mulberry cultivars were very sensitive to ascorbic acid and metabisulfite with IC50 values lower than 1 mM. Moreover, both inhibitors exhibited complete inhibition of DHPPA oxidation at 1 mM and 2 mM concentrations, respectively. 5 mM SDS is required for the fully active diphenolase from Morus nigra L. It can be concluded from the present study that the crude enzymes prepared from the ripe mulberry fruits of two cultivars possess diphenolase activities sharing similar functional properties.

PP-986
High affinity receptor for IgE gene polymorphism in Turkish asthmatic patients

S. Baturoglu[1], F. Yildirim[2], G. Unluguzel[1], G. Haklar[1] and B. Ceyhan[2]
[1]*Department of Biochemistry, Faculty of Medicine, Marmara University, İstanbul, Turkey,* [2]*Department of Internal Medicine, Subdepartment of Pulmonary Medicine, Faculty of Medicine, Marmara University, İstanbul, Turkey.*
E-mail: sunabaturoglu@yahoo.com

The gene for the β subunit of the high affinity receptor for IgE (FcεRI-β) on chromosome 11q13 is linked with clinical asthma and certain mutations have been identified in some studies while some other studies are not supporting this linkage. Our aim was to find out whether a Turkish allergic asthmatic population sample has polymorphism in the FcεRI-β gene or not. DNA samples from 67 subjects with atopic asthma and 67 control subjects were analysed. The median age for atopic asthmatic patients were 39 ± 14.1 and for control group 39.0 ± 13.8 yrs. Allergic status of the patients and controls were also assessed with serum IgE measurements and eosinophil counts in peripheral smears. The complete coding region of the FcεRI-β gene were sequenced. DNA samples were studied with polymerase chain reaction (PCR), artificial refractory mutation detection system (ARMS), single strand confirmation analysis (SSCP) methods were used. Serum IgE concentrations and eosinophil counts of asthmatic patients were significantly increased in comparison with the control subjects (401 ± 731 IU/l vs. 150 ± 73 IU/l, $P < 0.001$ and %3.37 ± 3.26 vs. 1.17 ± 1.34, $P < 0.001$). The three previously reported mutations (Ile181L/Val183L and I181L) were not detected in the subjects studied. We found wild type FcεRI-β genes in all of our study population. These results suggest that, in the population studied, linkage of asthma and atopy to 11q13 is not shown by mutations in the FcεRI-β gene. Other mutations in the non-coding region of this gene or in adjacent genes may be studied in further studies in large populations.

PP-987
Modified bovine serum albumin that shows acetylcholinesterase activity

S. Evran and A. Telefoncu
Department of Biochemistry, Faculty of Science, Ege University Izmir, Turkey. E-mail: serap.evran@ege.edu.tr

Acetylcholinesterases (AChE; EC 3.1.1.7) are widely distributed in the nervous system, where they catalyze hydrolysis of the neurotransmitter acetylcholine (ACh) (Rosenberry et al., 1975; Massoulié et al., 1993). Bioimprinting technique enables the introduction of new binding sites into proteins in the presence of a template (e.g. a substrate or a transition state analog) (Liu et al., 2004). In this way new types of enzymatic activity are obtained, although the starting proteins had either no activity (Wuff et al., 2002). In this study, we have developed a novel strategy for generating imprinted protein which mimic the catalytic activity of acetylcholinesterase. Free amino acids which are found in the active site of acetylcholinesterase were mixed with acetylcholine and then cross-linking of amino acids was performed. The starting protein (bovine serum albumin) without enzyme activity was added and cross-linking step was repeated to fix the new conformation of the protein. Dialysis step was required for removing the template molecules. The catalytic activity and the kinetics of the imprinted protein were investigated.

PP-988
Serum cystatin C concentrations were elevated in cirrhotic patients

C. Ulusoy[1], A. Tiftikci[2], N. Imeryuz[2], C. Celikel[3] and G. Haklar[1]
[1]*Department of Biochemistry, School of Medicine, Marmara University, Istanbul, Turkey,* [2]*Department of Internal Medicine, subdepartment of Gastroenterology, Marmara University, Istanbul, Turkey,* [3]*Department of Pathology, School of Medicine, Marmara University, Istanbul, Turkey. E-mail: cemalettinu@yahoo.com*

Liver diseases are among important health problems as they can lead to inflammation, necrosis, cirrhosis, or hepatoma. Liver fibrosis is an important process in liver diseases and has been shown to result from an imbalance between degradation and synthesis of extracellular matrix. Various enzymes, including serine proteinases, cysteine proteinases and metalloproteinases have been found to be involved in the degradation of extracellular matrix. Thus cystatin C, a member of protein inhibitors of cysteine proteinases could be involved in the progression of hepatic disease. In our study, we aimed to investigate the relationship between the serum levels of cystatin C and liver disease. In our study, 38 patients were enrolled and they were grouped as cirrhotic patients ($n = 8$, median age 57 ± 10.6) and others ($n = 30$, median age 54 ± 13.2) which include hepatitis B, hepatitis C, autoimmune hepatitis, and non-alcoholic steatohepatitis patients. Serum cystatin C levels were determined by N Latex Cystatin C kit (Dade Behring Marburg GmbH, Germany). Serum AST, ALT, albumin, ALP, GGT, total and direct billirubin concentrations were measured spectrophotometrically in an autoanalyser (Roche Modular, Germany). Biopsy specimens were obtained and evaluated pathologically by conventional histochemical dyes. Serum cystatin C levels were significantly elevated in cirrhotic group in comparison with others (1.68 ± 0.78 vs. 0.88 ± 0.16 mg/l, $P < 0.0001$). Pathological examinations revealed stage 4 fibrosis in all cirrhotic patients. Serum ALT

levels were also significantly elevated. Our results showed that serum cystatin C levels increased with the progression of chronic liver disease and is a potential marker for liver fibrosis.

PP-989
Effect of diphtheria toxin on cytoskeleton and protein synthesis

B. Günçer, M. Bektaş and R. Nurten
Department of Biophysics, Faculty of Medicine, Istanbul, Turkey. E-mail: esin_basak@hotmail.com

Diphtheria toxin is an exotoxin that is produced by bacteria named Corynebacterium diphtheriae. Diphtheria toxin can be cleaved into two fragments by furin or trypsin. Fragment A (FA) catalyzes transfer of ADP-ribose moiety of NAD into a covalent linkage with eukaryotic elongation factor 2(eEF2). ADP-ribosylation results in inactivation of eEF2 and inhibition of protein synthesis. Fragment B(FB)is the receptor binding domain. Diphtheria toxin-induced cytotoxicity is yet versatile and includes DNA cleavage and degradation of actin filaments. The relationship of these cellular events to inhibition of protein synthesis remains so far unknown. In this study, FA was purified from Sephadex G-100 column after treatment with trypsin. In order to determine the amount of toxin which was taken inside by the cell lines K562 and HL-60, FA was incubated in the presence of sodium boro-[3H]hydride. We investigated the effect of toxin (FA) on viability, cytoskeleton degradation, *in vitro* protein synthesis and DNA fragmentation in these cell lines. In viability experiments it was seen that HL-60 cell lines are not resistant to toxin and on the other hand; DNA fragmentation occurred in both cell types in the presence of FA. In K562 cell lines despite inhibition of protein synthesis the cells were resistant to toxin. This situation shows that protein synthesis inhibition itself may not be sufficient to induce apoptosis, and additional mechanisms likely come into play to unfold the cytotoxic effect.

PP-990
Identification of several dipeptidyl peptidase-IV activity and/or structure homologues in human brain tumors

J. Stremenova[1], E. Krepela[1], V. Mares[2], V. Dbaly[3], J. Marek[4], J. Trim[5], Z. Vanickova[1] and A. Sedo[1]
[1]*Laboratory of Cancer Cell Biology, Charles University in Prague, 1st Faculty of Medicine, Prague, Czech Republic,* [2]*Laboratory of Cancer Cell Biology, Institute of Physiology, Academy of Sciences, Prague, Czech Republic,* [3]*Department of Neurosurgery, Hospital Na Homolce, Prague, Czech Republic,* [4]*Department of Pathology, Hospital Na Homolce, Prague, Czech Republic,* [5]*Ferring Research Ltd., Southampton, UK. E-mail: jstrem@lf1.cuni.cz*

Dipeptidyl peptidase-IV (DPP-IV)-enzymatic activity is a common attribute of most members of the novel group of multifunctional "DPP-IV Activity and/or Structure Homologue (DASH)" molecules. Numerous local mediators involved in the regulation of cell growth have their biological effects controlled by DASH. Hydrolysis determines not only their half-lives but also modifies their receptor preference and downstream signaling. Association of dysregulated DASH pattern with cancer development and progression has been suggested by several authors. Real time RT-PCR, histochemistry, immunocytochemistry and biochemical assays exploiting advantage of highly specific inhibitors discriminating among the DASH members were used to determine expression and distribution of several DASH members in bioptic material from human brain tumors of different origin and WHO grade. Compared to the low-grade astrocytomas and control tissues, significantly increased DPP-IV enzymatic activity was observed in both non-astrocytic and high-grade astrocytic brain tumors. However, while in the later case the DPP-IV activity increment was dominantly attributable to the "canonical" DPP-IV/CD26, non-astrocytic tumors upregulate namely DPP8/9.Our results, demonstrating varying DASH distribution, suggest possibility of their targeting by specific inhibitors, diminishing risk of side effects caused by nonspecific interference with other DASH molecules.

Acknowledgement: Work was supported by IGA NR/8105-3 and MSMT 0021620808 grants.

PP-991
Effect of net charge of the regulatory domain on catalytic properties of L-pyruvate kinase

I. Faustova, N. Oskolkov and J. Järv
Institute of Organic and Bioorganic Chemistry, Tartu University, Tartu, Estonia. E-mail: ilona.faustova@ut.ee

Activity of L-type pyruvate kinase (L-PK, EC 2.7.1.40) is regulated via phosphorylation of the Ser residue, located in position 12 of the N-terminal regulatory domain of the protein molecule. This phosphorylation decreases affinity of L-PK against phosphoenolpyruvate (PEP) and causes sigmoidal rate vs. concentration plot for this substrate, but has no influence on the hyperbolic rate-concentration plot for the second substrate ADP. As phosphorylation of L-PK alters the net charge of the N-terminal domain of L-PK, it can be proposed that appearance of cooperativity can be caused by this change. To explore this possibility the net charge of the N-terminal domain of L-PK molecule was changed by point mutations in positions 9, 10 and 13 of the protein primary structure, located around the phosphorylation site at Ser 12. These mutations included replacement of the Arg(9) , Arg(10) and Val(13) residues by Ala, Lys, Gln, Leu or Glu, and the catalytic properties of the mutants as well as the phosphorylated enzyme were studied. We have found that in some of these three positions both catalytic activity and enzyme stability can be significantly affected by the mutations. However, the cooperative behaviour of the substrate reaction cannot be induced by replacement of the amino acids, but needs phosphorylation of the Ser(12) residue.

PP-992
Analysis of the coding sequence of the *CCHCR1* gene in the genome of cervical cancer cells

J. Pacholska-Bogalska[1], K. Ciepluch[1], A. Jozefiak[2], W. Kedzia[2], M. Myga-Nowak[1] and A. Gozdzicka-Jozefiak[1]
[1]*Department of Molecular Virology, Adam Mickiewicz University, Poznan, Poland,* [2]*Department of Gynaecology and Obstetrics, Poznan University of Medical Sciences, Poznan, Poland. E-mail: joanna_pacholska@yahoo.com*

The oncogenic HPV (human papilloma virus) types are etiological agents in the development of cervical carcinoma. However, cellular factors engaged in the process of cancer development in cervical epithelial cells are poorly known. Using yeast two-hybrid system we have previously shown that the CCHCR1 protein interacts with the E2 protein of human papillomavirus type 16 in the normal epithelial tissue. CCHCR1 (coiled-coil alpha helical rod protein 1) is a protein of unknown function in the cell. Nuclear localization of the CCHCR1 and its possible interactions with DNA suggest that CCHCR1 regulates cell differentiation or proliferation. It was found to be overexpressed in keratinocytes in psoriatic skin lesions, therefore it was suggested to be involved

in psoriasis susceptibility. The *CCHCR1* gene, encoding 782 amino acid protein, is located on the chromosome 6 and consists of 18 exons. The *CCHCR1* gene is highly polymorphic. The aim of our study was to analyse the coding sequence of the *CCHCR1*gene in the cervical cancer and HPV positive and negative dysplastic cells. The DNA fragments of gene were amplified by PCR and analyzed by SSCP method and sequencing. Preliminary results of our study revealed that exons 7,8 and 11 of the *CCHCR1* gene are the most polymorphic. We suggest that the changes in the gene coding sequence and secondary structure of the CCHCR1 protein may affect its biochemical or antigenic properties.

PP-993
aPTT test interference in a patient treated with heparin + steroid

E. Serin[2], S. Kuyucu Yildiz[1], G. Buğdaycı[2] and N. Yildiz[1]
[1]*Department of Neurology, Abant Izzet Baysal University, Izzet Baysal Medical Faculty, Bolu, Turkey,* [2]*Department of Biochemistry, Abant Izzet Baysal University, Izzet Baysal Medical Faculty, Bolu, Turkey. E-mail: erdincserin@yahoo.com*

A 27-years-old female patient with developed cerebral venous thrombosis while on steroid treatment, and anticoagulation with heparin was initiated, followed by coumadine. aPTT values and then after INR values were analysed to determine the optimum drug dosage. 1000 U/h after a 5000 U bolus heparin (routine procedure) administration caused a severe increase in aPTT values (> 120 sec) in the plasma obtained 5 hours later. After the heparin dosage was decreased (500 U/h), then the aPTT values had fallen down (29 sec) in between the reference limits (21–35 sec). During the management of patient these aPTT fluctuations continued and sensitive titration of heparin was needed. After the technical errors (blood sample drawn from the heparin infusing extremity or coagulameter calibration errors, vs) were eliminated, difficulty in optimizing the heparin dosage and the wide fluctuations in aPTT values were thought to be related to a drug interference, probably to steroids. The aimed thrombolitic therapy was achieved and the thrombus was resolved by the sensitive titration and frequent aPTT analyses although the lack of optimal anticoagulation occurred many times in this patient. If the aPTT values obtained from a patient under heparin treatment with common dosages were over 120 seconds, one must be suspicious about a possible drug interference with the aPTT testing.

PP-994
Role of capacitative calcium influx (CCI) in mouse VSM expressing the α2-isoform of the Na-k-atpase (NKA)

M. Tosun[1], T. J. Pritchard[2], R. M. Lynch[3] and R. J Paul[2]
[1]*Pharmacology, Ege University, College of Pharmacy, 35100, Izmir, Turkey,* [2]*Molecular and Cellular Physiology, University of Cincinnati, College of Medicine, Cincinnati, OH, 45267-0576, USA,* [3]*Physiology, University of Arizona, Tucson, AZ, 85724-5051, USA. E-mail: metiner.tosun@ege.edu.tr*

Various roles have been suggested for the two α-isoforms of NKA in VSM as well as their differential regulation in disease states such as hypertension. Our previous results support the hypothesis that the α2-subunit is important in vascular contractility and blood pressure regulation. To extend these studies, we expressed the α2-isoform in mouse using the smooth muscle specific, α-actin promoter. PCR and Southern blot analyses were used to identify mice carrying the transgene for α2-isoform NKA (α2sm+). Western blot analysis showed that α2-isoform is increased 2-8 fold in α2sm+ mice. Mouse aorta was incubated in Ca-free solution with CPA to inhibit SERCA and unload SR stores. Readdition of Ca increased force whose peak was greater in intact than endothelium-denuded aorta, while the plateau was smaller; these responses were suppressed in aorta from TG mouse. CCI estimated from Ca measurements in primary cultured α2sm+ VSM cells was also suppressed by 20–50%. Our results suggest a diminished CCI influx in TG mice which may be related to their significantly lower systolic blood pressure. Our findings support the hypothesis that the α2-isoform is linked with NCX to modulate SR Ca loading. Thus, α2sm+ mice provide unique models in analyzing the relative contribution of the α2-isoform to VSM Ca homeostasis under pathological conditions.
Acknowledgment: Supported by NIH HL66044 (RJP, RML), Ege University& Novartis Turkey (MT).

PP-995
Evaluation of the influenza virus-inhibitory and antioxidant activities of Bulgarian and Turkish medicinal plants

J. Serkedjieva[1], R. Tzvetkova[2], M. Nikolova[2], M. Sokmen[3] and A. Sokmen[3]
[1]*Institute of Microbiology, Bulgarian Academy of Sciences, 26, Acad. G. Bonchev St., 1113 Sofia, Bulgaria,* [2]*Institute of Botany, Bulgarian Academy of Sciences, 1, Acad. G. Bonchev St., 1113 Sofia, Bulgaria,* [3]*Faculty of Art and Science, Cumhuriyet University, 58 140 Sıvas, Turkey.*
E-mail: jserkedjieva@microbio.bas.bg

It is accepted that acute viral infections are accompanied by profound changes in cell/tissue metabolism, which lead to intensive generation of reactive oxygen species. It has been found that the main cause of mortality from influenza virus-induced pneumonia is the cytotoxicity, which in its turn is determined by the substantially increased levels of •O2- (Akaike et al., 1996). Thus the use of antioxidants could be of great value in preventing the onset or the progression of the disease. We tested the inhibitory effect of 134 plant products – extracts, fractions and pure substances-obtained from 67 Bulgarian and Turkish medicinal plants on the replication of selected influenza virus strains in MDCK cells. The reduction of virus-induced CPE and infectious virus yields were used as measures for viral inhibition. Fifteen samples (11.2%) inhibited influenza virus replication. The most effective products were tested further for their antioxidant capacities by three separate and complementary methods – DPPH assay, b-carotene-linoleic acid assay and NBT-reduction assay. The antiviral effect was connected with antioxidant activity in 100%. There are few reports on the comparative evaluation of the antioxidant and the antiviral activities of plant extracts and plant-derived substances: Euphorbia thymifolia L. (Lin et al., 2002) and Crataegus sinaica L. possessed antioxidant and anti-HSV action (Shahat et al., 2002).

PP-996
Protective effect of melatonin on membrane bound enzymes in brain ischemia/reperfusion injury

M. Tuncer[1], B. Pehlivanoğlu[1], D. Balkancı[1] and T. İsbir[2]
[1]*Department of Physiology, Faculty of Medicine, Hacettepe University, Ankara, Turkey,* [2]*Department of Molecular Medicine, Institute of Experimental Medical Research, İstanbul University, İstanbul, Turkey. E-mail: metuncer@hacettepe.edu.tr*

Na^+-K^+/Mg^{+2}ATPase and Ca^{+2}/Mg^{+2}ATPase are membrane bound enzymes and are essential for functional continuity of the

membrane. Ischemia/reperfusion (I/R) injury effects the activity of these enzymes which have a role in the pathophysiology of the cerebral ischemia. The aim of this study is to evaluate the potential effect of melatonin on activities of Na^{+}-K^{+}/Mg^{+2}ATPase and Ca^{+2}/Mg^{+2}ATPase. Forebrain ischemia was induced by two-vessel occlusion plus hypotension method in Wistar albino rats ($n = 42$) under urethane anesthesia. The activities of the membrane bound enzymes during I and R periods were evaluated in control, ischemia (15 min) and I/R (15/75 min) groups. The protective effect of melatonin was investigated in three groups where melatonin (iv) was applied at the beginning of reperfusion at doses of 400 μg/kg, 1200 μg/kg and 2400 μg/kg. Enzyme activities were measured in the tissue samples removed from frontoparietal cortex. All the data analyzed statistically by SPSS 10.0 for Windows program. Enzyme activities were attenuated in I and I/R groups, being more significant in I/R group ($P < 0.005$). Melatonin improved enzyme activities in all the groups compared to I/R groups ($P < 0.005$). Melatonin treatment increased Na^{+}-K^{+}/Mg^{+2}ATPase activity in a dose-dependent manner which is not observed in Ca^{+2}/Mg^{+2}ATPase activity. These results suggest that; melatonin acts as a neuroprotective agent in reperfusion injury via stabilizing membrane.

