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Effect of angiotensin II type 1 receptor blocker on osteoporotic rat femurs

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Abstract:
Background: Osteoblasts and osteoclasts are known to express Ang II type I (AT1) receptor in cell cultures, suggesting the existence of local renin-angiotensin system (RAS) in bone. This study was designed to investigate the effects of losartan as AT1 receptor blocker on ovariectomized rats' femur.
Methods: Losartan (5 mg/kg/day) was administered *via* oral gavage for 8 weeks. Bone mineral density (BMD) was measured using dual energy X-ray absorptiometry, while tensile and three-point bending tests were performed for evaluation of biomechanical properties of bone. The trabecular porosity was analyzed by scanning electron microscopy.
Results: There was a significant decrease in BMD values of ovariectomized rats' femurs which were reversed by losartan treatment. According to tensile test results, ultimate tensile strength and strain values of losartan treated ovariectomized rats' femurs increased and decreased, respectively, when compared to that of ovariectomized animals. Losartan treatment also caused a significant recovery in flexural strength and modulus parameters regarding respective control values, which mean losartan treated ovariectomized rats' femur had more force tolerance until break than ovariectomized rats' femur. Quantitative microscopic analysis showed larger trabecular porosity in ovariectomized rats than control rat femurs and it was significantly decreased after losartan treatment.
Conclusion: Blockage of AT1 receptor increased strength, mass and trabecular connections of ovariectomized rat femurs. Therefore, it is tempting to speculate that drugs, including AT1 receptor blockers, may be used for the treatment of osteoporosis or reduction of its detrimental effects by in the future.

Key words:
osteoporosis, biomechanics, bone, angiotensin, ovariectomy

Abbreviations: ACE – angiotensin converting enzyme, Ang II – angiotensin II, AT1 – angiotensin II type 1, AT2 – angiotensin II type 2, BMD – bone mineral density, LOS – losartan, OVX – ovariectomy, RAS – renin-angiotensin system, ROI – region of interest, SEM – scanning electron microscopy

Introduction

Osteoporosis is a major global health problem especially for women [12, 40]. It is a systemic skeletal disease that is characterized by low bone mass and microarchitectural deterioration of bone tissue with an associated increase in fragility and susceptibility to fractures [7, 10, 16, 20, 25, 31, 33, 36, 44]. Despite some current therapeutic interventions, the treatment of fractures associated with osteoporosis has not been adequately addressed. Additionally, 20% of osteoporotic patients die in the first year after fracture due to long term hospitalization [4, 8, 12].

Recent clinical studies indicate that β blockers and antihypertensive drugs reduce the risk of bone fractures in the elderly populations [24, 30, 43]. The renin-angiotensin system (RAS) is found not only systemically but also locally in several tissues, and has also been studied in bone microenvironments [24, 27, 35]. Recent findings showed RAS to play an important role in bone tissue suggesting a detrimental effect of angiotensin II (Ang II) in bone [43]. This has led to the theory that angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers may have an indirect effect on bone mineral density (BMD) and fracture risk [43]. Moreover, osteoblasts and osteoclasts are shown to express Ang II type I (AT1) and type II (AT2) receptor in cell cultures [21, 23, 24, 37], suggesting the existence of local RAS in bone. Ang II has been suggested to promote bone resorption *via* AT1 or AT2 receptors [24, 37] and blockage of either of these receptors was proposed to inhibit differentiation and bone formation in cell culture and in ovariectomized animal models [18, 24]. However, current findings are still controversial and no significant effect was observed by the AT2 receptor inhibitors in rat calvarial osteoblasts [18] or in the co-cultures of human osteoblast and osteoclast precursor cells [24, 37]. Despite these contradictory results about the receptor it activates, the importance and modulatory effect of Ang II in osteoblastic bone formation and osteoclast-mediated bone resorption is highly significant, and needs further investigation [21, 23, 26].