PP-997
Molecularly imprinted polymers for urease recognition

A. Baran Teke, M. Teke and A. Telefoncu
Department of Biochemistry, Faculty of Science, Ege University, İzmir, Turkey. E-mail: aysebaran2000@hotmail.com

Recognition sites for molecules have been created in synthetic polymers with a technique called molecular imprinting. The technique is based molecularly imprinting the protein of the interest into the polymer during the polymerization process. When the template protein is subsequently removed, protein specific cavities remain within the polymer matrix. The cavities can be re-loaded with a new sample of the template protein, which then binds specifically within the cavities to the exclusion of other proteins. In this study, we introduced monomers containing amine and/or carboxyl acid groups into polymer units to create either an amphoteric copolymer chain network or negative and positive charged polymer chain network. We selected urease as a template protein. The enzyme is utilized for diagnostic purposes, in the determination of urea in biological fluids. Urease, as the template protein, was used to orient monomers prior to polymerization. Imprinted polymers were synthesized by polymerization of the functional monomers with N,N'-methylenbisacrylamide as the crosslinker in the presence of the template protein. 4-Vinylprydine (VP) and methacrylic acid (MAA) are ionizable monomers, which frequently will act as acid, base, nucleophile or electrophile units. The free radical polymerization was initiated by a water-soluble initiator ammonium persulfate. The recognition ability of the amphoteric, basic and acidic polymers were investigated and compared each others.

PP-998
Preparation and characterization of biosensor based on imprinted urease

M. Teke, M. K. Sezgintürk, E. Dinçkaya and A. Telefoncu
Department of Biochemistry, Science Faculty, Ege University, İzmir, Turkey. E-mail: mustafateke@mail.ege.edu.tr

Immobilized enzymes are widely used for various biochemical and biomedical processes. Urea, the end product of nitrogen metabolism, has a considerable significance in clinical chemistry, where blood urea nitrogen analysis gives an important indication of possible kidney diseases. Increased levels of blood urea nitrogen occur in cases of renal failure. The analysis of urea is also important in other fields, such as agricultural chemistry, where urea is used in fertilizers, for determination of water quality, and in seawater analysis. Urease (urea aminohydrolase, EC 3.5.1.5), which catalysis the hydrolysis of urea to ammonia and carbon dioxide, has been used in immobilized form in artificial kidney for blood detoxification. According to one report approximately half a million patients worldwide are being supported by haemodialysis.

In this study, the enzyme of urease was first complexed by using a substrate analogue, thiourea, in aqueous medium and then this enzyme was immobilized on gelatin by crosslinking with glutaraldehyde on a glass electrode surface. Similarly, urease noncomplexed with thiourea was also immobilized on a glass electrode in the same conditions. The aim of the study was to compare of two biosensors in terms of their repeatability, pH stability and thermal stability, and also, linear ranges of two biosensors were compared with each other.

PP-999
Study of horizontal gene transfer from genetically modified food into gastrointestinal tract of animal model

S. Stuchlik[1], Z. Bargárová[2], P. Celec[1], S. Natarajan[1], R. Pálffy[1] and J. Turna[1]
[1]*Dept. of Molecular Biology, Faculty of Natural Sciences Comenius University, Bratislava, Slovak Republic,* [2]*Dept. of Molecular Biology, Faculty of Natural Sciences Comenius University, Bratislava, Slovak Republic. E-mail: turna@fns.uniba.sk*

Several studies to determine possible horizontal gene transfer (HGT) from genetically modified organisms (GMO) into microbial flora have been published recently. In this work we have developed unique rescue system based on the complementation of CP4 aroA gene present in GM corn maize (encoding 5-enolpyruvylshikimate-3-phosphate synthase – CP4 EPSPS) to study this phenomenon. We prepared several mutants of CP4 aroA gene with deletions in range from 50 to 200 bp in the central part of the sequence. These deletion mutants will be used in complementation experiments to determine frequency of HGT. We have also studied surviving of naked plasmid DNA carrying the whole CP4 aroA gene in different parts (mouth, stomach, ileum, colon ascendens) of gastrointestinal tract in rats. The set of primers was designed to amplify 200 bp product from 5' end, 3' end and central part of the gene. This experiment will be necessary for adjustment of proposed HGT study experiment. During the HGT study of the CP4 aroA gene from genetically modified feed through gastrointestinal tract to bacteria living in animal gut we have also observed functional N-terminal truncated CP4 EPSPS protein forms present in bacteria. We can conclude from our results that some truncated forms of EPSPS can confer full function of native protein and these findings also should be taken into account in risk assessment of possible HGT from GM food/feed.

Acknowledgment: This work was supported by the grant APVT-20-17102.

PP-1000
Genetic variation of natural populations of forest tree species *Fraxinus angustifolia* by the use of molecular markers

R. M. Papi[1], K. A. Spanos[2] and D. A. Kyriakidis[3]
[1]*Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece,* [2]*Forest Research Institute, National Agricultural Research Foundation, 57006, Vassilika, Thessaloniki, Greece,* [3]*National Hellenic Research Foundation, 48 Vas. Constandinou, 11635, Athens, Greece. E-mail: rigini@chem.auth.gr*

The study of genetic variation of *Fraxinus angustifolia* natural populations was the main objective of this work. For the purpose of the work, eight native populations of *F. angustifolia* were identified and selected all over continental Greece. The molecular markers FEMSATL4, FEMSATL11, FEMSATL16, FEMSATL19 and M2-30 of nuclear microsatellite DNA were used to study genetic diversity. From the results' analysis, heterozygosity levels within populations and genetic diversity within and among populations were estimated. High polymorphism within populations and high total gene diversity (HT = 0.784) were recorded for all molecular markers. Molecular markers FEMSATL4, FEMSATL11 and M2-30 were more polymorphic in comparison to FEMSATL16 and FEMSATL19. Mean total heterozygosity (all populations) was found high for all molecular markers (Ho = 0.721–0.909, total mean Ho = 0.799). Genetic differentiation among populations was found low (FST 0.024–0.133, total mean FST = 0.059), fact which explains only a small proportion of the total genetic diversity.

Acknowledgment: The research project was founded under the Fifth Framework Programme (Biodiversity and Ecosystems) of the European Union (FRAXIGEN, EVK2-CT-2001-00108).

PP-1001
Development of a novel biosensor for homocysteine determination

S. Alaçam and A. Telefoncu
Department of Biochemistry, Faculty of Science, Ege University, İzmir, Turkey. E-mail: sencer_1@hotmail.com

Homocysteine is a sulphur containing amino acid. It is not found in natural protein structure but because of methionine metabolism it can be found in all cell types. High levels of homocysteine in plasma is associated with coronary artery disease, cerebrovascular disease, Alzheimer disease, etc. In last decade homocysteine becomes a marker for atherosclerosis and myocardial infarction. Many methods have been developed for clinical determination of homocysteine such as immunoassay, HPLC and enzymatic assay. In this study, development of cheap and fast determination method for homocysteine was aimed. For this purpose, homocysteine desulfhydrase (E.C. 4.4.1.2), (systematic name: L-homocysteine hydrogen-sulfide-lyase) was immobilized on eggshell membrane by crosslinking with glutaraldehyde. As our knowledge this enzyme has been used for first time in biosensing system. Extracted eggshell membrane was modified with homocysteine desulfhydrase and then modified membrane was placed to the surface of ammonium selective electrode which served as homocysteine biosensor. Homocysteine desulfhydrase catalyses reaction below:

L-homocysteine + water → Sulfide + ammonia + 2-oxobutirate

Formed ammonia was detected by pH-ionmeter and by this way homocysteine concentration was detected. For further characterization of biosensor, the effects of enzyme loading, glutaraldehyde concentration, temperature, pH and ionic strength on the response of biosensor have been investigated.

PP-1002
NDRG1 is regulated in human glioblastoma *in vitro* as a consequence to the changing oxygen concentrations of the microenvironment

H. M. Said[1], S. Stein[2], A. Staab[1], A. Katzer[1], M. Flentje[1] and D. Vordermark[1]
[1]*Department of Radiation Oncology, University of Würzburg, Germany,* [2]*Department of Hematology and Oncology, Medical Clinic III, University of Mainz, Germany.*
E-mail: said_h@klinik.uni-wuerzburg.de

NDRG1 (N-myc downregulated gene 1) is a gene, regulated during cell proliferation, differentiation & response to stress(s) ex. Hypoxia. NDRG1 regulation depends onHIF-1α , but, HIF-1α-independent pathways are also involved in NDRG1 regulation during chronic hypoxia.

Materials & methods: Human U251, U373, GaMG & U87-MG glioma cells were exposed to (5%–0.1%) O2, for 1 h, 6 h, 24 h in addition to 24 h with reoxygenation over 24 & 48 h. NDRG1 expression was detected via western blots & mRNA northern blots, HIF-1α via western blots.β-actin, 18S RNA & β-Tubulin served as loading controls, respectively.

Results: With exemption of U87-MG (where NDRG1 was strongly expressed, independent of O2 level, no NDRG1 expression of was displayed in a normoxic environment in all other cell lines & at 5% O2. A substantial degree of NDRG1 expression, with a maximum at 0.1% and relative stability during reoxygenation emerged at (1–0.1)% O2. HIF-1α was (moderately–strongly) expressed after 1 h 0.1% O2, & stable up to 48 h reoxygenation after 24 h at 0.1% O2.

Conclusion: Determination of NDRG1 protein levels in cancers aid in the diagnosis of the disease. Differences of NDRG1 expression levels in different tumours should be considered within this context. NDRG1 has a relatively long stability. Only glioma cells with minimal O2 concentrations display a substantial NDRG1 expression. HIF-1α is a major regulator of this gene in gliobstoma.

PP-1003
Cytotoxic effects of four new thiosemicarbazones on K562 and ECV 304 cells

T. Bal[1], **B. Atasever**[2], Z. Solakoglu[2], S. Erdem-Kuruca[2] and B. Ulkuseven[1]
[1]*Department of Chemistry, Istanbul University, Istanbul, Türkiye,* [2]*Department of Physiology, Istanbul Medical Faculty, Istanbul University, Istanbul, Türkiye. E-mail: belkisatasever@yahoo.com*

Many years, thiosemicarbazones and their metal complexes have been the subject of medicinal studies because of their biological properties. Thiosemicarbazones were found that have antibacterial, antifungal, antimalarial, and antioxidant properties in literature. We investigated effects of four (1–4) new N1,N4-diarylidene-S-methyl-thiosemicarbazone chelates with iron(III) and nickel(II) on K 562 myeloid leukemic cell line and ECV 304 endothelial cell line. The cells were incubated with six different doses of the thiosemicarbazones (50, 10, 5, 1, 0.1, 0.01 μg/ml) for 3 days. Cell cytotoxicity was evaluated by colorimetric MTT test. Study results show that Fe containing thiosemicarbazone derivatives (1 and 2) efficiently cytototoxic for K562 cells and mildly proliferative for ECV 304 cells at same doses. LC 50 dose of Fe chelate (1) was < 5 μg for K562 cell lines. On the other hand, cytotoxicity results of Ni chelate (4), denoted reverse effects on two cell lines, toxic for ECV 304, but proliferative for K562. Experimental results imply that presence of Fe and methoxy

substituent position have definitive effects on cytotoxic properties of studied four new thiosemicarbazones in K 562 and ECV 304 cell lines.

PP-1004
Characterization of polyphenoloxidase (PPO) of medlar (*Mespilus germanica* L.) fruit throughout ripening

H. Torun[1], Y. Kolcuoglu[2], F. Ayaz[1] and A. Colak[2]
Departments of [1]Biology and [2]Chemistry, Karadeniz Technical University, 61080 Trabzon, Turkey.
E-mail: torun80@yahoo.com.tr

Characterization of polyphenoloxidase (PPO) enzyme during fruit ripening of medlar (Mespilus germanica L.) was studied in the present study. Three samplings were conducted from late autumn, when the fruit's pulp whitish brownish-half soft (193 DAF, stage 1), brownish-fully soft (207 DAF, stage 2) and dark brown-fully soft (214 DAF, stage 3). During ripening, substrate specificity, optimum pH and temperature, optimum enzyme and substrat concentrations were determined. Among five mono- and di- phenolic substrates (PHPPA, L-DOPA, Katechol, 4-Methylcatechol and Tyrosine), due to the highest enzyme activity, 4-methylcatechol was chosen as substrate for all stages. For determining optimum pH, the range of pH 3.0–9.0 were tested. The highest enzyme activity was measured at pH 7.0 throughout ripening. Temperature optimum for each stage was determined by measuring the enzyme activity at various temperatures over the range of 10–70 °C with 10 °C increments. Among these, optimum temperatures were found 30, 20 and 30 °C, respectively. Optimum enzyme and substrate concentrations were found 0.1 mg/ml and 40 mM, respectively. During ripening, Vmax and Km value were 476 U/mg protein and 26 mM at 162 DAF, 256 U/mg protein and 12 mM at 177 DAF, 222 U/mg protein and 8 mM at 184 DAF. Accordingly, it can be concluded that the present data showed that as the fruit ripening progressed, there was no significant changes in the optimum values of PPOs, although the kinetic parameters changed.

PP-1005
NMP-22 in the diagnosis of bladder cancer

I. M. Bolayirli[1], H. Balci[1], B. Ozkan[2], B. Tunc[2], Z. Talat[2], M. Hacibekiroglu[1] and A. Oner[2]
[1]Fikret Biyal Central Research Laboratory, Istanbul University Cerrahpasa School of Medicine, Istanbul, Turkey, [2]Department Of Urology, Istanbul University Cerrahpasa School of Medicine, Istanbul, Turkey. E-mail: mrtbola@yahoo

Objectives: Urinary bladder transitional cell carcinoma (TCC) is a common cancer encountered in urinary system. Several biological urinary markers for TCC have been investigated. Several investigators found the sensitivity of NMP-22 in range of 62–92% and the specificity in range of 65–72%. The objective of current study was to assess the sensitivity of NMP-22 for the detection of bladder carcinoma as well as to correlate the NMP-22 with multiplicity of tumor, tumor size, configuration, stage, grade and tobacco usage respectively.
Methods: Eighty-one patients (13 female, 68 male) aged between 22 and 88 (mean ± SD: 62.26 ± 14.41) having clinical suspicious of bladder cancer or in follow- up due to a previous one voided urinary sample prior cystoscopy are stabilized by NMP-22 Urine Stabilizing Solution (DPC Cat No:LSB25) and studied by NMP-22(DPC Cat No:LKMP1)on DPC IMMULITE autoanalysor. A positive test was defined as NMP-22 greater than 10 U/ml. We calculated sensitivity, specificity, positive and negative predictive value for NMP-22.
Results: Sensitivity was 44.8%, specificity was 76.9%, positive predictive value was 52% and negative predictive value was 71%. There was no correlation between NMP-22 and tumor size, stage, grade and tobacco usage ($P > 0.05$).
Conclusions: In the population tested, urine NMP-22 determination was not effective in the diagnosis of bladder carcinoma with regard to the sensitivity and specificity values, therefore control cystoscopic evaluation cannot be avoided.

PP-1006
Experimental studies for production of cytoplasmic and periplasmic thermostable glucose isomerase in recombinant *E. coli*

B. Öztürk, B. Kırdar and A. Hortaçsu
Department of Chemical Engineering, The Institute of Science and Engineering, Istanbul, Turkey. E-mail: oezburcu@yahoo.com.tr

D-Glucose isomerase (GI), which catalyzes the reversible isomerization of D-Glucose to D-Fructose, is of commercial importance in the production of high fructose corn syrup. At high temperature, equilibrium for the isomerisation reaction is shifted toward fructose. The low expression level of thermostable GI in the thermophilic microorganisms and the difficulties encountered during cultivation of the thermophilic microorganisms and purification of the enzyme from its native host make obtaining large amounts of the thermostable enzymes from the natural host impractical. To overcome these problems, GI from thermophile *Thermus thermophilus* was cloned into two different pET vectors, and expressed in *Escherichia coli*. In the first part of the study, the GI gene was cloned into pET-20b (+) vector containing pelB signal sequence for the periplasmic localization of the recombinant protein. The ratio of the translocated enzyme to the total produced enzyme changed with time. The maximum volumetric and specific activities of GI in periplasm and cytoplasm of the recombinant cells were calculated as 50.6 U/l culture and 42.8 U/mg protein, 36.6 U/l culture and 19.6 U/mg protein, respectively. In the second part of the study, the same gene was cloned into pET-28a (+) vector for high level cytoplasmic expression of the GI enzyme. The maximum volumetric and specific activities were calculated as 1474.9 U/l culture and 366.0 U/mg protein, respectively.

PP-1007
Experimental studies to determine the effect of protein characteristics on translocation of fusion proteins in *E. coli*

F. Ö. Ütkür[1], A. Hortacsu[1] and B. Sarıyar[2]
[1]Chemical Engineering, Bogazici University, İstanbul, Turkey, [2]Chemical Engineering, Marmara University, İstanbul, Turkey.
E-mail: ozde.utkur@boun.edu.tr

Previous experimental work to translocate maltose binding protein-glucose isomarase fusion protein from the cytoplasmic to the periplasmic space have shown that the fusion protein mainly remained in the cytoplasm and only one per cent of the expressed fusion protein was transported to the periplasm. In the present study, genetic engineering techniques are exploited to investigate the effect of protein size on translocation. For this purpose, two industrial enzymes of different sizes were cloned using the pMAL-p2 vector. These two enzymes are: pullulanase and putative serine protease of Th. thermophilus HB8. The fusion proteins MBP-serine protease and MBP-pullulanase were expressed in recombinant XL1 cells and the cellular distribution of the fusions were determined in cytoplasmic and periplasmic compartments by

the appropriate enzyme assays and SDS-PAGE analysis. The experimental results showed that most of the MBP-serine protease fusion expressed was translocated to the periplasm with the highest value of 77 per cent and almost all of the MBP-pullulanase fusion expressed was translocated to the periplasm with the highest value of 99 per cent. On the other hand, the results for MBP-GI case was not satisfactory and not more than 1 per cent of the total protein produced was translocated to the periplasm. Therefore, it was obvious that the length of the protein to be translocated didn't have any effect on translocation.

PP-1008
Determination of genetic anomalies by RT-PCR method, and the importance of prognosis in childhood leukemia

M. Kömür[1], F. Erbey[1], G. Leblebisatan[1], İ. Bayram[1], H. Bolat[2], B. Antmen[1] and A Tanyeli[1]
[1]*Division of Pediatric Hematology and Oncology,* [2]*Haematology Laboratory, Cukurova University Medical Faculty, Adana, Turkey. E-mail: drmustafakomur@yahoo.com*

Studies within the last 30 years have shown that etiology of the genetic anomalies have an important role in childhood acute leukemia. These anomalies are important for the clinical process and the prognosis of the illness. In this study we used RT-PCR method to determine these genetic anomalies and aimed to find out the ratio of anomalies in children with leukemia who were referred to our hospital and the effects of these anomalies on prognosis. It includes 132 patients (98 ALL, 34 AML). In ALL patients we examined t(1;19), t(12;21), t(9;22) and t(4;11), in AML patients t(8;21), t(15;17), t(9;22) translocations and inv(16). Results of ALL patients are as follows; %17.4 of the patients with t(12;21), %11.2 with t(1;19) and the %5.1 with t(9;22) positive. Results of AML patients are %8.8 with t(8;21), %8.8 with t(15;17) and %3 with inv(16) positive. We observed that t(12;21) and t(9;22) had a bad effect on prognosis whereas t(1;19) had a positive effect on prognosis in ALL patients. We also observed that t(15;17), t(8;21) and inv(16) found in AML patients had a positive effect on prognosis as well. We found that in childhood leukemia determination of genetic anomalies is important for diagnosis and prognosis. However we need more cases to evaluate better the frequency of genetic anomalies seen in childhood leukemia and importance of its prognosis.

PP-1009
Developing and running a clinical database for inherited disorders and a candidate nationwide study

A. E. Yalin, S. Yuzbasioglu, E. D. Yenilmez, S. M. Yildiz, A. Tuli, M. A. Curuk, G. Attila and K. Aksoy
Department of Biochemistry, Çukurova University, Medical Faculty, Adana, Turkey. E-mail: eyalin@cu.edu.tr

Hemoglobinopathies are known to be most common genetic abnormality and as in the worldwide, constitutes important public health problem especially in Çukurova region of Turkey. The present study investigated that a prenatal diagnosis registration system was a safe management of data for couples involved in prenatal diagnosis. An in-house relational database was developed on a personal computer by MS Access and around 1800 couples for both thalassemias and abnormal hemoglobins were registered to date. The database includes data entry forms for demographic and clinical findings of couples and chorionic villus, and uses a search facility that allows users to query data according to selected parameters among records. The system was developed in normalised manner. Therefore, the system and database are effective in raising safety of data and an increase in efficiency.

PP-1010
Evaluation of the antiherpetical activities of two traditional Greek medicinal plants

M. Matta, G. Sylignaki, D. Lazari and C. Panagiotidis
Department of Pharmaceutical Sciences, Aristotle University of Thessaloniki, GR 54 124 Thessaloniki Greece. E-mail: pchristo@pharm.auth.gr

Herpes simplex viruses (HSV) are ubiquitous pathogens which cause a variety of diseases ranging in severity from mild to severe, and in certain cases, they can even become life threatening, especially in immunocompromized patients. HSV becomes latent mainly in trigeminal ganglia, after primary infection, and persists for the lifetime of the host with periodic reactivations. Nucleoside analogues, such as aciclovir (ACV), are the only approved drugs for the treatment of HSV infections. However, the widespread use of nucleoside-based drugs has led to the emergence of resistance in HSV. Medicinal plants have been traditionally used for different kinds of ailments including infectious diseases. The present study focuses on the evaluation of extracts derived from two Greek plants, i.e. Sideritis perfoliata subsp. perfoliata and Thymus longicaulis, either for their virucidal activity or their abilities to inhibit HSV-1 propagation. Air-dried and powdered aerial parts of the above mentioned plants were extracted at room temperature with a series of solvents of increasing polarity, petroleum ether, CH2Cl2, MeOH, mixture of MeOH-H2O 1:1 and H2O. The dried extracts were dissolved in DMSO and tested for their ability to inhibit infection or delay the virus lytic cycle. Anti-HSV activities were found in extracts from both plants and the mechanism of action is being evaluated.

PP-1011
Prion protein gene polymorphisms in four Slovenian autochthonous sheep breeds

J. Zabavnik[1], M. Cotman[1], P. Juntes[1], M. Pogačnik[1] and I. Ambrozic[2]
[1]*Veterinary Faculty of the University in Ljubljana, Slovenia,* [2]*Veterinary Administration of the Republic of Slovenia, Ljubljana, Slovenia. E-mail: jelka.zabavnik@vf.uni-lj.si*

Scrapie is a fatal degenerative disease of the central nervous system known as the transmissible spongiform encephalopathy (TSE) occurring naturally in sheep. Three main scrapie-linked polymorphisms in the prion protein gene (Prnp) located at the codon positions 136, 154 and 171 modulate the susceptibility to classical type of scrapie. In order to evaluate and characterize the Prnp polymorphisms in Slovenian autochthonous sheep breeds and to evaluate their genetic susceptibility we have analyzed the Prnp from 171 sheep of four breeds (Bela Krajina Pramenka, Bovska sheep, Istrian Pramenka, Jezersko-Solcava). Polymorphisms at codons 136, 154 and 171 were determined by nucleotide sequencing of the Prnp and by alelic discrimination assay. Four allelic variants were determined in Bela Krajina Pramenka and Istrian Pramenka, and five and eight different genotypes have been determined, respectively. The examined Jezersko-Solcava sheep and Bovska sheep have five allelic variants and eleven and eight genotypes, respectively. The most frequent genotype in the examined sheep population is ARQ/ARQ (28.65%). Animals carrying this genotype are moderately susceptible to scrapie. The allelic variant VRQ, known to carry very high risk of scrapie is only poorly represented in the population of the examined sheep.

Only 5.85% of examined sheep population has genotype VRQ/VRQ. Slightly more abundant is the allelic variant ARR that is typical for sheep resistant to scrapie (6.43% of ARR/ARR).

PP-1012
Yeast protein kinase CK2 holoenzyme requires both regulatory β and β′ subunits for its activity

K. Kubiński, K. Domańska, E. Sajnaga, R. Zieliński and **R. Szyszka**
Department of Molecular Biology, Catholic University of Lublin John Paul II, Lublin, Poland. E-mail: szyszkar@kul.lublin.pl

CK2 is a pleiotropic constitutively active serine/threonine protein kinase composed of two catalytic α- and two regulatory β-subunits, whose regulation is still not well understood. Yeast CK2 is composed of two catalytic (α and α') and two regulatory (β and β') subunits. It seems to play an essential role in regulation of many cellular processes. Four active forms of CK2, composed of αα'ββ', α_2ββ', α'_2 ββ', and a free α'-subunit were isolated from wild-type yeast and strains containing a single deletion of the catalytic subunit. In addition in yeast strains having single deletion of the regulatory β or β' subunit, a holoenzyme CK2 activity was not observed. Those activities were restored when strains having deletions were transformed with plasmids carrying CK2β or CK2β' encoding genes respectively. Each species of protein kinase exhibits properties typical for CK2, but they differ in substrate specificity and sensitivity to inhibitors. Presented results confirm that both β or β' regulatory subunits are required for formation of yeast holoenzyme and its catalytic activity. Different substrate specificity and sensitivity to inhibitors suggests that each CK2 isomer may regulate different processes or may differ in the way of its regulation.

PP-1013
BNP levels in patients undergoing continuous ambulatory peritoneal dialysis and their relationship to mortality rate

P. Tombaklar Bilgi[1], A. Coşkun[1], H. Aral[1], M. Besler[2], G. Güvenen[1], S. Trablus[2], R. Ataman[3] and F. Sametoglu[4]
[1]Central Clinical Chemistry Laboratory, Ministry of Health Istanbul Training and Research Hospital, İstanbul, Turkey, [2]Department of Nephrology, Ministry of Health Istanbul Training and Research Hospital, İstanbul, Turkey, [3]Department of Nephrology, Istanbul University Cerrahpaşa Medical Faculty, Istanbul, Turkey, [4]Ministry of Health İstanbul Training and Research Hospital, Internal Medicine Clinics, Istanbul, Turkey. E-mail: drhalearal@yahoo.com

Chronic inflammation, as evidenced by increased levels of pro-inflammatory cytokines and C-reactive protein (CRP), is a common feature in dialysis patients and is associated with an increased cardiovascular morbidity and mortality. It is well known that plasma brain natriuretic peptide (BNP) concentration is elevated in end stage renal diseases (ESRD). We examined BNP and CRP levels in patients undergoing continuous ambulatory peritoneal dialysis (CAPD) and their relationship to prognosis of heart failure, mortality, after one year. Sixty-four patients (27 male, 37 female) made up our study group. CRP levels were measured by nephelometric method, (Beckman Coulter Inc., USA) and BNP concentrations were measured by fluorescence immunoassay (Biosite Inc., USA). Two patient subgroups were made up; mean BNP levels were found as 445 ± 589 pg/ml in patients with CRP > 0.8 mg/dl and 245 ± 291 pg/ml in patients with CRP ≤ 0.8 pg/ml ($P > 0.05$). Four of 64 patients died in one year; only one had BNP over 500 pg/ml, the others had BNP below 100 pg/ml. Although, increased BNP levels in parallel with CRP was seen in dialysis patients, it was not similar in mortality rate. This may be because our group was so small, further research is needed to investigate the role of BNP in predicting cardiovascular risk factors in patients undergoing CAPD.

PP-1014
The effect of MMP26 gene expression in tumorigenesis and invasiveness of gastric carcinoma

T. Karabiyik[1], G. Dogusoy[2], M. Paksoy[2], A. K. Belli[2], F. Aydogan[2] and **M. B. Irmak Yazıcıoğlu**[1]
[1]Department of Biology, Fatih University, Istanbul, Turkey, [2]Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey. E-mail: life.scientist@gmail.com

The matrix metalloproteinases (MMPs) contribute to the invasive and metastatic abilities of a variety of malignant tumors. Among these MMPs, MMP-26 has been described to take role in invasion and metastasis of cancers of epithelial origin including choriocarcinoma, breast and prostate carcinoma. Moreover, MMP-26 gene, along with MMP-7 gene, is a target of Wnt-signaling pathway, being regulated by β-catenin. There is a striking relation of Wnt pathway and gastric carcinogenesis. Alterations of the genes in this pathway, such as β-catenin and E-cadherin, have been illustrated in gastric carcinoma cases, indicating deregulation of this pathway during gastric carcinogenesis. In this study, the involvement of MMP-26 gene in invasion and metastasis of gastric tumors has been analyzed, and the roles of MMP-7 and β-catenin were also studied. Tumor and normal biopsies of nineteen patients have been studied. The expression levels of MMP-26, MMP-7, and β-catenin were investigated by quantitative RT-PCR. Out of nineteen tumor samples, two, which are invasive, showed MMP-7 expression. Differences in β-catenin mRNA expression between normal and tumor samples were observed in eight patients. There was no MMP-26 expression in both normal and tumor samples, which indicates that MMP-26 expression does not occur in gastric epithelial tissue, and MMP-26 does not play a role in the invasion of gastric tumors.

PP-1015
Lysosomal and extralysosomal enzyme activities during starvation

L. Begic
Department of Biochemistry, Faculty of Pharmacy, University of Tuzla, Tuzla, Bosnia and Herzegovina. E-mail: lejla.begic@untz.ba

Starvation is an excellent model for research of enzyme activities which take part in catabolism of myofibrillar proteins. Due to the fact that most protein reserves in the body are stored within skeletal muscles, the mobilization of amino acids from muscular proteins represents the basic adaptation to starvation. The aim of this work is to determine the impact of starvation on activity of lysosomal enzymes cathepsin D, cathepsin L, as well as of extralysosomal calpain in skeletal soleus and semimembranosus muscles of rats, which have been starved in periods lasting one to seven days. After seven days of starvation, the weight loss of 22% was evident in fast glycolytic semimembranosus muscle and of 23.5% in slow oxidative soleus muscle. The increase of lysosomal enzyme activities was especially evident in m.soleus. Cathepsin L activity was increased after the first day of starvation, whereas the cathepsin D activity was increased on the second day in both muscles. It shows that cathepsin L takes part in the initial phase of myofibrillar protein degradation. Activity of extralysosomal

enzyme calpain was significantly increased on fourth day of starvation in m. soleus, while in m. semimembranosus no significant changes in enzyme activity have been noticed. These results suggest that the lysosomal proteinases and calpain have role in the higher protein degradation process during starvation.

PP-1016
Structural and functional studies of lipocalin-type prostaglandin D synthase by X-ray small angle scattering

K. Inoue[1], T. Inui[2], T. Okubo[3], K. Goda[4], Y. Urade[5] and N. Yagi[1]
[1]*SPring-8/JASRI, Hyogo, Japan,* [2]*Osaka Prefecture University, Osaka, Japan,* [3]*Osaka University, Osaka, Japan,* [4]*Computer-Aided Molecular Modeling Research Center Kansai, Hyogo, Japan,* [5]*Osaka Bioscience Institute, Osaka, Japan.*
E-mail: katsuino@spring8.or.jp

Lipocalin-type prostaglandin D synthase (L-PGDS) is a member of the lipocalin superfamily. The tertiary structure of L-PGDS showed an eight-stranded β-barrel architecture. L-PGDS has some interesting properties. One of them is that L-PGDS has hydrophobic part inside the β-barrel structure and it can bind a large kind of hydrophobic low-molecular compounds, e.g., retinoic acid, some kind of dyes and amyloid b-protein. Such a characteristic of L-PGDS aroused our interest in studying the binding process with some substrates. So, in order to clarify the binding process, especially to study the conformational change of L-PGDS in the solution, we have performed the small angle X-ray scattering (SAXS) measurements. The SAXS measurements were done with three kinds of samples, that is, L-PGDS/retinoic acid (RA), L-PGDS/bilirubin (BR) and L-PGDS/biliveldin (BV) system. From the SAXS measurements, only in the small-angle region, deviation was observed on scattering curves. When a ligand binds to L-PGDS, the radius of gyration (Rg) becomes smaller. Rg differs depending on the size of the ligand. Those structural changes may be ascribed for the conformational flexibility of L-PGDS molecule; leading its unique property that L-PGDS exhibits a broad selectivity of ligand binding. As the future perspective of our study, our ultimate goal is to take advantage of L-PGDS as a kind of 'Micro carrier' for lipophilic drugs.

PP-1017
Crystal structure of a B-DNA/Z-DNA junction

S. Ha[1], Y. Kim[2], A. Rich[3] and **K. Kim**[1]
[1]*Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine, Suwon 440-746, Korea,* [2]*Department of Chemistry, Sungkyunkwan University, Suwon 440-746, Korea,* [3]*Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.*
E-mail: kkim@med.skku.ac.kr

Left-handed Z-DNA is a higher energy form of the double helix, stabilized by negative supercoiling generated by transcription or unwrapping nucleosomes. Regions near the transcription start site frequently contain sequence motifs favorable for forming Z-DNA, and formation of Z-DNA near the promoter region stimulates transcription. Z-DNA is also stabilized by specific protein binding; several proteins have been identified with low nanomolar binding constants. Z-DNA occurs in a dynamic state, forming due to physiological processes then relaxing to the B form. Each time a DNA segment turns to Z-DNA, two B-Z junctions form. These have been examined extensively, but their structure was unknown. Here, we describe the structure of a B-Z junction as revealed by X-ray crystallography at 2.6 Å resolution. A fifteen base-pair segment of DNA is stabilized at one end in the Z conformation by Z-DNA binding proteins, while the other end remains B-DNA. Continuous stacking of bases between B-DNA and Z-DNA segments is found, with the breaking of one base pair at the junction and extrusion of the bases on each side. B-Z junctions are easily formed in biological systems due to the widespread occurrence of negative supercoiling. The knowledge that these are associated with extrusion of bases at the junction is likely to open many new avenues of research.

PP-1018
Investigation of the homocysteine effect on the glutathione peroxidase activity *in vitro* conditions

A. Durmaz and N. Dikmen
Department of Biochemistry, Faculty of Medicine, Cukurova University, Adana, Turkey. E-mail: aysendurmaz@yahoo.com

Homocysteine is a sulphydryl-containing amino acid derived from the metabolic demethylation of dietary methionine. Among fasting individuals, 'normal' total homocysteine (tHcy) commonly ranges from 5 to 15 μmol/l, and higher fasting values are classified arbitrarily as moderate (16–30), intermediate (31–100), and severe (> 100 μmol/l) hyperhomocysteinaemia. Glutathione peroxidase (EC 1.11.1.9) belongs to the family of selenoproteins and place an important in the defence mechanisms of mammals, birds and fish against oxidative damage by catalyzing the reduction of a variety of hydroperoxides, using glutathione as the reducing substrate. This study has done in order to examine how commercially available homocysteine affects the bovine erythrocyte glutathione peroxidase activity. With the low GSH concentration (1×10^{-4} M GSH) activity of GPx is inhibited severely by Hcys (with 50 μM Hcys 97% inhibition, with 500 μM Hcys 99% inhibition). It was observed that when the GSH concentration is increased this inhibition is decreased significantly (when GSH concentration is 10×10^{-4}M with 50 μM Hcys 38% inhibition and with 500 μM Hcys 45% inhibition). At the end of the study, it was observed that the enzyme activity was inhibited in severe hyperhomocysteinaemia (50–500 μM Hcy) conditions and high glutathione concentrations partially protected the enzyme from this inhibition. This results suggested that GPx inhibition may be effective on the physiopathology of the hyperhomocysteinaemia.

PP-1019
ADMA: Is it really a new marker for cardiovascular risk factor?

M. Cihan and M. Erbil
Department of Biochemistry and Clinical Biochemistry, Gulhane Military Medical Faulty, Ankara, Turkey.
E-mail: mcihan@gata.edu.tr

It is said that 'Coronary endothelial dysfunction is associated with elevated asymmetric dimethylarginine in patients with early atherosclerosis.' and 'asymmetric dimethylarginine has the roles in the pathophysiology of lots of diseases'. We know that finding a new marker for Cardiovascular Diseases is not so easy and There should b ea long time period and also retrospective data. From Framingham Study, We are sure that saying 'something is responsible for Cardiac Diseases ' is not so easy. In our poster, we combined the results of several studies that address a set of related research hypotheses about ADMA and its role in cardiac diseases.

PP-1020
Urinary methylmalonic acid levels in ischemic stroke

N. Kazan[1], **M. Şeneş**[1], Ö. Coşkun[2], L. İnan[2], G. Saydam[1] and D. Yücel[1]
[1]*Department of Clinical Biochemistry, Ankara Education and Research Hospital, Ministry of Health, Ankara, Turkey,* [2]*Department of Neurology, Ankara Education and Research Hospital, Ministry of Health, Ankara, Turkey.*
E-mail: senesmehmet@yahoo.com

In this study, urinary methylmalonic acid (MMA), red blood cell (RBC) folate, serum homocysteine, vitamin B12 and folate levels were measured in 40 patients (age = 64 ± 14; 21 male, 19 female) with ischemic stroke and controls (age = 65 ± 8; 14 male, 11 female). Mean total homocysteine level of ischemic stroke group was higher than that of control group but not statistically significant ($P = 0.239$). Vitamin B12 levels of patient group were lower than those of control group, but difference was not statistically significant ($P = 0.650$). Serum and RBC folate levels of patients were higher than those of control group but the difference between the groups was not statistically significant ($P = 0.294$ and $P = 0.145$). MMA concentrations of patient group were significantly higher as compared with controls ($P = 0.006$). We modified a previously used HPLC method for the measurement of MMA and determined the performance characteristics of the method. The results of the study show that serum homocysteine, vitamin B12, folic acid and RBC folate values are important in risk stratification of ischemic stroke in older population, but urinary MMA concentrations may better reflect vitamin B12 deficiency at the tissue level than plasma vitamin B12 concentrations. The modified HPLC method is suitable for routine use in clinical laboratory with respect to the performance characteristics, cost-effectiveness and practicability of the method.

PP-1021
Partial purification and characterization of arylamine N-acetyltransferases (NATs) from human breast tumor tissues

Y. S. Su
Geylan and T. Güray, Department of Biology, METU, Ankara, Turkey. E-mail: senemsu7@metu.edu.tr

Arylamine N-acetyltransferases (NATs) are found nearly in all species from bacteria to humans. They catalyse the acetyl transfer from acetyl coenzyme A (AcCoA) to an aromatic amine, heterocyclic amine or hydrazine compound. In humans, cytosolic N-acetyltransferases are involved in biotransformation of many arylamine and hydrazine drugs, including many carcinogens present in the diet, cigarette smoke and the environment. Arylamine N-acetyltransferases (NATs) were partially purified from human breast tumor tissues with complete separation of the isoforms in DEAE-Cellulose ion-exchange step. NAT with activity towards p-aminobenzoic acid (PABA) was isolated and purified from human breast tumor with 77% yield and a purification factor of 5-fold. NAT with activity towards sulfamethazine (SMZ) was isolated and purified from human breast tumor with 21% yield and a purification factor of 3-fold. Further purification attempts by Blue Sepharose affinity column chromatography resulted in the complete loss of both enzyme activities. The NAT1 purified from human breast tumor tissues had a molecular weight (Mr.) value of about 27 600 and an isoelectric point (pI) around 4.8, as confirmed by SDS-PAGE, IEF and Western blotting analysis. With immunohistochemical analysis, the level of intensity of NAT1 immunostaining was observed to be going from weak in reduction mammoplasty samples to strongest in malignant breast tissue.

PP-1022
Immunocytolocalisations of cytokeratin 7 and 8 in primary and metastatic lung adenocarcinomas

S. Oguztüzün and **A. Melekoglu**
Department of Biology, Kirikkale University, Kirikkale, Turkey.
E-mail: angelson2025@yahoo.com

The distinction of a primary lung adenocarcinoma from a metastatic lesion is important, because the treatment and prognosis differ for patients with these malignancies. Such a distinction can be difficult because of overlapping cytologic features. The cytokeratin 7 (CK7) and cytokeratin 8 (CK8) immunoprofiling has been helpful in the differential diagnosis of primary lung adenocarcinomas versus adenocarcinomas metastatic to the lung. In our study we have evaluated the staining with the expression of CK7 and CK8 and ALP in the discrimination between primary and metastatic lung adenocarcinoma in 111 cytologic specimens. Cytologic smears preparations from 77 primary lung adenocarcinomas and 34 metastatic lung adenocarcinomas were immunostained with monoclonal antibodies CK7 and CK8, and ALP. Positive immunostaining for CK7 and CK8, and ALP was based on cytoplasmic staining of the neoplastic cells. Positive staining with ALP was noted in 48 primary lung adenocarcinomas (79%). 20 metastatic adenocarcinomas (77%) were positive for ALP. CK8 is a common marker of primary lung adenocarcinomas (58% of cases) but it is also observed in 33% of metastatic adenocarcinomas. CK7 was noted in 11 metastatic lung adenocarcinomas (58%) and in 28 primary lung adenocarcinomas (51%). In conclusion, An adenocarcinoma is likely a primary lung adenocarcinoma when it is of CK8, ALP positive phenotype. It is also likely a metastatic lung adenocarcinoma when it is of CK7 positive phenotype.