Therefore, our study was designed to investigate the effects of losartan, an AT1 receptor blocker, on bone mass and strength of ovariectomized rats. Both tensile and three-point bending tests were performed and thus detailed mechanical and structural parameters evaluated, comparatively. Despite the above studies reporting RAS functions in bone formation, none of these treatments were applied after osteoporosis development. For this purpose, losartan administration was started at the 12th week after ovariectomy and was applied for the following 8 weeks. Consequently, this is the first study that focuses on the recovery of damage after a 3 month period that proves the appearance of osteoporotic complications. Our results showed that losartan treatment can improve ovariectomy-induced bone deformations.

Materials and Methods

Animals

Seventy-five female Wistar rats, 3 months old and 250–300 g body weight (Akdeniz University, Faculty of Medicine, Animal Laboratory, Antalya, Turkey) were used. The animals were exposed to a 12-h light and 12-h dark cycle at 22°C room temperature. The animals had access to standard laboratory chow and water *ad libitum*. Experimental procedures were approved and carried out in accordance with Akdeniz University Animal Care and Use Committee's guidelines.

The animals were divided into five groups: Control (n = 15) (CONT), sham operated (n = 15) (SHAM), losartan-treated control (n = 15) (CONT + LOS), ovariectomized (n = 15) (OVX), losartan-treated ovariectomized (n = 15) (OVX + LOS).

Thirty rats in OVX and OVX + LOS groups underwent bilateral ovariectomy after being anesthetized with ketamine (Ketalar, Pfizer-Eczacibasi Inc., Turkey) and xylazine (Alfazyne, Egevet Inc., Turkey). Ventral incision was carried out, and ovaries were removed after the ligation of the uterine horn. Losartan (Losartil, Drogsan Co., Ankara, Turkey) (5 mg/kg/day) was dissolved in water and administered *via* oral gavages after 12 weeks of ovariectomy induction and repeated for 8 weeks (CONT + LOS and OVX + LOS). The same amount of vehicle was administered to the age-matched groups; CONT, SHAM, and OVX,

via oral gavages for the same period. All animals were sacrificed by overdose of urethane anesthesia at the end of the 20th week. Femurs were collected for biomechanical evaluations, histomorphologic and bone mineral density (BMD) measurements.

BMD measurement

Femur BMD was measured using dual energy X-ray absorptiometry (Norland XR 46, Norland, USA) with a scan speed of 1 mm/s and a resolution of 0.5 × 0.5 mm. Before the measurements, the instrument was calibrated by means of a Norland phantom and the BMD was determined by the analysis of the femoral shaft.

Biomechanical tests

The biomechanical properties of bone were measured using a tensile test and three-point-bending test. Twelve and eleven femurs from each group were used for tensile test and three-point bending test, respectively. Both tests were performed using a computerized Shimadzu Autograph universal testing machine (AG-G series; Shimadzu Co., Kyoto, Japan). The biomechanical tests were conducted by a 5-kN load cell and at a crosshead speed of 2 mm/min at room temperature. Following removal of the soft tissues around the femur, the bones were wrapped in gauze soaked in isotonic saline, and frozen at –20°C until testing. Four hours prior to mechanical testing, the bones were thawed at room temperature.

Tensile testing methods

The femur was mounted vertically in the machine with the use of acrylic cement. During testing, isotonic solution was regularly applied to prevent the bones from drying. Tensile test was applied until a fracture occurred. System control and data analysis were performed using Trapezium software (Shimadzu Co., Kyoto, Japan). Typical force-displacement and