PP-1023
ACE1 and eNOS T-786C gene polymorphism in elite Turkish athletes

I. Peker[1], F. Ciloglu[2], Z. Kavuncuoglu[1], I. Sahin[3], E. Suel[3], B. Cotuk[3], I. Basoz[3], B. Tangurek[4] and **A. Pehlivan**[3]
[1]*Dept.of Chemical Engineering, Biotechnology Laboratory 314, Marmara University, Istanbul, Turkey,* [2]*Genlab Medical Diagnostics and Research Laboratory, Istanbul, Turkey,* [3]*Marmara University, Sport School, Istanbul, Turkey,* [4]*Siyami Ersek Hospital, Istanbul, Turkey.*
E-mail: ipeker@marmara.edu.tr

Genetics is gaining importance as a tool for determining sportive talent. We analyzed ACE1 (Angiotensin Converting Enzyme) genotype in elite athletes (football players, wrestlers, volleyball players and basketball players) and eNOS-786C (endothelial Nitric Oxide Synthase) genotype in elite wrestlers and compared them to sedentary controls. There were four ACE1 study groups: 1- Football: 33 elite football players and 50-controls, 2- wrestling: 49 elite wrestlers and 52 controls, 3- Volleyball: 64 elite volleyball players and 100 controls and 4-Basketball:58 elite basketball players and 100 controls. All the wrestlers also had their eNOS gene polymorphisms determined and compared to those of the control group. No meaningful relationship was found between ACE1 allele frequency distribution in elite athletes and those of the control group ($P > 0.05$). A statistically significant difference was found in the study of eNOS T-786C polymorphism (TT, TC, CC) between TT and TC genotypes in elite wrestlers and

controls, ($P = 0.005$). No such relationship could be determined in the study between CC genotype and the control group. Establishment of a meaningful relationship between eNOS gene polymorphism and elite wrestlers has provided hope for the possible use of performance genes as markers in the determination of sportive talent.

PP-1024
Serum leptin levels during the menstrual cycle in Iranian healthy women

N. Einollahi, N. Dashti and F. Nabatchian
College of Allied Health Sciences, Tehran University of Medical Sciences. E-mail: einolahn@tums.ac.ir

Leptin, the protein encoded by the ob gene is expressed in adipose tissue and is considered to act centrally as a signaling factor to regulate body weight homeostasis through the control of appetite and energy expenditure. it also appears that leptin is involved in reproductive physiology. Circulating leptin levels are closely related to the percentage of body fat and correlate with the body mass index(BMI).Significant correlations have been found between sexual hormones and leptin which has cytokine and hormonal properties. In this study, we investigated the possible changes in serum leptin concentration through out the menstrual cycle. Fasting blood samples were collected during the follicular phase, midcycle and luteal phase of the menstrual cycle from healthy women. Serum estradiol, progesterone, LH, FSH, and leptin serum levels were measured. Serum leptin concentrations differ during menstrual cycle in line with changes in gonadotropin steroid concentrations. Also leptin level were correlated with BMI.

PP-1025
Effect of steroid treatment on serum oxidant-antioxidant system and interleukin levels in pediatric patients with asthma

G. Mungan[1], B. Guven[1], M. Can[1], S. Acikgoz[1], G. Ustundag[2] and T. Tuncer[2]
[1]Department of Biochemistry, [2]Department of Pediatry, Zonguldak Karaelmas University School of Medicine, Zonguldak, Turkey. E-mail: agmungan@yahoo.com

Aim: We aimed to investigate the plasma levels of interleukin–6 (IL-6), Tumor Necrosis Factor-alfa (TNF-a), malone dialdehyde (MDA) and Total Antioxidant Status (TAS) which were assumed to have a significant role in pathogenesis of asthma in children and, to evaluate the effects of steroid treatment on these parameters.
Methods: The study groups included 27 children of 6–16 years with mild-medium persistent asthma and 25 healthy children in the same age. Symptom scoring, breathing function tests and the measurements of plasma levels of Ig E, eosinophylic cationic protein, IL-6, TNF-a, TAS and MDA levels are performed in the asthmatic group before and after the budenoside treatment of 4 weeks and were compared with the results of the healthy group.
Results: There was no significant difference between the pretreatment MDA and TAS levels of the asthmatic and control groups ($P > 0.05$). Post-treatment MDA levels were found to be significantly lower than the pretreatment levels ($P < 0.05$), but the TAS levels did not change. TNF-a levels of asthmatic group were significantly higher than those of control group ($P < 0.01$), but IL-6 levels were not significantly different. The TNF-a and IL-6 levels of asthmatic group did not change after budesonide treatment.
Conclusions: We conclude that budesonide can achieve clinical improvement in asthmatic children without affecting the oxidant stress, antioxidant defense and proinflammatory cytokins, although the inflammatory activity may continue.

PP-1026
The levels of lysyl oxidase, superoxide dismutase, ceruloplasmin and related trace elements in the patients with acute myocardial infarction

S. Himmetoglu[1], V. A. Vural[2], F. Ayan[2] and S. Kaya[1]
[1]Department of Biochemistry, [2]Department of Cardiology, Medical Faculty of Cerrahpasa, University of Istanbul, Istanbul, Turkey. E-mail: scerrahpasa@yahoo.com

The aim of this study, is to evaluate the lysyl oxidase (LOX), copper (Cu), zinc (Zn), superoxide dismutase (SOD) and ceruloplasmin (Cp) in the patients of acute myocardial infarction ($n = 25$) and healthy controls ($n = 20$). AMI patient group has been examined on the days of number 0, 7 and 30.for mentioned parameters. The LOX activity of the AMI patient group on the day number 0. was significantly higher than the other groups and was lowering in time; and on the day number 30. was at the same level with the control group. The plasma copper level of the AMI patient group that showed no significant difference with the control group, on the day number 0.,increased in time, reached the highest value on the day number 7 and was still higher than the level of the control group on the day number 30.While the plasma zinc level of the AMI group was on the same level of the control group on the day number 0,it lowered down rapidly till the day number 7 and even on the day number 30,it was still very low according to the control group. The plasma SOD activity showed no remarkable difference on the days of number 0, 7 and 30.The Cp levels in the AMI group on the day number 0, were higher according to the control group, and reached the highest level on the day number 7,and were found to be lowered a little bit on the day number 30.Data indicate that, serum LOX activity can be considered a valuable marker for diagnosis of the ischemic heart diseases and during the following of the prognosis.

PP-1027
The effect of atorvastatin on biomarkers of postmenopausal woman

L. Koldas[1], S. Himmetoglu[2], F. Ayan[1], M. T. Agac[1], **S. Kaya**[2] and M. Eryavuz Sarıdogan[3]
[1]Department of Cardiology, [2]Department of Biochemistry, [3]Department of Physical Medicine and Rehabilitation, University of Istanbul, Medical Faculty of Cerrahpasa, Istanbul, Turkey. E-mail: skaya@istanbul.edu.tr

Statins are the most commonly used drugs for prevention and therapy of cardiovascular diseases. Recently, besides their specific cholesterol-lowering effect, these agents have gained considerable interest via various pleiotropic effects, which probably contribute to the bone formation. The purpose of the present study is to investigate the effects of statin treatment on bone formation and resorption markers, and to evaluate its effect which is still under debate on osteoporosis.29 postmenopausal women who are given statin for the first time, because of hypercholesterolemia were enrolled to the study. Normocholesterolemic 16 postmenopausal women were chosen as the control group. Bone formation markers such as Bone ALP, Osteocalcin, PIIINP, PINP and bone

resorption marker such as ICTP were measured in the patient group before and after the statin treatment. There were no significant differences in the levels of bone formation and resorption markers which are measured before and after the treatment ($P > 0.05$) in the patient group. It has been found that osteocalcin levels were higher in the control group than the patient group before and after the treatment respectively ($P < 0.001$ and $P < 0.001$). After statin treatment, correlation was found between bone formation and resorption markers. As respect with the determined correlation it can be concluded that statins may have a role on maintenance of the balance of bone formation and resorption.

PP-1028
Investigation of thrombin activatable fibrinolysis inhibitor levels in essential hypertension

A. Yildiz[1], B. Aslan[1], N. Yucel[1], S. Cigerli[1], F. Karakoyunlu[1], N. Eren[1] and S. K. Okutur[2]
[1]*Department of Biochemistry, Sisli Etfal Education and Research Hospital, Istanbul, Turkey,* [2]*Internal Medicine Clinic Sisli Etfal Education and Research Hospital, Istanbul, Turkey.*
E-mail: nezaketeren@yahoo.co.uk

This study involves totally 80 cases that is new diagnosed and previously non-treated Essential hypertension (EHT) patients (*n*:40) and normotansive control group (*n*:40). Our cases previously did not use antilipidemic drugs. We separated these two groups as with hypercholesterolemia (>200 mg/dl) and without hypercholesterolemia (<200 mg/dl) to observe TAFI (Thrombin activatable fibrinolitic inhibitor) values of the patients with EHT how to react independent from hypercholesterolemia. In this study BMI (Body Mass Index), systolic blood pressure, diastolic blood pressure values, Total, LDL, VLDL, HDL-Cholesterol, Trygliceride, Fibrinogen and TAFI Ag (antigen by ELÍSA method) values were measured. All parameters were compared between each other and the groups. TAFI Ag values were significantly higher in hypertensive groups than the control groups ($P < 0.01$). Contrary to the literature we followed that TAFI Ag values were not affected from hypercholesterolemia and TAFI Ag values increased in hypertensive groups independent from Total-C. Contrary to the literature we didn't establish correlation between two groups when we compare TAFI Ag with BMI, Trygliseride, HDL-, LDL, VLDL-Cholesterol, Fibrinogen. By the light of these outcomes, we can conclude that elevated TAFI Ag values independent of coexisting hypercholesterolemia may play a role in the pathogenesis of EHT and may be considered as a new hemostatic factor.

PP-1029
Plasma homocystein and platelet aggregation levels in stone and paper workers

H. Yapislar[1], S. Aydogan[1], A. Tuncay[1] and **N. Simsek**[2]
[1]*Physiology Department, Medical Faculty, Kayseri, Turkey,* [2]*Hematology Laboratory, Medical Faculty, Kayseri, Turkey.*
E-mail: handeyapislar@hotmail.com

Introduction: In industrial societies, occupational diseases are very important for workers who works in risky business branches. Enlargement of industrialization in different areas cause workers to expose many different chemicals and dusts. Inhalation of these chemicals and dusts for a long time cause many pulmoner diseases. By the time, these chemicals and dusts also cause some cardiovascular diseases, too. Today, it is known that hyperhomocysteinaemia is an independent risk factor for atherosclerosis, thrombosis and other cardiovascular diseases. Platelets also play an important role in the process of homeostasis and thrombosis. Activation of platelets perturbs haemostatic balance, which eventually leads to thrombotic events. In this study, we aimed to study plasma homocystein levels and platelet aggregation levels in workers who works in stone and paper industry and examine the cardiovascular disease risk of these workers.
Method: Thirty-eight paper workers, 35 stone workers and 30 office workers for control included in this study. Blood samples were collected from subjects. Plasma homocystein levels were detected by HPLC and platelet aggregation levels were measured with aggregometer.
Results: Although plasma homocystein levels found increased in stone and paper workers according to control group, this increase was not significant ($P > 0.05$). However, platelet aggregation levels in stone and paper workers were increased significantly according to control subjects ($P < 0.05$).
Conclusion: In this study, homocystein levels of these workers may be affected by the duration of working period and workers ages. However, elevation of platelet aggregation elucidate the risk of cardiovascular diseases in stone and paper workers.

PP-1030
Potential function of Matrilin-2 in rat skeletal muscle regenerating from notexin-induced necrosis

M. Kiricsi[1], E. Korpos[2], L. Mátés[2], L. Mendler[1], F. Deák[2], I. Kiss[2] and L. Dux[1]
[1]*Department of Biochemistry, Faculty of Medicine, University of Szeged, Szeged, Hungary,* [2]*Institute of Biochemistry, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary. E-mail: kiricsim@hotmail.com*

A large number of factors are crucial for the regeneration of skeletal muscle, however little is known about the role of the extracellular matrix in this process. Matrilin-2 is expressed in the connective tissue covering skeletal muscle. Our goal was to identify the potential role of matrilin-2 during regeneration. To characterize the major events of regeneration we measured the expression of some myogenic factors, myogenin and myo D. Necrosis of the soleusmuscle of Wistar rats was achieved with an injection of the venom notexin. Muscles were dissected at 6, 12 and 24 hours, days 2, 4, 7, 14 of regeneration and from control animals. Northern blot analysis showed that the mRNA level ofmatrilin-2 was elevated on day 2 as compared to the control muscle and reached the maximum level between 4–7 days of regeneration similarly to the myogeninmRNA expression. Using immunofluorescent staining we localized matrilin-2 in thematrix surrounding proliferating myoblasts 2 days after necrosis. Strong staining was observed around differentiating myoblasts and myotubes on day 4 of regeneration. We co-localized matrilin-2 withalpha-bungarotoxin in the neuromuscular junctions between day 4–7, when the motor endplates are reformed. Matrilin-2 facilitates muscle regeneration by influencing the differentiation of myoblasts and by inducing the innervations of muscle fibers.
Acknowledgment: This work was supported by grants from OTKA TO34399, TO49608, ETT 256/2003, RET-08/04 OMFB-0066/2005.

PP-1031
Electrochemical investigation on the ligand binding by hemoglobin

S. Rezaei-Zarchi[1], **A. A. Saboury**[1], H. Ghourchian[1], N. Nouroozi[2], A. A. Moosavi- Movahedi[1], M. R. Ganjali[2] and A. Javed[3]
[1]*Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran,* [2]*Department of Chemistry, Faculty of Science, University of Tehran, Tehran, Iran,* [3]*Department of Chemistry, University of Agriculture, Faisalabad, Pakistan.*
E-mail: srezaei@ibb.ut.ac.ir

Recently, the electrochemical method is used for investigating the influence of some materials and drugs on the structure and stability of redox proteins. In this study, the effects of 2,3-diphosphospho-D-glyceric acid (DPG) and phytic acid (IHP) on the structure of hemoglobin were investigated by electrochemical method. Cyclic voltametry (CV) was performed with a PAR 263 potentiostate/Galvanostate (EG and G, USA). The working electrode was an iodide modified silver electrode that was prepared by the way of M. S. Sibbald. A saturated calomel electrode (SCE) was used as the reference electrode. A platinum electrode served as the counter electrode. Whole experiment was performed in 30×10^{-5} M bovine hemoglobin solution in 1 mM KNO3 at pH 7.0. In this condition, anodic and cathodic peaks were observed at 257 mV and 57 mV, respectively. Titration of this solution was done with DPG and IHP as the hemoglobin ligands. Potentiometric response of hemoglobin was determined for these effectors. The effect of DPG and IHP concentrations were determined on the anaerobic redox reaction showed that DPG and IHP induced stabilization of the reduced state and destabilization of R-like [Met-Fe (III)] state of Met-hemoglobin. Also this experiment is showed that, in spite of many electrochemical investigation that need fixing of protein on the surface of electrode, by using of iodide modified silver electrode one can investigate the effect of a ligand on hemoglobin in a solution.

PP-1032
Molecular markers in the cryopreservation of olive and garlic tissue

S. Trigwell[1], R. Howden[2], P. T. Lynch[1], A. J. Hargreaves[2] and **P. L. R. Bonner**[2]
[1]*Biological Sciences Research Group, School of Science, University of Derby, Derby, DE22 1GB, UK,* [2]*School of Biomedical and Natural Sciences, Nottingham Trent University, Nottingham, NG11 8NS UK. E-mail: philip.bonner@ntu.ac.uk*

Successful cryopreservation of plant material is important for a variety of economic and ecological reasons, but the molecular basis of its success or failure is poorly understood. A previous study by our group using Helianthus tuberosus suspension cultures showed that significantly higher transglutaminase activity was associated with the 0.5 M sucrose pre-freeze treatment, which in turn resulted in better post-thaw recovery (1). Transglutaminases (E.C.2.3.2.13) have been detected in both the membrane and soluble fractions of plants, and have been shown to utilise a number of substrates, including cytoskeletal proteins. They form covalent protein to protein crosslinks, which result in higher molecular weight polymers and they can also catalyse the incorporation of polyamines into proteins. The CRYMCEPT project has sought to determine molecular markers of successful cryopreservation focusing on changes in transglutaminase activity and cytoskeletal proteins in extracts of *Allium sativum* L. (garlic) stem discs and *Olea europaea* L. (olive) somatic embryos. Evidence will be presented to support the view that changes in the levels and/or activity of cytoskeletal proteins and transglutaminase represent useful markers of cryopreservation status.

Reference:

Harris W., Lynch PT., Hargreaves AJ. and Bonner PLR. Cryoletters (2004);25: 213–217. (European Commission: CRYMCEPT (Establishing Cryopreservation Methods for Conserving European Plant Germplasm Collections) Project.)

PP-1033
Determination of interleukin (IL) 1β-511(T/C) gene polymorphism in glial tumors of central nervous system

O. Tubay Bagdatoglu[1], G. Polat[1], Z. N. Dogruer[1], H. Tuna[2], C. Bagdatoglu[3] and U. Atik[1]
[1]*Department of Biochemistry, Faculty of Medicine, Mersin University, Mersin, Turkey,* [2]*Department of Neurosurgery, Faculty of Medicine, Ankara University, Ankara, Turkey,* [3]*Department of Neurosurgery, Faculty of Medicine, Mersin University, Mersin, Turkey. E-mail: otbagdatoglu@mersin.edu.tr*

Glial tumors are the most common tumors in central nervous system. Several risk factors have been associated with tumors. Genetic predisposition is an important risk factor of developing malignancy. Cytokines play an important role in tumor development via acting tumor angiogenesis and regulating immune responses. IL-1? is an proinflammatory cytokine. Single nucleotide polymorphisms (SNP) occur in the promoter regions of proinflammatory cytokine genes influence cytokine production. The aim of this study was to determine the association of IL-1β-511(T/C) gene polymorphism with glial tumors. The whole bloods of 59 glial tumor patients and 103 healthy controls were collected in EDTA-containing tubes. DNA was extracted by high pure template preparation kit (Roche Diagnostics, GmbH, Mannheim, Germany). SNPs were genotyped using polymerase chain reaction (PCR) technique. and finally the genotypes were designed as follows: CC, CT, and TT. CC genotype was not seen in control group. TT genotype was detected as 8.47% in patients while 0.97% in controls ($P < 0.05$) with an Odds ratio (OR) of 9.44 (95%CI 1.03–223.01). These results suggest that there may be an association between TT genotype of IL-1β-511, which is related with high expression of this cytokine, and glial tumors.

PP-1034
Antioxidative and antiproliferative effects of Turkish *Rheum ribes* aqueous extract

P. Uyar[1], F. Ozgokce[2], N. Coruh[3] and M. Iscan[4]
[1]*Graduate Program of Biotechnology, Middle East Technical University, Ankara, Turkey,* [2]*Department of Biology, Yuzuncu Yil University, Van, Turkey,* [3]*Department of Chemistry, Middle East Technical University, Ankara, Turkey,* [4]*Department of Biology, Middle East Technical University, Ankara, Turkey.*
E-mail: pembegul@metu.edu.tr

Rheum ribes L. is an annual herb of the Polygonaceae family and found mostly in Eastern Turkey. Its fresh stems and petioles are consumed as vegetables and used as a medicinal plant to promote digestion, used against hemorrhoids and measles. This study was designed to investigate the antiproliferative and antioxidative properties of *Rheum ribes* young shoots as its edible parts. *Rheum ribes* extracts (RRE) were prepared as dry sample to water ratio of 1:12. Antioxidant capacity of the extract was determined by the ability to scavenge hydrazyl radical, DPPH, and the result was expressed as fifty percent inhibitory concentration (IC_{50}) of 0.128 ± 0.081 mg/ml. Human Myeloid Leukemia (HL-60) cell line was used as a model system for the proliferation studies. HL

60 cells were cultured in the presence of various concentrations of RRE, and exposed over 72 h. The percentage of cell viability was evaluated by metabolization of the tetrazolium salt XTT.RRE displayed a dose-dependent inhibition of cell proliferation with an fifty percent effective dose (ED_{50}) of 20.15 ± 0.86 μg/ml. These investigations suggested that the aqueous extract from *Rheum ribes* can be considered as a potent antioxidant and a strong antiproliferative agent. As a result, *Rheum ribes* exerts various activities with dose dependent as well as exposure-time dependent manners; in this sense, it has a potential for cancer chemoprevention.

PP-1035
The spectrum of abnormal hemoglobins in Antalya province, Mediterranean region of Turkey

I. Keser[1], E. Aydemir[1], O. Kayisli[1], A. Sanlioglu[1], M. Simsek[2], I. Mendilcioglu[2], A. Yesilipek[3] and G. Luleci[1]
[1]Department of Medical Biology and Genetics, [2]Department of Perinatology, [3]Department of Pediatric Hematology, Medical Faculty, Akdeniz University, Antalya, Turkey.
E-mail: keser@akdeniz.edu.tr

Talassemia is the most common with the frequency of 12% in Antalya Province that is located in the central part of the Mediterranean region in Turkey. Therefore, it is one of the target areas for carrier screening. This study aims to find the prevalence/spectrum of abnormal hemoglobins. We studied 600 postnatal and 200 prenatal cases with the disorder over 6 years. Laboratory analyses of blood samples were carried out following standard procedures. We have identified and characterized an abnormal hemoglobins with the novel and rare beta-thalassemic mutations. Four different abnormal hemoglobins were found such as Hemoglobin Antalya, Hb G-Coushatta, Hb Knossos, Hb D-Punjab in Antalya Province, Turkey. These abnormal hemoglobins are unstable and cannot be detected by simple electrophoretic examinations. These findings suggest that the abnormal hemoglobins is more frequent than expected and is an important to give a genetic counseling to the families with risk for thalassemia.

PP-1036
Soluble endothelial cell protein C receptor affecting the development of arteriovenous fistulae thrombosis in hemodialysis patients

N. Bayraktar[1], A. Bilgic[2], S. Karakus[3], M. Turan[1] and F. N Ozdemir[2]
[1]Department of Biochemistry Laboratory, [2]Department of Nephrology, [3]Division of Hematology, Baskent University, Ankara, Turkey. E-mail: drnbayraktar@yahoo.com

Arteriovenous fistulae (AVF) thrombosis is the most common cause of morbidity in hemodialysis (HD) patients. It has been postulated that increased sEPCR levels may be prothrombotic and associated with an increased risk of venous thrombosis in nonnumeric patients. Here we aimed to investigate possible effects of sEPCR levels on the development of AVF thrombosis in HD patients. Sixty patients who were being followed by HD and 22 healthy controls were included. Patients with diabetes mellitus, amyloidosis, and vasculitis were excluded. Patients were divided into two groups: Group I (n = 29), no vascular access thrombosis within 5 years and Group II (n = 31), AVF thrombosis >2 times. Groups were analyzed to evaluate any relationship between sEPCR levels and development of AVF thromboses. Plasma levels of sEPCR were significantly higher than healthy controls. Group II and I was compared to evaluate the sEPCR level that influences AVF thrombosis which result in no statistical significance. A Pearson bivariate correlation analysis which was done to evaluate any relationship between sEPCR and clinical and laboratory parameters in HD patients, revealed that; increased plasma sEPCR levels were negatively correlated with age, duration of ESRD and HD. In conclusion, this is the first study, analyzing sEPCR levels in HD patients. We could not find any relationship between plasma sEPCR levels and AVF thrombosis, but still further studies are needed to evaluate factors affecting AVF thrombosis.

PP-1037
Probing Taxol™ (paclitaxel)-cell membrane interactions with Langmuir-blodgett Monolayer, Fourier Transform Infrared Spectroscopy and differential scanning calorimetry

Y. Manavbasi[1] and E. Suleymanoglu[2]
[1]Dept. of Pharmacology, [2]Dept. of Pharmaceutical Chemistry and The Central Laboratory, Gazi University, Faculty of Pharmacy, Hipodrom, 06330-Ankara, Turkey. E-mail: yaaseemin@gmail.com

The interaction of Taxol™ (paclitaxel), a potent agent employed in cancer therapy, with model phospholipid structures is presented. For the physicochemical characterization of surface, structural and thermodynamic properties of various drug-lipid complexes, Langmuir-Blodgett Monolayer, Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC) were used trying to clarify its effects on cell membrane properties. The effect of sample preparation, lipid type and drug concentration have a detrimental impact on the observed features. Recognition of Taxol™ (paclitaxel) with phosphatidylcholine moieties results in alterations of physical parameters of the model membrane, such as surface recognition with lipid headgroups, onset, pre-transition and main phase transition temperatures, as well as on acyl chain packing. The drug has a fluidizing effect in the gel phase of saturated phospholipids. Our study focuses mainly on Taxol™ (paclitaxel) effects on model cellular membranes, but the described biophysical aspects of its mode of action are also employed in liposomal drug delivery in tumour treatments trials. Both of these aspects are described based on our experimental designs as a preliminary hypothesis and proposals.

PP-1038
Comparison of anti cyclic citrullinated peptide levels with other markers in rheumatoid arthritic patients

H. Çürüksulu[1], D. Gültekin[1], H. Aral[2], L. Özgönenel[3], F. Nartop[1], E. Çetin[3], G. Güvenen[2] and F. Sametoglu[2]
[1]Central Clinical Chemistry Laboratory, Ministry of Health İstanbul Göztepe Training and Research Hospital, Istanbul, Turkey, [2]Central Clinical Chemistry Laboratory, Ministry of Health İstanbul Training and Research Hospital, Istanbul, Turkey, [3]Physical Medicine & Rehabilitation Clinic, Ministry of Health İstanbul Training and Research Hospital, Istanbul, Turkey.
E-mail: drhalearal@yahoo.com

We investigated anti cyclic citrullinated peptide (ACCP) levels in patients with rheumatoid arthritis (RA) in presence and absence of rheumatoid factor (RF). Seronegative (n = 30) and seropositive (n = 30) two groups with similar demographic were obtained

among the patients with RA of at least 5 years duration, using American Rheumatism Association Criteria. The control group (n = 20) comprised of healthy individuals. Serum ASO, CRP, RF levels were measured by nephelometric method (Beckman Coulter, USA) and ACCP levels were measured by ELISA (Euroimmun Medizinische Labordiagnostika GmbH, Germany). In comparison with the seronegative patients, the seropositive patients had higher ESR levels (24.53 ± 6.35 vs. 23.56 ± 10.94 mm/h), higher CRP (0.86 ± 0.55 vs. 0.62 ± 0.42 mg/dl), higher WBC (7.5 ± 1.9 vs. 7.2 ± 1.9 /μl) higher hemoglobin (12.59 ± 1.47 vs. 12.13 ± 1.55 gr/dl) and lower ASO levels (65.42 ± 40.83 vs. 94.17 ± 73.62 U/l) ($P > 0.05$). Anti-CCP antibodies were found to be positive in 96.7% of the seropositive and 26.7% of the seronegative patients ($P < 0.01$). No positive result was found in control group. In conclusion our study support the hypothesis that in diagnosis of rheumatoid arthritis anti-CCP is a better indicator than RF especially in patients with RF negative. Using anti-CCP also helps in differential diagnosis of RF positive patients with other rheumatoid diseases.

PP-1039
Genomic plasticity is frequent in HPV-associated carcinogenesis

C. Bleotu[1], G. Anton[1], A. Plesa[1], M. Chivu[1], I. Alexiu[1], R. Dragusin[2], I. Popa[2] and **E. Cernescu**[1]
[1]Stefan S Nicolau Institute of Virology, [2]Coltea Clinical Hospital. E-mail: cbleotu@yahoo.com

Human papillomavirus (HPV) was implicated in laryngeal cancers with variable ratio in different area. The aim of this project was to study the role of HPV in the development of epithelial cancer. Samples from 55 patients presenting tumors of the tonsil, pharynx, and larynx were analyzed. The group mean age was 63 ± 11 years and male/female ratio = 49/6.The viral DNA detection was done with degenerate primers in single and nested PCR. The positive samples were genotyped in PCR for HPV type 16. To determine the HPV 16 genomic integration, positive samples were processed with specific E2 viral gene primers comparative with hybridization (a new ELISA system). Measurement of DNA contents was performed on paraffin-embedded tissue by flow cytometry and allowed us to estimate cancer ploidy level. Our results showed high positivity for HPV (especially 16 type) in tonsils and laryngeal cancer detected by nested PCR. HPV infection was not related to age/gender/stage/differentiation grade/TNM rates/ alcohol-tobacco use. Integration of HPV into the host genome was over 50% in HPV16 positive cases and aneuploidy was a frequent event, probable because the promotion of carcinogenesis by HPV increases genomic modification. Our results underline that HPV induce changes at the genomic level during carcinogenesis at laryngeal and pharyngeal sites and differences in relation to other studies may be geographical and/ or methodological.

PP-1040
The association between GST gen polymorphisms and pancreatic cancer

L. Ayaz[1], H. Yildirim[1], Z. N. Dogruer[1], K. Ocal[2], B. Ercan[1], L. Tamer[1], S. Aydin[2] and U. Atik[1]
[1]Mersin University Biochemistry Department of Medical Faculty, Mersin, Turkey, [2]Mersin University Surgery Department of Medical Faculty, Mersin, Turkey. E-mail: lokmanayaz@yahoo.com

Pancreatic cancer is an aggressive disease that is almost uniformly fatal, with the incidence rate approaching the mortality rate. Pancreatic cancer has been linked to the exposure of environmental chemicals, which generally require metabolic activation to highly reactive toxic or carcinogenic intermediates. The GSTs are a family of phase-2 isoenzymes believed to protect cells from reactive chemical intermediates and oxidative stress resulting from a wide range of electrophilic xenobiotics and endogenous intermediates. We aimed to investigate whether profiles of GST M1, T1 and P1 genotypes may be associated with the risk of pancreatic cancer. We examined adults 30 with pancreatic cancer and 70 healthy controls. DNA was extracted from whole blood, and the GSTM1, GSTT1 and GSTP1 polymorphisms were determined using LightCycler Instrument. Associations between specific genotypes and the development of pancreatic cancer were examined by use of logistic regression analyses to calculate odds ratios and 95% confidence intervals. Gene polymorphisms at GSTM1, GSTT1 and GSTP1 in subjects with pancreatic cancer were not significantly different than in the controls ($P > 0.05$). Also the combinations of different GSTM1, GSTT1 and GSTP1 genotypes were not an increased risk of pancreatic cancer ($P > 0.05$). We could not demonstrate any significant association between the GSTM1, GSTT1 and GSTP1 polymorphism and pancreatic cancer in this population.

PP-1041
Intragenic suppressors of Ycf1-S908A mutant, carrying a non-phosphorylatable version of the yeast ABC transporter Ycf1

P. Eraso, F. Portillo and M. J. Mazón
Bioquímica y Genética de Levaduras, Instituto de Investigaciones Biomédicas Alberto Sols CSIC-UAM, Madrid, Spain. E-mail: peraso@iib.uam.es

ATP-binding cassette (ABC) proteins mediate the translocation of a variety of substances across biological membranes. Eukaryotic ABC transporters include the multidrug resistance associated proteins (MRP1, MRP2), the yeast cadmium factor (Ycf1), the cystic fibrosis transmembrane conductance regulator (CFTR) or the multidrug-resistance protein (MDR1). Ycf1 is a vacuolar membrane protein involved in heavy metal and drug detoxification. Ycf11 contains two TMDs and two NBDs as a member of the ABC superfamily, a third N-terminal TMD present only in the MRP subfamily, and a hydrophilic R-like domain common to the CFTR and MRP subfamilies. Although the regulatory significance of the R domain is only well established in CFTR, it has been shown that phosphorylation of Ser908 and Thr911 in Ycf1 R domain is necessary for transport activity. We performed an intragenic suppressor analysis of S908A mutation to understand the mechanism by which this mutation alters Ycf1 function. Random mutagenesis of the mutant gene was performed and revertants were selected by their ability to detoxify cadmium ions or diamide. Mapping and sequencing revealed 22 different changes that suppress S908A mutation. Four of them were located in the cytoplasmic loop connecting TMD0 and TMD1, four in TMD1, two in NBD1, four in the R domain and eight in TMD2 indicating either physical proximity or functional interactions between R and the other domains. Characterization of the suppressor mutants will be presented.

PP-1042
Comparison between the soluble transferrin receptor and serum ferritin as a marker of iron state in hemodialysis patients

Y. Doventas[1], F. Basinoglu[1], M. Koldas[1], **D. Yildiz**[2], A. Doventas[3], A. Kural[1] and H. Seval[1]
[1]Department of Biochemistry, Haseki Education and Research Hospital, Istanbul, Turkey, [2]Department of Biochemistry, Gaziosmanpasa Hospital, Istanbul, Turkey, [3]Department of Internal Medicine, Anadolu Health Center & Johns Hopkins Hospital, İstanbul, Turkey. E-mail: yasemined@hotmail.com

Objective: In hemodialysis (HD) patients, an adequate iron management is important in the treatment of anemia. Serum ferritin, serum amiloid-A protein (SAA) and transferrin saturation (TS) may be influenced by the presence of inflammation. Recently, the soluble transferrin receptor (s-TfR) has been used to be a marker of functional iron stores in HD patients.
Methods: In this study, we examined the s-TfR, ferritin and SAA levels of 100 HD patients. We separated the patients into two groups with iron deficiency anemia and without iron deficiency anemia. Iron deficiency anemia had diagnosed by routine laboratory methods (ferritin < 50 microg/l and transferrin saturation $< 16\%$) in 50 patients. Serum ferritin, SAA and transferrin receptor levels were measured with nephelometric methods. There were no differences between the two groups with and without iron deficiency anaemia with respect to mean age, body weight, haemodialysis duration and serum creatinine levels ($P > 0.05$). Although elevated levels of ferritin and SAA there were no difference between the two groups ($P > 0.05$); the iron deficiency group had higher s-TfR values than the non-iron deficiency group ($P < 0.001$).
Conclusion: We conclude that the measurement of s-TfR levels may be useful in the diagnosis of functional iron deficiency in haemodialysis patients. Usage of ferritin levels as a marker of iron deficiency anaemia in HD patients is not useful because of affected by inflammation.

PP-1043
Association of CYP2C9 gene polymorphisms after heart valve replacement with anticoagulant therapy

H. Yildirim[1], L. Tamer[1], N. Sucu[2], O. Gungor[2], M. Dikmengil[2] and U. Atik[1]
[1]Department of Biochemistry, [2]Department of Cardiovascular Surgery, Mersin University Faculty of Medicine, Mersin, Turkey. E-mail: haticeyldrm@yahoo.com

Valve replacement with valvular heart disease inevitable in certain conditions. Mechanical heart valves require anticoagulant therapy to lower the thromboembolic risk. Coumadin is an anticoagulant agent used for the prevention of thromboembolic events after heart valve replacement. Bleeding is major adverse effect of coumadin. Coumadin dose must be adjusted very well. The International Normalized Ratio (INR) is used for coumadin dose adjustment. Coumadin is metabolized by CYP2C9. Gene polymorphisms of CYP2C9 decrease coumadin metabolisation and then are able to increase bleeding risk. The aim of the present study was to investigate the association of CYP2C9 gene polymorphisms after heart valve replacement in group of patients who are taking coumadin. The study subjects consisted of 74 patient with heart valce replacement. Blood was collected in EDTA-containing tubes and DNA was extracted from the leucocytes. CYPC9*2, CYP2C9*3 alleles were detected by using real time PCR with LightCycler instrument. In our study, we found that patients having CYP2C9*2 and CYP2C9*3 genotype have lower dose of coumadin according to patients having wild type genotype (CYP2C9*2 $P = 0.0296$, CYP2C9*3 $P = 0.022$). In addition to combine association of CYP2C9*2 and CYP2C9*3 genotype coumadin dose and INR levels were investigated and we found that patients having both forms of CYP2C9*2 and CYP2C9*3 heterozygous (CYP2C9*2/*3) require lower coumadin dose than patients having wild type genotype ($P = 0.001$).

PP-1044
Hyperthermophilic DNA polymerase I from *Geobacillus anatolicus*

M. Caglayan and N. Bilgin
Department of Molecular Biology and Genetics, Bogazici University, Istanbul, Turkey. E-mail: melikecaglayan@yahoo.com

The DNA polymerase I gene of a newly discovered *Geobacillus* species, *Geobacillus anatolicus* from a terrestrial hydrothermal vent at 98 °C has been identified and sequenced. The entire DNA polymerase I gene excluding the start codon was cloned into pCR-T7/NT-TOPO expression vector and was expressed in *Escherichia coli*. The recombinant *Geobacillus anatolicus* DNA polymerase I fusion protein including an His (6)-tag at its N terminal part was obtained. The recombinant protein was purified using Ni-affinity and gel filtration chromatography and biochemically characterized. *Geobacillus anatolicus* DNA polymerase I gene contains a long open reading frame of 2637 bases that encodes 878 amino acid residues. Calculated molecular weight of the DNA polymerase I is 99.3 kilo Dalton. Similarity analyses suggested that *Geobacillus anatolicus* DNA polymerase I may not contain a putative 3'-5' exonuclease activity. However, the conserved regions related to 5'-3' exonuclease activity were present in the amino acid sequence of *Geobacillus anatolicus* DNA polymerase I.

PP-1045
BNP levels in patients undergoing continuous ambulatory peritoneal dialysis in malnutrition

A. Coşkun[1], G. Güvenen[1], H. Aral[1], P. Tonbaklar Bilgi[1], M. Besler[2], M. Trablus[2], H. Balcı[3], R. Ataman[4] and F. Sametoglu[5]
[1]Central Clinical Chemistry Laboratory, Ministry of Health İstanbul Training and Research Hospital, Istanbul, Turkey, [2]Nephrology Department, Ministry of Health İstanbul Training and Research Hospital, Istanbul, Turkey, [3]Central Clinical Chemistry Laboratory, İstanbul University Cerrahpaşa Medical Faculty, Istanbul, Turkey, [4]Nephrology Department, İstanbul University Cerrahpaşa Medical Faculty, Istanbul, Turkey, [5]Internal Medicine Clinic. Ministry of Health İstanbul Training and Research Hospital, Istanbul, Turkey. E-mail: drhalearal@yahoo.com

Atherosclerotic cardiovascular disease and malnutrition are widely recognized as leading causes of the increased morbidity and mortality observed in uremic patients. We examined BNP levels and dialysis sufficiency criteria of Kt/Vurea, nPCR and creatinine clearance in malnutrition. Sixty-one patients (28 male, 33 female) made up our study group. Nutritional status was assessed by serum albumin; the presence of an inflammatory reaction was assessed by CRP, ESR, fibrinogen, WBC. Lipid parameters were also determined. BNP concentrations were measured by fluorescence immunoassay (Biosite Inc., USA). BNP levels were found as 303 ± 276 pg/ml in patients with albumin ≤ 3.5 g/dl ($n = 24$) and 415 ± 601 pg/ml in patients with albumin > 3.5 g/dl ($n = 37$) ($P > 0.05$). Malnourished patients had higher CRP

levels (1.89 ± 2.33 vs. 1.18 ± 1.54 mg/dl), higher ESR (93 ± 31 vs. 85 ± 37 mm/h), higher fibrinogen (770 ± 29 vs. 672 ± 290 mg/dl), higher WBC (9.5 ± 3.1 vs. 8.7 ± 2.6 /μl), lower Kt/Vurea (2.04 ± 0.54 vs. 2.22 ± 0.56) lower nPCR (0.89 ± 0.26 vs. 0.95 ± 0.24) lower CCl (58 ± 14 vs. 65 ± 20) higher cholesterol (199 ± 53 vs. 173 ± 55) and higher triglyceride (227 ± 110 vs. 197 ± 98) compared with well-nourished patients ($P > 0.05$). Although differences were not significant, we found decreased BNP levels in malnourished patients with higher inflammation markers and lipid levels. Further research is needed to investigate the role of BNP in predicting cardiovascular risk factors in uremic patients, with larger study groups.

PP-1046
Levels of cholinesterases measured in patients with epileptic seizures

L. Kenar[1], H. Ulas[2], T. Karayilanoglu[1], M. Ortatatli[1] and **H. Yaren**[1]
[1]*Department of Medical NBC Defence,* [2]*Department of Neurology, Gulhane Military Medical Academy, Ankara, Turkey.*
E-mail: lkenar@gata.edu.tr

Objective: Seizures might be one the manifestations of epilepsy. The ethiopathogenesis of the seizures is still on debate and one of the accused is considered disorder signalling mechanism in which an acetylcholine-mediated event is concerned. Thus, we investigated the levels of acetylcholinesterase (AchE) and butyrylcholinesterase (BTC) in plasma and serum, respectively.
Materials and methods: Cholinesterase's were studied on blood drawn from the patients ($n = 15$) with seizures and healthy controls ($n = 10$). All patients did not receive an antiepileptic treatment. The blood withdrawn during the seizures and after the seizure (2 h) was analyzed. Ellman's method was used to determine the activities of the enzymes.
Results: In blood taken from the patients who were on seizure, BTC and AChE levels were found reduced when compared to controls ($P = 0.067$ and $P < 0.001$ respectively). Median values were 3794 U/l and 55.9 U/ml for BCE and ACHE respectively. When compared to controls, the levels of BTC and AChE were found decreased in those obtained after seizure with mean values of 3205 U/l and 87.9 U/ml respectively ($P = 0.012$ and $P < 0.001$ respectively).
Conclusion: It was found that the levels of both enzymes were decreased in patients with seizures despite a slight increase on 2nd h following seizure than that measure on seizure. These results also indicated that seizures on epilepsy, a very complex and systemic disorder, may also be originated from the inhibition of AChE release.