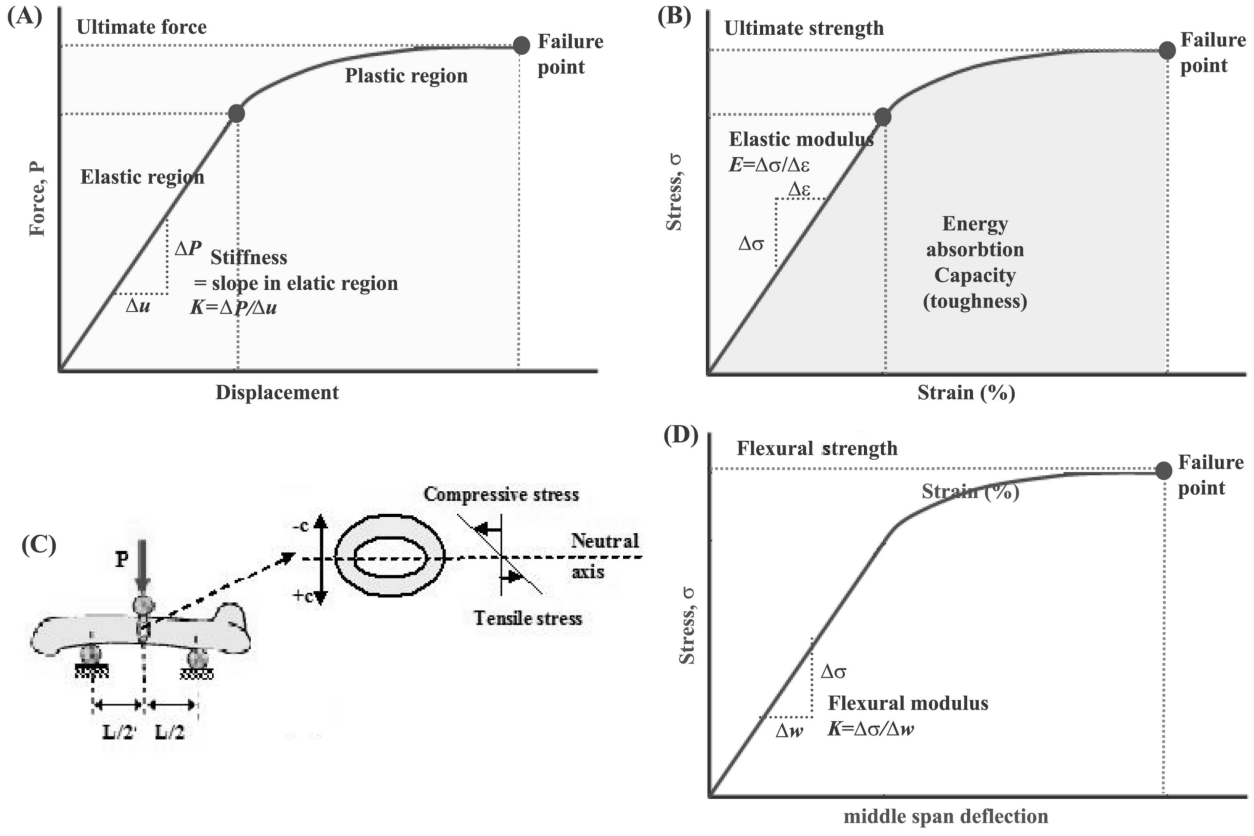

Fig. 1. (**A**) Force-displacement curve. (**B**) Stress-strain curve for bone. (**C**) Three point bending test fixture. (**D**) Three-point bending test (Bending causes tensile and compressive stresses. The value of stress is greatest at the surface of the bone and is zero at the neutral axis)

stress-strain curves obtained from the tensile test are shown in Figure 1A and B. With the tensile test, ultimate tensile load, ultimate tensile strength, strain, stiffness, tensile modulus (Young's modulus) and energy absorption capacity (toughness) were measured. After fracture, the thinnest region of the femoral shaft was cut horizontally and pictured. Cortical surface areas were calculated from the pictures as square millimeters by using Image-J Software (US National Institutes of Health, 2008, Bethesda, Maryland, USA).

Stiffness (*K,* N/mm) is determined from the slope of the elastic region of the load-displacement curve (Fig. 1A) which represents the extrinsic stiffness by means of the following equation; $K = \Delta P/\Delta u$. The ultimate stress was calculated from the equation: $\sigma = P/A$ where σ is the ultimate stress (MPa), P is the failure load (N) and A is the cortical area (mm^2). The tensile or Young's modulus is a measure of the intrinsic stiffness of the material. The tensile modulus (E) can be calculated from the slope of stress-strain curve within the elastic region. The tensile modulus (MPa) was then calculated as follows: $E = \Delta\sigma/\Delta\varepsilon$ where ε is the strain. The strain (% displacement) was obtained with the following equation; $\varepsilon = \Delta L/L_0$ where ΔL is the change in the length and L_0 is the original length. Energy absorption capacity (toughness) is a measure of the amount of energy needed to cause material failure and is determined by the area under the stress-strain curve (Fig. 1B).