PP-1047
Estimation of glomeruler filtration rate in patients with diabetes mellitus

F. Basinoglu[1], **Y. Doventas**[1], F. Aksak[2], M. Koldaş[1], R. Kazancıoğlu[2], H. Seval[2], A. Kural[1] and M. Gungor[1]
[1]*Department of Biochemistry,* [2]*Department of Nephrology, Haseki Education and Research Hospital, Istanbul, Turkey.*
E-mail: filizbasinoglu@hotmail.com

Objectives: We studied the correlation of cystatin C, beta2mikroglobulin(B2M), creatinin, GFR the Cockcroft-Gault(C&G),the Modification of Diet in Renal Disease(MDRD)GFR,GFR Cystatin (GFR cyc)with each other and creatinine clearance that is often used in our daily clinical practices in patients with Type 2 DM.
Material and methods: A total of 46 patients 29 females, 17 males, aged 40–82 recruited from Nephrology outpatient clinic of Haseki Education and Research Hospital. After 12–14 h of fasting blood samples were taken for detecting serum cystatin C, B2M, creatinine, BUN, albumin, HbA1c and thyroid function values. Creatinine Clearance ,GFR C&G, GFR MDRD, GFR cys calculated. They were sub grouped in two as euthyroid and thyroid dysfunction group.
Results: Creatinine clearance showed significant correlation with, GFR MDRD ($P < 0{,}000$), GFR C&G ($P < 0.000$), GFR cys ($P < 0.000$) in all type2DM but there was no correlation between creatinine clearance, GFR C&G,GFR MDRD,GFR cys in subgroup with thyroid dysfunction. In subgroup with thyroid dysfunction there was only significant correlation between creatinine clearance and serum creatinine ($P < 0.008$) but none between serum cystatin C, B2M.
Conclusion: As a summary, patients with type 2 DM cystatin C, B2M, creatinine values significantly estimates reduced creatinine clearance but serum cystatin C and B2M is not a better renal marker than creatinine.

PP-1048
Protein synthesis elongation factors Tu and Ts from *Geobacillus anatolicus*.

T. Sahin*, **B. Akalin*** and N. Bilgin
Department of Molecular Biology and Genetics, Bogazici University, Istanbul, Turkey. E-mail: baris.akalin@boun.edu.tr

Geobacillus anatolicus is a newly identified hyperthermophilic bacteria isolated from a hydrothermal vent at 98 °C. The genes encoding protein synthesis elongation factor Tu (EF-Tu) and its nucleotide exchange factor Ts (EF-Ts) from *Geobacillus anatolicus* were identified and sequenced. *Geobacillus anatolicus* EF-Ts contains 294 aminoacids with a calculated molecular weight of 32.6 kDa, and *Geobacillus anatolicus* EF-Tu contains 395 aminoacids with a calculated molecular weight of 43.3 kDa. Sequence comparisons of the corresponding genes from other thermophilic and mesophilic bacteria were used to identify the molecular characteristics leading to thermostability. EF-Tu and EF-Ts encoding genes from *Geobacillus anatolicus* were cloned and expressed as His-tagged proteins in *Escherichia coli*. Purified EF-Tu and EF-Ts proteins were biochemically characterized and cross-reactivities to the corresponding *Escherichia coli* factors were investigated. Hyperthermophilic EF-Ts were found to be fully active in complex formation with mesophilic *Escherichia coli* EF-Tu. Kinetic parameters for EF-Tu and EF-Ts from *Geobacillus anatolicus* in nucleotide exchange and in protein synthesis *in vitro* were measured.
* Equally contributed to this work

PP-1049
Two year screening research on discriminating value of carbohydrate antigen 15-3 and prolactin in breast cancers

F. Kuralay[1], S. Uysal[1] and T. Canda[2]
[1]*Department of Biochemistry,* [2]*Department of Pathology, Dokuz Eylul University School of Medicine, Izmir, Turkey.*
E-mail: Filiz Kuralay filiz.kuralay@deu.edu.tr

Carbohydrate antigen 15-3 (CA 15-3) and prolactin (PRL) are important circulating markers for predicting newly diagnosed

breast cancer. The aim of the present study was to determine the discriminating value of CA 15-3 and PRL levels in serum in benignant (n = 187) and malignant (n = 204: n = 189 nonmetastatic, n = 15 metastatic) breast cancer cases who were newly histologically proven as a firstly diagnosed or a metastatic case and not taken any therapy between January 2004 and January 2006 in Dokuz Eylul University Hospital. CA 15-3 and prolactin levels were quantified by chemiluminescent enzyme immunometric assay using Immulite Systems. Statistics, diagnostic usefulness tests and receiver operating characteristics (ROC) curve analysis were evaluated by SPSS 11.0 programme. CA 15-3 levels as U/ml and mean ± standard error of mean, in metastatic group (393.17 ± 194.63) were significantly higher than both benign (33.12 ± 8.96) and nonmetastatic malign group (51.62 ± 8.41); while prolactin showed no difference. The accepted cutoff values gave low accuracy results, whereas cutoff levels calculated by ROC demonstrated a significantly higher diagnostic accuracy (sensitivity-specificity pairs: for CA 15-3 0.60-0.56, for prolactin 0.57-0.53 respectively). Area under the ROC curve was 0.625 for CA 15-3, 0.543 for prolactin. Hence, the diagnostic usefulness tests including ROC analysis of CA 15-3 and prolactin improves the biochemical discrimination of benign and malign breast cancers.

PP-1050
Clinical usefulness of cross-linked N-telopeptide of type I collagen (NTx) as a bone metastatic marker in patients with lung cancer

E. Emine[1], Y. Doventas[2], M. Tekesin[3] and H. Balci[4]
[1]Biochemistry,Yedikule Chest Diseases and Chest Surgery Education and Research Hospital, İstanbul, Turkey, [2]Department of Biochemistry, Haseki Education and Research Hospital, Istanbul, Turkey, [3]Chest Diseases, Yedikule Chest Diseases and Chest Surgery Education and Research Hospital, İstanbul, Turkey, [4]Biochemistry,Istanbul University Cerrahpasa Medical School, Central Laboratories, Istanbul, Turkey.
E-mail: eminealtunkaynak@hotmail.com

Objective: Type I collagen cross-linked N-telopeptide (NTx) in urine, the degraded form of type I collagen cross-linked in bone, has been evaluated as a marker of bone resorption. In this study, the clinical usefulness of NTx as a marker of bone metastasis of lung cancer.
Material and methods: We assessed 56 cases of lung Ca in which the diagnosis had been confirmed pathologically. The patients were 25 patients with lung cancer with bone metastasis (Group 1);); and 31 patients, with localized lung cancer and no evidence of bone metastasis (Group 2). For comparison, Type I collagen cross-linked N-telopeptide (NTx) in urine, serum alkaline phosphatase and calcium were simultaneously measured. NTx was measured by ELİSA methods.
Results: Concentration of NTx in urine were compared between the two groups with the Mann–Whitney U–test, with p values less than 0.05 considered significant. Urine NTx concentrations in Groups 1 and 2 were 167 ± 121, 105 ± 74 nMBCE/mMCr, respectively. The differences between the Group 1 and Group 2 were significant ($P < 0.05$) On the other hand, in the serum ALP there was no significant difference between the two group ($P > 0.05$)
Conclusion: These bone metabolic markers are promising clinical markers of bone metastatic and may be useful for prediction of therapeutic efficacy and recurrence in bone and quantification of the extent of bone metastases.

PP-1051
Telomerase activity and viral DNA status - markers in cervical lesions?

A. Plesa, A. Nastasie, V. Dragodan and G. Anton
Stefan S. Nicolau Institute of Virology.
E-mail: adrianaplesa@yahoo.com

Human papillomavirus (HPV) infection is an important event in the malignant transformation of human cervical epithelium. Several high-risk HPV subtypes might lead to CIN and invasive carcinoma. The reason for this phenomenon seems to be related to physical state of viral DNA, tumor suppressor genes inactivation and immortalize factors activation. High risk viral oncogene E6 is capable of inducing hTERT and telomerase activity; the increased hTERT expression is correlated to an immortal phenotype. Recent data incriminated viral genome integration as an important event in hTERT activation. The aim of this study is to identify new biomarkers for cervical cancer. 30 HPV16 positive biopsies selected from 120 samples presenting CIN I-III Pap smears, were tested by PCR/Southern blot for viral physical genome status and by RT-PCR for hTERT expression.. In 17 of these sample, viral DNA was presented in episomal status. The integrated form was noted in 13 cases (3/9CIN I, 3/9CIN II and 5/12 CIN III). hTERT expression was detected in 12 cases (1/9CIN I, 4/9 CIN II, 7/12 CIN III). It is to be mentioned that only in seven cases the viral physical status is correlated with hTERT expression. As negative control we used biopsies from 15 patients presenting CIN I-III but without detectable HPV infection. Among these samples, three presented hTERT activity (associated with the most severe lesions/ CIN III). A higher number of processed cases might lead to more pertinent data.

PP-1052
Glutathione S-transferases against drought stress in plants

C. Yilmaz[1] and **M. İşcan**[2]
[1]Graduate Program of Biochemistry, [2]Department of Biological Sciences, Middle East Technical University, Ankara, Turkey.
E-mail: cnylmz@metu.edu.tr

Plants have mechanisms to defend themselves against abiotic factors like chemical compounds generated by stress conditions such as drought. Glutathione S-transferases (GST) are thought to have roles in those conditions because the conjugation of glutathione (GSH) to such molecules by the activity of GST increases their solubility and facilitates further metabolic processing. Our aim is to investigate the role of GSTs in drought stress. We used *Pinus brutia* which is a member of Gymnospermae; class Coniferae and Genus Pinus as a model organism. In this study, the needles of 30 different individuals of *Pinus brutia*, located in METU-Yalıncak, were collected once in every month from June to September. The osmotic pressure values, which were used as the indicator of soil humidity, were measured and air humidity and temperature values were recorded. Highest drought stress was observed in September. The needles collected from individual trees were crushed in liquid nitrogen separately, homogenized in the 0.1 M Tris-HCl buffer, pH 7.8, containing 2-mercaptoethanol (20 mM), PVP-K30 (5%), EDTA (2 mM), Nonidet-P40 (0.5%), GSH (5 mM) and Pepstatin (3 μ/ml) by ultra-turrax, and centrifuged at 15 000 rpm. The cytosolic protein amount and GST activity against CDNB were determined individually. We observed slightly higher GST activity in September probably indicating the response of *Pinus brutia* against drought conditions.

PP-1053
DNMT1 expression in CD4+ T cell of patients with systemic lupus erythematosus

R. Januchowski[1], M. Wudarski[2], H. Chwalińska-Sadowska[2] and P. P. Jagodziński[1]
[1]*Department of Biochemistry and Molecular Biology, Karol Marcinkowski University of Medical Sciences, Poznań, Poland,* [2]*Department of Connective Tissue Diseases, Institute of Reumatology, Warsaw, Poland. E-mail: fagm13@wp.pl*

The etiology of Systemie Lupus Erythematosus (SLE) is still unclear. The abundant production of autoantibodies leads to immune complexes formation that can be deposited in various tissues resulting in dysfunction of organs and clinical manifestation of SLE. T cells defects can be responsible for alternation in immune system of SLE patients. T cells from patients with SLE exhibit low expression of DNA methyltransferase 1 (DNMT1), DNA hipomethylation and changes in genes expression.
We compared protein level of DNMT1 in CD4+ T cells of SLE patients (n = 14) with different clinical disease activity scored in SLE Disease Activity Index (SLEDAI) scale.
The CD4+ cells were isolated by positive biomagnetic separation technique. The protein level of DNMT1 in the CD4+ T cells was determined by western blotting analysis. Spearman correlation analysis suggests that protein level of DNMT1 in CD4+ T cells may reversely correlate with SLE activity scored in SLEDAI scale (R = -0.779, P = 0.001). The low level of DNMT1 protein may result in DNA hypomethylation, changes in genes expression of signal transduction molecules that alternate CD4+ T cells function in SLE patients.
Acknowledgment: Supported by a grants No. 6P05B01927, 2P05B18929 from the State Committee for Scientific Research.

PP-1054
Investigation of lambda-cyhalothrin effect on *Helicoverpa armigera* glutathione S-transferases

M. Konus[1], S. Ugurlu[2] and **M. Iscan**[3]
[1]*Graduate Program of Biochemistry, Middle East Technical University, Ankara, Turkey,* [2]*Plant Protection Central Research Institute, FSM Avenue, Ankara, Turkey,* [3]*Department of Biological Sciences, Middle East Technical University, Ankara, Turkey. E-mail: konus@metu.edu.tr*

Helicoverpa armigera is a major pest of the cotton, maize, sorghum, pigeon pea, chickpea, soybean, groundnut, sunflower, and a range of vegetables. *H.armigera* has developed resistance to all of the insecticides that have been deployed against it at any quantity. As pyrethroids are known safe on human health, they are used commonly and excessively by farmers in Turkey. Thus, they have caused increased resistance development in the field populations of the *H.armigera*. It has been thought that one of the factors that cause insecticide resistance in *H.armigera* could be the induction of the detoxification enzymes like glutathione S-transferases (GST). In this study, gut sections of *H. armigera* were obtained from Adana and Antalya field populations and susceptible populations from Israel. Cytosolic GST activity of each individual from Adana, Antalya and susceptible populations were determined using CDNB as a substrate. The mean of GST activity in Adana population (n = 50) and Antalya population (n = 50) were found 391.3 nmol/min/mg and 479.3 nmol/min/mg, respectively. The mean of GST activity in susceptible population (n = 50) was determined as 163.6 nmol/min/mg. GST activities of Adana and Antalya field populations' were statistically (t-test) higher (P < 0.05) than that of susceptible *H. armigera* populations. GST isozyme composition of field populations and susceptible population were compared on SDS-PAGE by western blotting.

PP-1055
Composition of the ribonucleoprotein complex of ribonuclease P from *Dictyostelium discoideum*

A. Vourekas[1], D. Kalavrizioti[1], O. Theodorakopoulou[1], V. Stamatopoulou[1], C. Toumpeki[1], C. Stathopoulos[2] and D. Drainas[1]
[1]*Department of Biochemistry, School of Medicine, University of Patras, Patras, Greece,* [2]*Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece. E-mail: vourekas.a@med.upatras.gr*

Ribonuclease P (RNase P) is an essential enzyme that matures the 5' ends of all primary tRNA transcripts. RNase P enzymes contain a similar in size RNA subunit which is absolutely required for catalysis. However, the size and number of protein subunits of the holoenzyme varies significantly, from one small subunit in bacteria to ten subunits in human RNase P. Bioinformatic analysis of D. discoideum sequencing data returned five ORFs homologous to previously characterized RNase P protein subunits from human. The encoded proteins (DRpp30, DRpp40, DRpp29, DRpp25, DRpp20) exhibit significant similarity as well as notable variation to their counterparts from other species characterized so far. Their association with the RNase P holoenzyme is investigated using immunobiochemical methods and presented herein. The RNA component of RNase P evaded until recently conventional bioinformatic approach. A putative RNA subunit has been identified and is currently under characterization. According to earlier evidence, extensively deproteinized D. discoideum RNase P preparations exhibit catalytic activity probably attributed to the RNA subunit. The aim of this work is the structural and functional characterization of a ribozyme that could be used in RNA-mediated gene therapy applications.
Acknowledgement: The first two authors have contributed equally to this work. We thank the Program HERAKLEITOS (European Social Fund, Operational Program for Educational and Vocational Training II) for funding our work.

PP-1056
Comparison of two methods for hemoglobin A2 measurement

G. Daglioglu, **A. Eraslan**, D. Temiz, M. Tahiroğlu, A. Curuk and K. Aksoy
Department of Biochemistry, Medical Faculty, Cukurova University, Adana, Turkey. E-mail: gdaghoglu@cu.edu.tr

Hemoglobinopathy is very common genetic disease in the world. Beta thalassemia and sickle cell anemia are two most famous among hemoglobin disorders. Beta thalassemia is a condition in which reduction or absence of beta globin chains found in hemoglobin. Control of beta thalassemia can be achieved through screening for carriers identification, genetic counseling and prenatal diagnosis. Hemoglobin A2 is considered as one of the most useful parameters for beta thalassemia trait. Therefore, HbA2 quantitation procedure is very important for identify heterozygous beta thalassemia. Microchromatografic procedures played a significant role for screening until High Performance Liquid Chromatography (HPLC) was developed. Recently HPLC is used for premarital screening center for beta tahalassemia trait. HPLC is fast, reproducible and fully automated. In addition, HPLC can quantify HbA2, F and most common Hb variants in single step. Thirty-seven normal (HbA2 < 3.7%) and 48 beta thalassemia

carriers (HbA2 > 3.7%) were selected by using microcolumn chromatography. When the results were compared significant differences were found between the two groups. The same samples were analysed by HPLC. Results were similar between two methods in beta thalassemia trait and normal. Generally, HbA2 level is higher than 3.7% in sickle cell trait by HPLC. For this reason, fifty samples analysed by microcolumn chromatography. The results were significant between HPLC and microcolumn.

PP-1057
GDF5 signaling and bone formation: molecular characterization of GDF5 mutants

C. Sieber[1], P. Seemann[2], R. Schwappacher[1], F. Ploeger[3], Y. I. Henis[4], J. Pohl[3], S. Mundlos[2] and P. Knaus[1]
[1]*Institute for Chemistry/Biochemistry, FU Berlin, Berlin, Germany,* [2]*Max Planck Institute for Molecular Genetics, Berlin, Germany,* [3]*Biopharm GmbH, Heidelberg, Germany,* [4]*Department of Neurobiochemistry, Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel. E-mail: knaus@chemie.fu-berlin.de*

Growth and Differentiation Factor 5 (GDF5) is a member of the Transforming Growth Factor β superfamily. It has several functions, such as regulating the development of cartilage, synovial joints and bone. Mutations in Gdf5 cause Hunter-Thompson type chondrodysplasia or Brachydactyly type C (BDC) in human. We describe two GDF5 mutations that alter ligand-receptor binding affinity. R438L and L441P cause symphalangism and BDA2, conditions normally associated with the BMP (Bone Morphogenetic Protein) antagonist NOGGIN and the high affinity receptor of GDF5, BMP Receptor type Ib (BRIb), respectively. GDF5 L441P is almost inactive. However, GDF5 R438L shows elevated biological activity caused by its increased affinity to BRIa. GDF5 is a homodimeric protein, which is highly homologous to BMP2. It is stabilized by a single disulfide bridge between cysteine 465 on the respective monomers. To create monomeric GDF5 we replaced cysteine 465 by alanine (GDF5 C465A). Surprisingly, the monomeric variant of GDF5 is as potent *in vitro* as the dimeric form. This could be confirmed by functional assays. Furthermore, dimeric and monomeric GDF5 show comparable binding to their high affinity receptor BRIb. Studies on live cells showed that dimeric and monomeric GDF5 induce homomeric BRIb and heteromeric BRIb/BRII oligomers. Our results suggest that GDF5 C465A has the same biological activity as wild type GDF5 in respect to binding to, oligomerization of and signaling through BRIb.

PP-1058
The effects of chronic ETA/ETB receptor blockade in the isolated rabbit aorta

B. Reel[1], G. Oktay[2], G. Ozsarlak Sozer[1] and Z. Kerry[1]
[1]*Pharmacology, Ege University, School of Pharmacy, Izmir, Turkey,* [2]*Biochemistry, Dokuz Eylul University, School of Medicine, Izmir, Turkey. E-mail: buket.reel@ege.edu.tr*

Endothelin (ET), an endothelium-derived vasoactive peptide regulates basal vascular tone via ETA and ETB receptors. ETA receptors in vascular smooth muscle cells (SMCs) mediate vasoconstriction. Endothelial ETB receptors lead to vasorelaxation by stimulating nitric oxide (NO) and prostacyclin production. Furthermore, ET was shown to stimulate superoxide anion (O2-) production by ETB receptors. The objective of this study was to investigate the effects of chronic blockade of ETA/ETB receptors on vascular responses and also basal O2- generation in isolated rabbit aortic rings. Rings from placebo (0.9%NaCl, s.c., $n = 8$) or TAK-044 (an ETA/ETB receptor antagonist, 5 mg/kg/day, s.c., 21 days, $n = 10$) treated white rabbits were used to study vascular reactivity. Organ chamber experiments were constructed in Krebs solution in presence or absence of N-nitro L-arginine (LNA, 10^{-4} M), an inhibitor of NO synthase (NOS). Nitrotyrosine levels (ng/mg protein) as marker of O2- production in aortic tissues were measured by ELISA. LNA increased maximum contractions (Emax) to 5-HT (1.40 ± 2.58, -LNA, 2.61 ± 3.12, +LNA, mean ± SEM, $P < 0.05$), and pD2 values of phenylephrine (6.13 ± 0.02, -LNA, 6.34 ± 0.11, +LNA, $P < 0.05$). TAK-044 significantly decreased pD2 values of 5-HT (6.15 ± 0.06, -LNA, 6.39 ± 0.07, +LNA, $P < 0.05$). However responses to acetylcholine were not affected by TAK-044. TAK-044 did not alter nitrotyrosine levels. In conclusion, TAK-O44 may be useful in antagonizing endogenous vasoconstrictor responses.