Three-point bending test methods

The flexural strength and modulus were analyzed by a three-point bending test on the entire femur. A sliding roller three-point bending fixture with a loading pin (diameter 6.4 mm) and two supporting pins (diameter 3.2 mm) were used for three-point bending test. The distance between the two supports was 18 mm. The loading pin compressed the middle of the tibia shaft (Fig. 1C). The bones were subjected to three-point bending on a universal testing machine until fracture occurred. Load-displacement curves were recorded using advanced software (Trapezium software). Typical force–middle span deflection curves obtained from the flexure tests are shown in Figure 1D. After fracture, the thinnest region of the femoral shaft was cut horizontally and pictured. All pictures were analyzed, and then outer diameter and inner diameter were measured in millimeters, whereas cross sectional moment of inertia was calculated in mm^4 by using image J.

Flexural strength is derived from the simple beam theory as follows; $\sigma_f = PLc/4I$ where σ_f denotes the flexural strength; P is the applied load that leads the specimen to rupture; L is the support span; I is cross sectional moment of inertia and c is a distance from cross sectional center of mass (neutral axis).

Flexural modulus may be determined from the slope of the initial straight-line part of the load-middle span deflection curve by means of this equation; $E_f = (L^3/48I)(\Delta P/\Delta\delta)$ where $\Delta P/\Delta\delta$ represents the slope of the force-middle span deflection curve; and E_f is the flexural modulus.

Histological procedures

Seven femurs from each group were used for scanning electron microscopy (SEM) analysis. For SEM evaluation, femurs from all groups were cut vertically from the proximal metaphysis area. The specimens were immersed in 1% Triton-X-100 for 20 min at room temperature in an ultrasonic cleaner (Bandelin Sonorex RK156, Berlin, Germany) in order to remove soft tissues. All specimens were fixed with 2.5% glutaraldehyde solution, then washed with 0.1 M Sorensen's phosphate buffer at 4°C for two hours, and post-fixed with 1% osmium tetraoxide. After dehydrating within ascending alcohol gradients, SEM protocol was performed. After dehydrating with isoamyl acetate again, the specimen was dried using a critical point dryer. All specimens were analyzed under a scanning electron microscope after being coated with a layer of gold/palladium. The surface morphology of trabecular bone (above neck of the femur) specimens was examined using a SEM (Zeiss, Leo 1430 SE, Oberkochen, Germany) operated at 15000 kV. The microscopic images were analyzed to determine total trabecular area, and trabecular porosity in square millimeters by defining regions of interest (ROIs) in the image analysis software (ImageJ). Obtained data were given as percentages of trabecular porosity in trabecular bone.

Statistical analysis

Statistical analysis was performed by using SIGMA-STAT 3.0. For BMD and biomechanical data, one-way ANOVA test and Bonferroni test as a *post-hoc* test were used. For histomorphological data, Kruskal-

Wallis test and Dunn *post-hoc* test were used. The statistical significance level for all comparisons was set as $p < 0.05$.

Results

BMD values were significantly decreased in OVX ($p < 0.005$) compared to CONT group (Tab. 1 and Fig. 2A) confirming a decrease in the amount of bone in OVX rats. Moreover, we observed a pronounced and significant increase in BMD values of OVX + LOS group compared to OVX group ($p < 0.005$). However, the recovery did not reach the level of CONT group. Interestingly, a decreased BMD in LOS-treated control rats (CONT + LOS group) was seen compared to CONT group.

Biomechanical test results

Tensile test results

The ultimate tensile strength decreased significantly, while the strain increased in OVX group compared to that in CONT group ($p < 0.005$). In contrast, treatment of OVX rats with losartan caused an increase in the ultimate tensile strength value and a decrease in strain values compared to those in OVX group ($p < 0.005$) (Fig. 2B and E).

The mean stiffness of OVX group was lower than that of CONT group, corresponding to 24% decrease ($p < 0.005$) with respect to CONT group values (Fig. 2C). However, the losartan treatment reversed the effect of ovariectomy by increasing the stiffness to the level observed in CONT rats ($p < 0.005$). Similarly, a significant decrease in Young's modulus and EAC values of OVX group were observed compared to that of CONT group (Fig. 2F). Yet, losartan treatment elevated those values to the level of bones obtained from CONT rats ($p < 0.005$).

Three point bending test results

The flexural strength and modulus values of OVX group were significantly decreased compared to that of CONT groups ($p < 0.005$). However, losartan treatment revealed a pronounced recovery by increasing both parameters regarding OVX group.

Histomorphometric results

In the OVX rats, the percentage of the trabecular porosity was significantly increased compared to that of femurs in CONT group rats ($p < 0.005$). Percentage of the trabecular porosity in the OVX + LOS rat femurs was significantly decreased compared to OVX

Tab. 1. Bone mineral density, biomechanical and scanning electron microscopy parameters of each group

	CONT	SHAM	CONT-LOS	OVX	OVX-LOS
BMD (g/cm^2)	0.1331 ± 0.0012	0.1301 ± 0.0023	0.1266 ± 0.0019*	0.1150 ± 0.0008*	0.1215 ± 0.0011‡
Tensile test					
Tensile strength (MPa)	64.45 ± 6.89	58.89 ± 3.15	60.45 ± 5.26	48.12 ± 3.82*	64.90 ± 3.84 ‡
Tensile strain (%)	0.15 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.22 ± 0.02*	0.17 ± 0.01‡
E.A.C. (mJ)	634.71 ± 27.43	612.72 ± 12.92	597.49 ± 11.76	410.64 ± 9.57*	604.19 ± 14.11‡
Tensile modulus (MPa)	86.34 ± 2.07	86.77 ± 2.37	83.45 ± 2.50	62.86±2.16*	83.22 ± 2.09‡
Tensile stifness (N/mm)	180.38 ± 5.05	180.19 ± 5.20	181.11 ± 3.89	137.49 ± 3.33*	180.44 ± 3.33‡
Three point bending test					
Flexural strength (MPa)	218.64 ± 5.27	207.45 ± 3.34	201.80 ± 4.50	168.12 ± 3.38*	205.95 ± 5.69‡
Flexural modulus (GPa)	9.71 ± 0.46	10.26 ± 0.48	9.46 ± 0.45	6.33 ± 0.25*	10.50 ± 0.45‡
SEM					
% trabecular porosity	9.20 ± 0.28	10.80 ± 0.36	11.19 ± 0.33	16.48 ± 0.78*	10.73 ± 0.34‡

Values are given as the means ± SEM; * $p < 0.005$ *vs.* CONT and ‡ $p < 0.005$ *vs.* OVX