PP-1059
Effect of light on vitamin B12 and folate measurements

A. Kosem, M. Senes, C. Topkaya and D. Yücel
Department of Clinical Biochemistry, Ankara Education and Research Hospital, Ministry of Health, Ankara, Turkey. E-mail: arzukosem06@yahoo.com

Little information can be found in the literature on the effect of light on serum vitamin B12 and folate measurements. Additionally, different kits and instrument systems for the measurement of these analytes can be peculiarly affected by light. In this study, we investigated the effect of light on these analytes. We collected blood specimens from 11 healthy volunteers in SST (16 × 100 mm) Vacutainer tubes. All specimens were allowed to clot for 30 min at room temperature before centrifugation. Sera separated and aliquoted in two groups of plain polypropylene tubes capped and stored in dark (Group 1) and light (Group 2). Duplicate vitamin B12 and folate measurements were performed immediately (0 h), at 8 h and 24 h after drawing. The measurements were performed in an Immulite 2000 analyzer with reagents from the manufacturer. We used arithmetic means of the duplicates for statistical analysis. The significance of differences between and within groups was analyzed by repeated-measures ANOVA. The significance of differences between baseline analyte means of the groups was assessed by the Student's paired *t*-test. When the 0 h specimens were considered reference, B2 measurements were not significantly affected by light up to 24 hours ($P > 0.05$). Whereas folate measurements were significantly affected by light 24 h after drawing. In conclusion, there is no need to store the samples in dark for vitamin B12 and folate measurements which performed in the same working day of drawing.

PP-1060
Interleukin-10 (-1082) and tumor necrosis factor α (-308) gene polymorphisms and preeclampsia

P. Vural[1], N. Yildirim[1], A. Demirkan[2], E. Cigerli[3], C. Akgul[3], U. Ozbek[2] and M. Canbaz[1]
[1]*Department of Biochemistry, Istanbul Medical Faculty,* [2]*DETAM Genetics Department,* [3]*Department of Obstetrics and Gynecology, Istanbul Medical Faculty. Istanbul University, Istanbul, Turkey. E-mail: pervinvural@yahoo.com*

Preeclampsia (PE) is characterized by hypertension, proteinuria, oedema and increased systemic inflammatory response. Cytokines IL-10 and TNF α exert opposite functions in inflammatory reactions, IL-10 acting predominantly as an anti-inflammatory and

TNF α as a proinflammatory factor. Functional single nucleotide polymorphisms in the genes of IL-10 and TNF α are associated with gene expression and plasma levels of IL-10 and TNF α. The aim of this study was to assess whether these IL-10 and TNF α gene polymorphisms are related to the risk of PE. We determined the allelic frequency of these mutations in a population of well-characterized PE (n = 40) as compared with normotensive non-pregnant controls (n = 50). Genomic DNA from patients and controls was typed for IL-10 (-1082 G/A) polymorphism using an allel specific polymerase chain reaction (ASPCR) and for TNF α (-308 G/A) polymorphisms with a PCR based restriction fragment length polymorphism (RFLP). Results were analyzed with a chi-square test. The frequency of the IL-10 (-1082) G allele and of the TNF α (-308) A alleles (both associated with increased transcriptional activity) were not increased in PE women. We concluded that together with genetic polymorphism there are some posttranscriptional factors influencing plasma cytokine levels. However, further studies (as well as increase the number of the participants) are necessary to investigate potential genetic causes of PE.

PP-1061
Evaluation of urinary cystatin-C in patients with monoclonal gammopathy

E. Altekin, Z. Altun, F. Kuralay and H. Islekel
Department of Biochemistry, Dokuz Eylul University, School of Medicine, Izmir, Turkey. E-mail: emel.altekin@deu.edu.tr

Renal involvement is one of the most common manifestations in multiple myeloma. Various endogenous markers have been used as indicators of renal function impairment. Urine Cys-C levels can be used as a marker of renal function. The aim of this study was to assess urine cystatin-C (Cys-C) levels in patients with myeloma. The urinary excretion of Cys-C was evaluated in 25 patients with multiple myeloma. Patients were divided in three groups according to immunological phenotyping of monoclonal immunoglobulin in urine immunfixation electrophoresis. In Group I: IgA - Kappa (K), IgA - Lambda (L) excretion (n = 8), In Group II: IgG - K, IgG - L excretion (n = 8), In Group III: free - K or free - L excretion (n = 9) were observed in urine. Urine Cys-C levels (as Mean ± SD and mg/L) were significantly higher in Group I (1.43 ± 0.53) than those of both Group II (0.63 ± 0.30; $P < 0.05$) and Group III (0.25 ± 0.38; $P < 0.05$). These findings may be explained as the results of the different molecular weights of these immunoglobulins. Since the molecular weight of the Ig-A is higher than Ig-G, it may expected to cause greater renal impairment in Group I than other groups. We suggest that, possible kidney dysfunction caused by immunoglobulin nephrotoxicty in multiple myeloma might be assessed by urinary Cys-C levels.

PP-1062
Use of a special study module for undergraduate medical biochemistry education: basic medical laboratory techniques

F. G. Sagin, E. Y. Sozmen, Y. D. Akcay, E. Sezer, T. Onat and F. Z. Kutay
Dept. of Biochemistry; Ege University Medical Faculty, Izmir, Turkey. E-mail: ferhan.sagin@ege.edu.tr

Medical & laboratory knowledge expands with great speed, demanding more efficient teaching techniques in education. A meaningful learning helps the student to integrate the new-old knowledge thus is crucial in the long-term. Aiming this objective, we planned and implemented a Special Study Module (SSM); Basic Medical Laboratory Techniques for Phase II students (n = 12) in our faculty. This 2 week SSM included theoretical sessions every morning which were designed with student-centered teaching methods such as discussions, independent group studies, puzzles, etc. Constructing meaningful linkages and relevant hierarchy between topics through case studies and problems were highly preferred. Related laboratory practical which were structured objectively through the student performance guides took place every afternoon. The assessment methods for this SSM emphasized mastery and learning rather than grades. Students were evaluated on the basis of their active attendance in the classroom/laboratory as well as lab performance. Pre and post tests were used to assess the efficiency of the program. The mean % of correct answers increased from 36.92 (pre-test) to 78.47 (post-test). Students voiced their satisfaction and stated their benefit through the questionnaires. Biological sciences are best learned when they are presented in a relevant way and platform. Student active strategies emphasizing repetition, reinforcement and self-performance should be preferred in teaching whenever possible.

PP-1063
Purification and characterization of archaeal 6xHis tagged recombinant proteases

B. Koyuncu, B. Demirok and S. Kocabıyık
Department of Biology, METU, Ankara, Turkey. E-mail: bilsev@metu.edu.tr

Proteases are degradative enzymes which catalyze the hydrolysis of peptide bonds. They have crucial roles in metabolic processes and industry. Since they are physiologically essential molecules, they occur ubiquitously in a wide variety of organisms including viruses, *archaea*, *bacteria* and *eukarya*. Several thermophilic and hyperthermophilic *archaea* produce significant levels of intra- and extracellular proteolytic enzymes with high intrinsic molecular stability. Moreover, genome sequence data revealed even more expansive proteolytic genotypes of these organisms than can be inferred from biochemical analysis. However, the role of proteolysis in the metabolisms of thermophilic *archaea* is less clear. We have already cloned a Clp P-like periplasmic serine protease (PSP) and an aspartic protease (thermopsin) gene of the thermoacidophilic *archaeon Tp. volcanium*. PSP and thermopsin genes were also heterologously expressed in *E. coli* by adding 6xHis tag to the 5' ends of the respective proteins by using QIA-Expressionist Kit. Here we report the purification of the recombinant fusion proteins by employing nickel-nitrilotriacetic acid (Ni-NTA) metal affinity chromatography. Also some biochemical parameters associated with the PSP and thermopsin were determined.

PP-1064
Salvinorin A effect on rats when tested in a bioassay based on Hall's Open Field Test

S. S. Bolle[1], Ü. Sayın[1], N. Puralı[2], B. Keçeli[2] and **D. J. Siebert**[3]
[1]Institute of Forensic Science, Istanbul University, Istanbul, Turkey, [2]Biophysics, Medicine Faculty of Hacettepe University, Ankara, Turkey, [3]The Salvia divinorum Research and Information Center, Malibu, CA, USA. E-mail: sharonbolle@gmail.com

Salvinorin A, the first known naturally occurring non-nitrogenous full agonist at κ-opioid receptors, is the psychoactive component of the hallucinogenic mint *Salvia divinorum* (1). *Salvia divinorum*, or any of its active ingredients are not specifically listed in the Controlled Substances Act and it is currently used as a legal alternative to controlled substances. Usually smoking the

dried leaf or absorption in buccal mucosa by chewing the fresh leaves, doses of approximately 200 mcg can produce profound hallucinogenic effects of short duration. The mechanism of action of salvinorin A is at the κ-opioid receptor. Little data is available on the psychopharmacological effects of this substance so animal behavioral studies were undertaken to explore the open field locomotor activity effects of this substance in rats. Using Hall's Open Field Test, a dosage of 4 mg/kg purified salvinorin A was administered intraperitoneally to rats. Squares entered, rearing up on hind legs, holes explored, and length of immobility were recorded. The data was evaluated by Sigmastat Statistic Program, using paired t-test. Salvinorin A, caused a statistically significant decrease in open field locomotor activity, rearing and exploratory behaviour. These results show that, Salvinorin A inhibits the locomotor activity and exploratory behaviour.

Reference:

1. Chavkin *et al.* (2004). J Pharmacol Exp Ther , 308:1197–1203.

PP-1065
α-amylase production from food waste by using newly isolated *Halomonas* sp.

K. S. Uzyol[1], B. Sarıyar Akbulut[2] and D. Kazan[2]
[1]*Environmental Engineering Department,* [2]*Chemical Engineering Department. Marmara University, Engineering Faculty, Goztepe Campus, 34722 Kadıkoy-İstanbul-Turkey.*
E-mail: ksuzyol@yahoo.com

Amylases are important and valuable industrial enzymes, widely used in industrial applications such as food, textile, detergent, and brewery. alpha-amylases can be produced by a wide variety of microorganisms including fungi, yeast, archea and bacteria. Moderately halophilic bacteria are extremophilic microorganisms that grow optimally in media containing 3–15% NaCl. Moderately halophiles are receiving an increasing attention in biotechnological applications because of their resistance to extreme environmental conditions, such as high salinity. In this work, alpha-amylase was produced by newly isolated *Halomonas* sp. from Çamaltı Saltern Area, Izmir-Turkey. The optimal medium composition for *Halomonas* sp. to yield the highest amylase production from food waste was determined. Glucose, maltose, sucrose, lactose and starch were used as alternative carbon sources to find the best carbon source for amylase production by *Halomonas* sp. Similarly, the effect of different nitrogen sources on alpha-amylase production was also studied. Finally using Response Surface Methodology, the optimal concentrations of carbon and nitrogen sources were determined for alpha-amylase yield.

PP-1066
Drought-induced oxidative damage and antioxidant responses in lentil (*Lens culinaris,* M) under polyethylene glycol mediated water stress

U. Celikkol Akcay, M. Kavas, **O. Ercan**, E. Aksoy, M. Alshalalfa, O. F. Gerdan, M. Yucel and H. A. Oktem
Department of Biology, Middle East Technical University, Ankara, Turkey. E-mail: oercan@metu.edu.tr

Lentil is a nutritionally valuable pulse legume crop especially important for tropical and subtropical parts of the world. Turkey is also one of the major lentil producing countries and rate of annual consumption is around 2–3 kg/person. In the present study, changes in relative water content (RWC), chlorophyll fluorescence, lipid peroxidation, proline and enzymatic antioxidant system were evaluated to determine the effect of polyethylene glycol mediated water stress in root and shoot tissues of lentil (*Lens culinaris* M cv. Fırat-87). For induction of drought stress, seven days old lentil seedlings were treated with PEG 6000 (-0.80 Mpa) for 9 days. PEG treatment resulted in oxidative injury, as expressed in decreased wet weight, dry weight and increased level of lipid peroxidation, proline (up to three fold), H_2O_2 and RWC. Although no changes in the activities of catalase (CAT), ascorbate peroxidase (APX) enzyme were observed, SOD activity was increased in both shoot and root tissues. We also did not observe any change in the chlorophyll fluorescence level of leaves. Our data suggest that increased proline level and enhanced SOD activity are the two mechanisms taking place in the protection against water stress-induced oxidative damage in lentil.

PP-1067
Study of malate dehydrogenase from *Streptomyces*

D. Mikulasova, M. Koharyova, J. Maderova and M. Kollarova
Department of Biochemistry, Comenius University, Bratislava, Slovak Republic. E-mail: kollarm@fns.uniba.sk

The data concerning MDH of *Streptomyces* genes are very limited. The first complete functional characterization of *Streptomyces* MDH showed that the enzyme of *S.aureofaciens* is very similar in many respects to other bacterial MDHs. The enzyme showed a strong NADH specificity. It was more efficient for the oxaloacetate reduction than the malate oxidation. In cases involving NADPH, the specific activity does not exceed 1.5% of the specific activity when NADH was used. Unlike MDHs from other sources, it was not inhibited by excess oxaloacetate substrate. The aim of this work was to prepare *S. aureofaciens* crystals and collect data for structure determination of native MDH or for MDH-NADH and MDH-NADPH complexes, in order to better understand MDH functions involving the interaction of the enzyme with coenzymes. Purified malate dehydrogenase (MDH) of *S. aureofaciens* was crystallized either in the absence or in the presence of NADH or NADPH coenzymes by hanging-drop vapour-diffusion method. An X-ray study has shown, that MDH crystals belong to space group C2221 with unit-cell parameters a = 53.2 Å, b = 104.6 Å, c = 520.0 Å, α = β = γ = 90 °, MDH-NADH crystals to space group C2 with unit-cell parameters a = 51.5 Å, b = 51.5 Å, c = 256 Å, α = β = γ = 90 °, and MDH-NADPH crystals to space group C2221 with unit-cell parameters a = 72 Å b = 72 Å, c = 520 Å, α = β = γ = 90 °. The crystal of native MDH diffracted to 2.1 Å resolution.

PP-1068
VEGF-1154 (A/G) polymorphism and laryngeal squamous cell carcinoma

Zn. Dogruer[1], G. Polat[1], O. Tubay Bagdatoglu[1], M. Unal[2] and U. Atik[1]
[1]*Mersin Univ. Department of Clinical Biochemistry,* [2]*Mersin Univ. Department of Otorhinolaryngology.*
E-mail: nilzeynep@yahoo.com

Squamous cell carcinoma (SCC) of the larynx is the most frequent malignancy occurring in the head and neck region. Angiogenesis has been correlated with the potential for invasion and metastasis. Tumor vascularization is mediated by the release of angiogenic peptides from tumor cells, macrophages and extracellular matrix. Vascular endothelial growth factor (VEGF) is thought to be one of the most important angiogenic factors. Overexpression of VEGF is associated with increased angiogenesis and invasion in solid tumors. The aim of this study is to

investigate the relation between the VEGF-1154 (A/G) gene polymorphism and the laryngeal SCC. The study consisted of 45 patients with laryngeal SCC and 89 control subjects. Genotypes were detected using PCR technique from DNA. The genotypes were designed as; AA (low VEGF expression), AG and GG (high VEGF expression). AA genotype was not seen in both patient and control groups. AG genotype was 57.8% in patient group and 37.08% in control group respectively. GG genotype was detected in a rate of 42.2% in patient group, and 62.92% in control group. According to the high risk (GG) genotype, the difference between the patient and control groups were statistically significant (Odds ratio 0.43%95CI = 0.194–0.952, $P = 0.036$). In conclusion, laryngeal SCC is known to be a lower invasive behavior and metastasis potential than other solid tumors, and low frequency of VEGF GG genotype polymorphism in our study supports this hypothesis.

PP-1069
Effects of extremely-low-frequency pulsed electromagnetic fields on collagen synthesis in rat lung

S. M. Moshtaghioun[1] and S. Rezaei-Zarchi[2]
[1]*Biology Department, Yazd University, Yazd, Iran,* [2]*Institute of Biochemistry & Biophysics, Tehran University, Tehran, Iran. E-mail: moshtaghiun@yazduni.ac.ir*

To investigate the effects of extremely-low-frequency PEMFs (pulsed electromagnetic fields) on the synthesis of collagen, six groups of animals each consisting of eight mature male rats were selected randomly: one group for the control and five for the test. Using a parallel set of Helmholtz coils, a uniform field intensity of 2 mT at different frequencies of 25, 50 and 100 Hz yielded the most effective frequency to be 25 Hz. Then, at this frequency, two different field intensities of 1 and 4 mT were applied. The treatment time of 2.5 h/day lasted for 8 days, keeping the same procedure for the control group, except with the field turned off. On the ninth day, the rats were killed and lung samples from the same region were taken for collagen assessment by measuring hydroxyproline content using the Stegemann-Stalder [(1967) Clin. Chim. Acta 8, 267–273] method. The results indicated that a PEMF of 1, 2 and 4mT at 25Hz increased the collagen synthesis ($P < 0.05$). The other frequencies did not have any noticeable effect.

PP-1070
The complexes of enzymes with water soluble polymers

H. Kuzu, M. Altıkatoglu, O. Oztolan, A. Arslan, M. Celebi, C. Arıoz and Y. Basaran
Department of Bioengineering, Yildiz Technical University, Istanbul, Turkey. E-mail: huri@yildiz.edu.tr

In biotechnology, stabilities of the enzymes and proteins in vitro are still being one of the most important issues. Storage and operational stabilities are both important for the usage of enzymes. Covalent conjugation or formation of the complexes of the enzymes with water-soluble polymers can lead to improvement of the stability. In this study the purchased Aspergillus oryzae alpha-amylase (Taka amylase), Mucor Miehei Rennet, HRP (Horseraddish Peroxidase) and cellulases of Aspergillus Niger KK2 were purified. Enzyme-polymer covalent conjugates and complexes were prepared. These conjugates and complexes were analyzed by Viscotek and HPLC techniques. The activities of pure enzymes and the conjugates are measured in different temperatures and pH values. These processes caused the change of the activity pH and temperature ranges.

PP-1071
Genomic organization and functional analysis of *Cynara cardunculus* L. aspartic protease gene family

C. Pimentel[1,2], E. Pires[1,3], C. Faro[1,3] and C. Rodrigues-Pousada[2]
[1]*Departamento de Biologia Molecular e Biotecnologia, Centro de Neurociências e Biologia Celular de Coimbra, Coimbra, Portugal,* [2]*Genomics and Stress, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal,* [3]*Departamento de Bioquímica, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, Coimbra, Portugal. E-mail: pimentel@itqb.unl.pt*

A few aspartic proteinases (APs) have been isolated from the cardoon *Cynara cardunculus* L., whose flowers are traditionally used in Portugal in the manufacture of ewe's cheese such as Serra da Estrela. Beyond the already known cardosins A, B, the screening of a cardoon genomic library disclosed two new genes, named cardosin C and D. Comparison of the cardosins A, B and D genomic clones with the respective cDNAs obtained by 5'RACE revealed the presence of an intron in the 5'UTR of the genes. The alignment of cDNA clones, against genomic clones, strongly suggests that an intron in the leader region might be a common feature of among plant APs genes. Based on sequence differences among the genes, four sets of specific primers were designed and used to evaluate the expression of each gene by RT-PCR, at three different stages of floral development and in several parts of the plant. Our results show that the four genes exhibit distinct patterns of expression, suggesting that they might play different biological roles. To further investigate the spatial and temporal expression of cardosin genes, each of the 5' flanking regions, including the leader intron, was fused to the GUS reporter gene and introduced separately into *Arabidopsis thaliana*. The functional analysis of cardosin promoters revealed that within the flower cardosin genes are differentially expressed. In addition, analysis of promoter deletions of cardosin A and B uncovered important regions in gene regulation.

PP-1072
Purification and study of substrate kinetics of fructose 1,6 bisphosphate aldolase from human placenta

N. H. Aksoy and P. Doğan
Department of Biochemistry, Faculty of Medicine, Hacettepe University, Ankara, Turkey. E-mail: nha@hacettepe.edu.tr

Fructose-bisphosphate aldolase is a major glycolytic enzyme found in most cells. In mammalian tissues there are three isoenzymes of aldolase referred to as Type A, the major form is found in muscle; Type B in liver and kidney, and Type C in brain. The enzyme catalyzes the reversible aldol cleavage of one molecule D-fructose 1,6-bisphosphate (FBP), into dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-P (GA3P). In our study, we wanted to examine the presence of aldolase and effects of substrate kinetics in healthy human placenta. Fructose 1,6 bisphosphate aldolase (E.C. 4.1.2.13, FBPA), was purified 40.5-fold from healthy human placenta by phosphocelluse chromatography. Purity was controlled by polyacrilamide gel electrophoresis (PAGE). It was observed that, Lineweaver-Burk diagram line was appeared to be down. A new diagram was performed for the phases that separate this point. At low concentrations of substrate, value of Km of healthy placental aldolase was determined as 3.048 ± 1.39 mM and value of Vm was determined as

636.103 ± 196.165. At high concentrations of substrate, Vm 1885.457 ± 292.48 and Km 23.063 ± 6.845 mM.

Key words: Aldolase, placenta, purification, substrate kinetics
Acknowledgement: H.U.B.A.B. PhD Thesis Grant (05.T06.101.001).

PP-1073
Electrophoretic analysis of CSF proteins in patients with preeclampsia

F. Burak[1], **O. Kaynar**[2], A. Bay Karabulut[3] and A. Kafkasli[1]
[1]Department of Obstetic and Gynecology, Faculty of Medicine, İnönü University, 44069, Malatya/Turkey, [2]Department of Biochemistry, Faculty of Veterinary Medicine, Atatürk University, 25700, Erzurum/ Turkey, [3]Department of Biochemistry, Faculty of Medicine, İnönü University, 44069, Malatya/Turkey.
E-mail: okaynar@atauni.edu.tr

Preeclampsia is a common problem during pregnancy and can be fatal for mother and unborn baby. This condition is characterised by hypertension and proteinuria after 20 weeks of pregnancy. Possible effect of high blood pressure and other complications on cerebro-spinal fluid (CSF) proteins profiles were investigated in this study. CSF samples were taken from 4 healthy and 12 patients with preeclampsia. Proteins of CSF were separated by SDS-polyacrylamide gel electrophoresis and visualised by silver-staining. Densitometry scans of dried gels were performed using Desaga CD 60 laser densitometer with Gelscan software. All of the protein lines were scanned and results were determined either ratio or amount of each protein in total composition. A decrease in the protein compositions (1.19–2.81 folds) and for some certain proteins (proteins between 34.9–52.5 kDa and molecular weights higher than 103 kDa 6.96 and 9.97 fold respectively) were determined in CSF proteins of patients with preeclampsia when compared with healthy subjects. As a result, analysing of CSF protein profiles can support the diagnosis of the preeclamsia because of some decreases of certain proteins.

PP-1074
The effects of famotidine on colon anastomosis healing in rats

C. Koca[1], A. İnan[2] and M. Şen[2]
[1]Department of Medical Biochemistry, [2]Department of General Surgery, Fatih University School of Medicine, Ankara, Turkey.
E-mail: cemile_md@yahoo.com

Healing of colonic anastomosis is a complex process involving inflammation. Histamine participate in the regulation of immune reaction and inflammatory response. It plays an important role in cell proliferation and lymphocyte response via histamine-2 receptors (H2R). In regard of histamine's role on immune reaction, we aimed to investigate the effect of an H2R antagonist, famotidine (FAM), on the healing of colon anastomosis. 28 male Sprague-Dawley rats were used in this study. Rats were underwent distal colon resection and end-to-end anastomosis. FAM group received 2 mg/kg/day FAM while control group received same amount of saline intramuscularly each day. Rats were sacrificed on the 3rd and 7th postoperative days. Anastomotic healing was assessed by bursting pressure (BP) and the hydroxyproline (OH-Pro) content of the anastomotic tissues. BPs of the FAM group (79.57 ± 21.11 and 188.29 ± 14.26 mmHg on 3rd and 7th day, respectively) were lower than control group (131.43 ± 53.31 and 209.43 ± 18.14 mmHg on 3rd and 7th day, respectively). OH-Pro contents of the perianastomotic tissues of FAM group (2.34 ± 0.63 and 2.65 ± 0.28 microg/mg tissue on 3rd and 7th day, respectively) were lower than control group (2.92 ± 0.25 and 4.63 ± 0.41 microg/mg tissue on 3rd and 7th day, respectively). Our data indicate that the administration of FAM impairs anastomotic healing of colon. The reason of this effect might be due to FAM's antagonizing impact on histamine's beneficial role on healing.

PP-1075
The drosophila MRP is a high capacity organic anion transporter and may transport 20-OH ecdysone glucoronide

F. Szeri[1], A. Ilias[1], K. Liliom[1], A. Magyar[2], S. Robinow[3] and A. Varadi[1]
[1]Institute of Enzymology, Biological Research Centre, Hungarian Academy of Sciences, Budapest, Hungary, [2]Research Group of Peptide Chemistry at Eötvös Loránd University of Sciences, Hungarian Academy of Sciences, Budapest, Hungary, [3]Department of Zoology, University of Hawaii, Honululu, USA.
E-mail: szeri@enzim.hu

The Drosophila genome contains 56 ABC genes, fourteen of them belongs to the ABCC/MRP subfamily. The so-called 'long MRPs' (MRP1, MRP2, MRP3, MRP6 and MRP7 in human) are represented by only a single gene, *dMRP/CG6214* in Drosophila. To reveal the function of the protein encoded by this gene we used the Sf9/baculovirus expression system. Functional studies, such as vesicular transport assays, ATPase activity measurements, and vanadate trapping indicated that dMRP is a high capacity, ATP-dependent, vanadate-sensitive organic anion transporter, which is capable of transporting leukotriene C_4 and the estrogen-metabolite estradiol-17-β-D-glucuronide. We found that the major steroid moulting hormone, 20-OH ecdysone, a key regulator in the coordination of multiple developmental processes in insects is not a substrate of dMRP. However, transport inhibition experiments suggested that dMRP interact with or transport the 20-OH ecdysone metabolite, 20-OH ecdysone glucuronide. Based on these data we assume that dMRP might play a role in the downregulation of ecdysone response in Drosophila. Furthermore, baculoviruses infect Lepidoptera species and disrupt their host's hormonal balance by conjugating ecdysteroids with UDP-glucose thus arresting the development of the infected insect. Our finding raises the possibility that an insect MRP capable of transporting ecdysteroid conjugates might play a role in the pathomechanism of baculovirus infection.

PP-1076
Prolactin and exercise

A. Olgun, **E. Saruhan**, Y. Kurt, T. Özgürtaş, Ş. Akman and M. K. Erbil
Department of Biochemistry and Clinical Biochemistry, Gulhane School of Medicine, 06018 Etlik, Ankara, Turkey.
E-mail: esaruhan@gata.edu.tr

The acute change of blood prolactin levels induced by 3000 m running exercise was analyzed in young men (age 20–23). Prolactin measurements were carried out on the blood samples withdrawn before, and 0-1-2 h after exercise. Mean prolactin values after exercise at 0 and 1 h were significantly higher than baseline, 0 h values being very pronounced (up to 3-fold of baseline, $P < 0.001$, and up to 7-fold in some cases). The mechanism, and the effects on metabolism, of this very significant exercise induced increase of prolactin levels in this particular study group needs to be elucidated.

PP-1077
Chemopreventive efficacy of synthetic retinoid (fenretinide) and PPAR-gamma ligand combination in the *in vivo* mammary carcinogenesis model

H. Kocdor[1], M. Kocdor[2], T. Canda[3], D. Gurel[3], R. Cehreli[1], O. Yilmaz[4], M. Alakavuklar[5] and **G. Guner**[6]
[1]*Institute of Oncology,* [2]*School of Medicine, Dept. of Surgery,* [3]*School of Medicine, Dept. of Pathology,* [4]*Multidisciplinary Laboratory,* [5]*Dept. of Medical Oncology,* [6]*Dept. of Biochemistry, Dokuz Eylul University, Izmir, Turkey. E-mail: hilal.kocdor@deu.edu.tr*

The present study aims to investigate the chemopreventive properties of PPAR-γ ligand and synthetic retinoid fenretinide (4-HPR) combination on an *in vivo* DMBA-induced mammary carcinogenesis model. Fifty female SD rats divided into five groups (Control, DMBA, DMBA + HPR, DMBA + PPAR, DMBA + HPR + PPAR) were used. IGF-1 and IGFBP-3 were used as biochemical surrogate-endpoint-biomarkers (SEB). Also, serum E2 and prolactin levels were analysed using by RIA. Hormone receptor status (ER/PR), aromatase and apoptosis were evaluated in histopatological sections. Tumor frequency and multiplicity were significantly higher in DMBA group than the other groups. But, no significance was found between chemoprevention groups. All tumors and proliferative lesions were ER-negative. Progesteron receptor status was found to be similar in DMBA pre-treated groups. Apoptotic cells were significantly higher in DMBA + PPAR group when compared to other chemoprevention and DMBA groups. Serum IGF-1 and E2 levels were similar among the groups while IGFBP-3 levels were significantly higher in Fenretinide pre-treated group than in the others. In conclusion, both Fenretinide and PPAR gamma ligand were effective in mammary tumor prevention. The magnitude of the effect was not enhanced by the use of the combination. The increment of apoptosis plays important roles on the anti-tumoral efficacy of rosiglitazone and also, chemopreventive efficacy of Fenretinide was found to be related to IGFBP-3 levels.

PP-1078
Molecular and physiological responses to salinity stress in wheat (*Titicum aestivum*)

W. Zerrad[1], S. Hillali[2], S. El Antri[2], M. Ibriz[2] and **A. Hmyene**[1]
[1]*Laboratoire de Biochimie, d'Environnement et d'Agroalimentaire. FST de Mohammedia B.P. 146. Mohammedia 20650 Morocco,* [2]*Laboratoire de Biochimie. FST de Settat.*
E-mail: hmyeneaziz2002@yahoo.fr

Abiotic stress reactions, especially to water deficiency and high levels of salt, are complex morphological and physiological phenomena in plants. To investigate the defence system to the salinity of common wheat (Triticum aestivum). We evaluate the effect of the saline stress on certain physiological and biochemical characters of this plant and output estimate its yield. The results obtained showed a reduction of the height of vegetation, the chlorophyll a, b and (a + b) content and the relative water content (RWC). Its also causes an increase of the relative water losses (RWL). In addition, the tolerance to the salinity of the studied varieties was characterized by a significant accumulation of osmoregulation components (TSS and TAA). The influence of NaCl on the yield also resulted in a reduction of (WTG) of the studied varieties. In addition, the addition of significant NaCl amounts to the culture caused a variation of the biochemical composition of the cellular membrane of common Wheat in term of total lipids and proteins; thus affecting the membrane stability. This variation evolves not only according to the saline stress but also according to the variety used.

PP-1079
Abnormal hemoglobins in Çukurova: two rare variants HbE-Saskatoon and HbG-Coushatta

A. Genc, F. Zeren, D. Temiz and **M. A. Curuk**
Department of Biochemistry, Cukurova University Medical Faculty, Adana, Turkey. E-mail: akif@cu.edu.tr

Sickle cell anemia and beta thalassemia are very common genetic diseases in Çukurova region. We characterized 69 carriers for common and some rare hemoglobin variants. Forty-two cases were detected as sickle cell (HbS) or HbD trait by electrophoresis. The results were confirmed with DNA analysis by using ARMS technique. Twenty-seven cases were detected HbE or HbC by electrophoresis. Five of them couldn't be confirmed with allel specific PCR (ARMS) for HbE. These were diagnosed by DNA sequencing as HbAC. We analysed DNA samples from 39 individuals having sickle cell anemia. Most of them were homozygous for HbS. Using ARMS and RFLP techniques, 17 of them were determined as heterozygotes for sickle cell anemia. These were screened by ARMS for common beta thalassemia mutations. Six different mutations (ten; IVS1-110, three; Cod 39, one of each Fsc5, IVS1-1, IVS1-5 and IVS1-6) were determined. Five compound hereozygosity with HbD and beta thalassemia were detected. Four of them were IVS1-110, the other one was IVS1-1. Two cases were coexisted HbE and beta tahalassemia (IVS1-110 and IVS1-6) mutations. Also, we diagnosed one case as HbSD and five of them as HbSE. We received two blood samples from premarital screening centers. We detected abnormal hemoglobins by electrophoresis and HPLC. They were characterized by DNA sequencing as a HbE-Saskatoon homozygous and HbG-Coushatta trait. We performed prenatal diagnosis for the first mother. The fetus inherited HbE-Saskatoon.

PP-1080
Microsatellite analysis of some horse breeds in Turkey: usefulness for parentage testing

M. Nizamlioğlu[1], **E. Kurar**[2], Z. Bulut[1], S. İnal[2] and F. Erzurum[3]
[1]*Department of Biochemistry, Selcuk University, Faculty of Veterinary Medicine, Konya, Turkey,* [2]*Deparment of Zootechnics, Selcuk University, Faculty of Veterinary Medicine, Konya, Turkey,* [3]*TİGEM, Ministry of Agriculture and Rural Affairs, Ankara, Turkey. E-mail: ekurar@selcuk.edu.tr*

The use of DNA technology for verifying parentage in breed registrations and identifying individual animal in forensic science is increasing every day. Therefore, different test panels were developed and reported for different animal species. In order to test efficacy of a microsatellite panel, a total of 189 blood samples was collected from different horse breeds in Turkey. As a preliminary study, we selected five horse microsatellite loci, LEX33, HMSO6, HMS02, HTG10 and AHT04, and used to amplify genomic DNA by polymerase chain reaction (PCR). The resulting PCR products were separated on polyacrilamide gels. Allele identification was based on their base-pair size by comparing a size standard. A total of 53 alleles was determined ranging from 9 to 11 at each locus. The observed heterozygosity (HO) and expected heterozygosity (HE) were ranged from 0.496 to 0.880 and from 0.800 to 0.851, respectively. Polymorhic information content (PIC) values were observed between 0.774 and 0.832. Probability of exclusion (PE) at each microsatellite locus ranged from 0.619 to 0.702, resulting in a total PE value of 0.99060. These preliminary results indicate that this set of microsatellite is useful for

horse parentage testing in Turkey. Due to possible high level of inbreeding in some breeds, the use of increased number microsatellite loci will thereby be appropriate for avoiding a false parenting and misidentification.

PP-1081
Exogenous abscisic acid increases stability of polysomes in embryos of *Triticale caryopses* during germination

M. Kazarnowicz, E. Szypulska and **S. Weidner**
Department of Biochemistry, Faculty of Biology, University of Warmia and Mazury in Olsztyn, 10-957 Olsztyn-Kortowo, M. Oczapowskiego St. 1A, Poland. E-mail: weidner@uwm.edu.pl

Some posttranslational processes that occur in embryos of germinating *Triticale caryopses* treated with different concentrations of abscisic acid (ABA) were examined. ABA increased the ratio of cytoskeleton-bound polysomes in the total population of polysomes and depressed the share of free and membrane-bound polysomes. Using exogenous RNase, stability of the total polysomal population as well as each polysomal fraction was investigated. The total extractable polysomes isolated from embryonic tissues of germinating *Triticale caryopses* treated with ABA were more stable than the polysomes isolated from the control sample caryopses. The contribution of the polysomes that were not digested by RNase was increased by higher concentrations of ABA applied during germination. At high concentrations of ABA, the quantitative contribution of polysomes in the total ribosomal fraction was almost 100% of the amount of polysomes before digestion and the modifications observed consisted mainly of the shift of the so-called heavy polysomes towards light polysomes, containing a few ribosomes. Within each polysomal population, cytoskeleton-bound polysomes (CBP and CMBP) were the most stable, which may imply that the bonds between polysomes and these protein filaments, created in all eukaryotic cells increased their stability.

PP-1082
Ouabain-sensitive colonic H/K-ATPase: isolation, purification and characterization

J. R. del Castillo
Instituto Venezolano de Investigaciones Científicas (IVIC), Centro de Biofísica y Bioquímica, Lab. Fisiología Gastrointestinal, Caracas, Venezuela. E-mail: jdelcas@ivic.ve

Distal colon absorbs K through a Na-independent, ouabain-sensitive H/K exchange, which has been associated to a K-ATPase located at the apical membrane of the colonocyte. A putative gene for this ATPase has been cloned and co-expressed with Na/K-ATPase β1 or gastric H/K-ATPase βg subunits. However, the native K-ATPase has not been isolated nor identified as an unique biochemical entity. Here, we describe a procedure to purify the ouabain-sensitive H/K-ATPase from guinea pig distal colon; how to preserve the enzymatic activity under freezing/defrosting conditions and the partial characterization of the purified enzyme. This ATPase is a heterodimer α/β of 100 and 50 KDa, respectively. The enzyme needs 10% DMSO to preserve its activity under freezing/defrosting conditions. The purified K-ATPase is Mg-dependent and preferentially hydrolyzes ATP. The enzyme is activated by K, Cs, and NH4 but is insensitive to Na and Li and independent of the K-accompanying anion. The pH optimum of the enzyme is 7.4. K-ATPase is inhibited by ouabain (IC50: 2.5 μM) and vanadate (IC50: 1.6 μM) but insensitive to SCH-28080 and bafilomycin-A. In the absence of potassium, the purified enzyme has an ouabain-sensitive activity, with an optimum pH of 6.6, suggesting that the enzyme could mediate a H/H exchange. The Na-independent, ouabain-sensitive K-ATPase of the apical membrane of colonocytes is an unique enzyme that could represent the biochemical entity of the colonic H/K-pump.

PP-1083
The epithelial tumor markers can be used in adenocarcinoma of esophagus but not in squamous cell carcinoma

A. Cebi-Ilhan[1] and **M. Ilhan**[2]
[1]Department of Medical Biology, [2]Department of Internal Medicine and Division of Medical Oncology. Yuzuncu Yil University, Faculty of Medicine. E-mail: cebiaysegul@hotmail.com

In this study, we aimed to detect the frequency of elevation CA 125, CEA, CA 199 and CA 153 in the different histologic type of esophageal cancer. The files of 238 esophageal cancer managed in the medical oncology policlinic of Yuzuncu Yil University, School of Medicine were screened for CA 125, CA 199, CEA and CA153 and levels of these markers were noted. Furthermore, the age, gender, histologic subtype and stage during diagnosis were noted. Finally, The elevation more than cut-off values of these markers were researched in the two different histologic subtype. The frequency of markers and importance of difference was calculated by SPSS 11.5 software. The median age 53, 58.8% of the patients female and 41.2% of the patients male were detected. 85.5% of patients had squamous cell carcinoma (SCC) and 14.5% adenocarcinoma (AC). The elevation more than cut-off values were detected to be respectively 61.5% and 10.4 for CA 199, 58.8% and 16.1 for CEA, 54.5% and 37.5 for CA 125 and 27.3% and 8.3 for CA 153 in AC and SCC cases. Although all of four tumor marker levels were increased in AC cases more than SCC cases, the difference between levels was found to be as statistical important only in CEA and CA 199. The epithelial tumor markers are more commonly secreted in AC cases when we compared with SCC cases. So, we can probably use these tumor markers to manage AC cases but value of these markers in SCC cases is low. The further studies are required.

PP-1084
Zinc status in infants with acute bronchiolitis

S. Hatipoglu[1], A. Kaya[2], V. Arıca[1], H. Dag[1], H. Balcı[2] and G. Karatekin[3]
[1]Dept. of Pediatrics, Bakirkoy Teaching and Research Hospital, Istanbul, Turkey, [2]Cardiology Institute, Biochemistry Laboratory, Istanbul University, Istanbul, Turkey, [3]Dept. of Neonatology, Sisli Etfal Teaching and Research Hospital, Istanbul, Turkey. E-mail: sahat55@gmail.com

Aim: Zinc is an essential micronutrient for human growth, development, and immune function. Zinc deficiency impairs overall immune function and resistance to infection. This study was done to investigate the association of zinc status in infants with acute bronchiolitis.
Material and methods: In this study, estimation of serum zinc was performed in 25 infants with acute bronchiolitis who admitted to hospital and 10 healthy infants, of age 2–18 months.
Results: The birth weight ($P = 0.106$), birth gestational age ($P = 0.278$) and admitted age to hospital ($P = 0.840$) were similar between the acute bronchiolitis and control group. The infants with acute bronchiolitis had a mean plasma zinc level

significantly lower than that of healthy children group (26.41 ± 29.95 micromol/dl vs. 54.96 ± 29.99 micromol/dl, ($P = 0.004$).

Conclusion: We concluded that Turkish infants suffering from bronshioloitis have decreased serum Zn levels. The deficiency of Zn was correlated to improper nutritional management. Therefore; zinc supplementation can be useful for the bronshiolitis infants. Further research should be conducted to determine the long-term developmental importance of these differences with zinc supplementation.