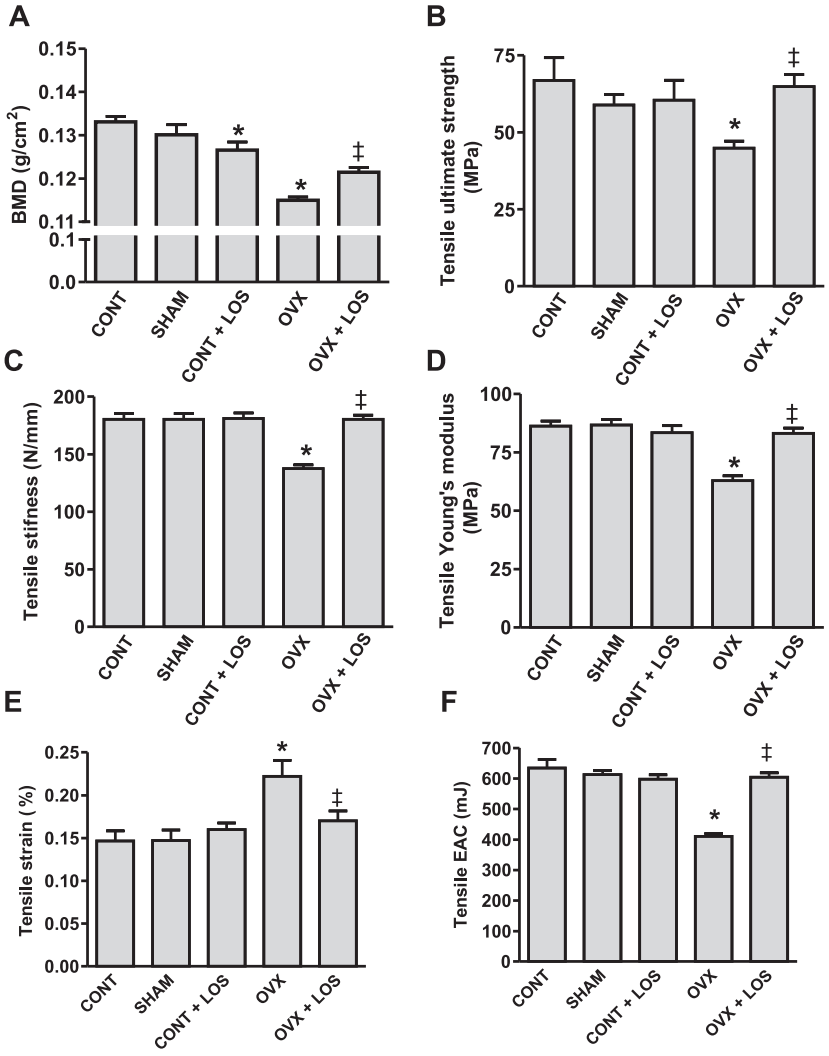

Fig. 2. Effects of losartan on BMD and tensile test in OVX rats. BMD values were significantly lowered in OVX group compared to CONT group and losartan treatment reversed this decrement (**A**). Ovariectomy disrupted ultimate strength (**B**), stifness (**C**), Young's modulus (**D**), strain (**E**), energy absorption capacity (EAC) (**F**) while losartan ameliorated all biomechanical parameters. * $p < 0.005$ *vs.* CONT group and ‡ $p < 0.005$ *vs.* OVX group

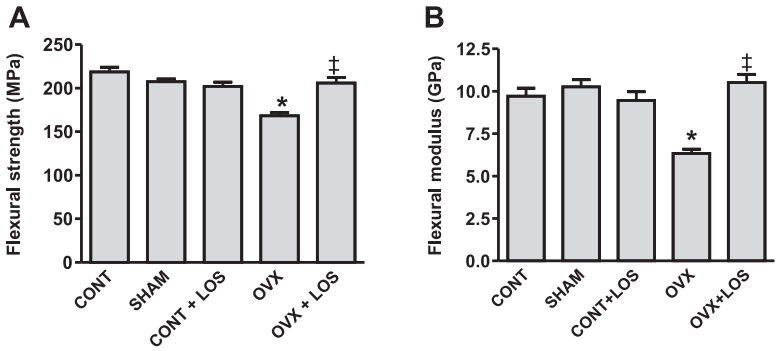

Fig. 3. Three points bending test results of all groups. Flexural strength (**A**) and flexural modulus (**B**) of OVX rat femurs are smaller than CONT. Losartan treatment (5 mg/kg/day) for 8 weeks returned biomechanical parameters to CONT group level. * $p < 0.005$ *vs.* CONT group and ‡ $p < 0.005$ *vs.* OVX group

rat femurs ($p < 0.005$). Because of decreased bone mass, the ratio of the porosity of trabecular bone resulted in an increase in OVX group while it was decreased in OVX + LOS group. Figure 4 A, B, C, D and E shows SEM images of trabecular porosity in all groups. In OVX group (Fig. 4D) decreased trabecular

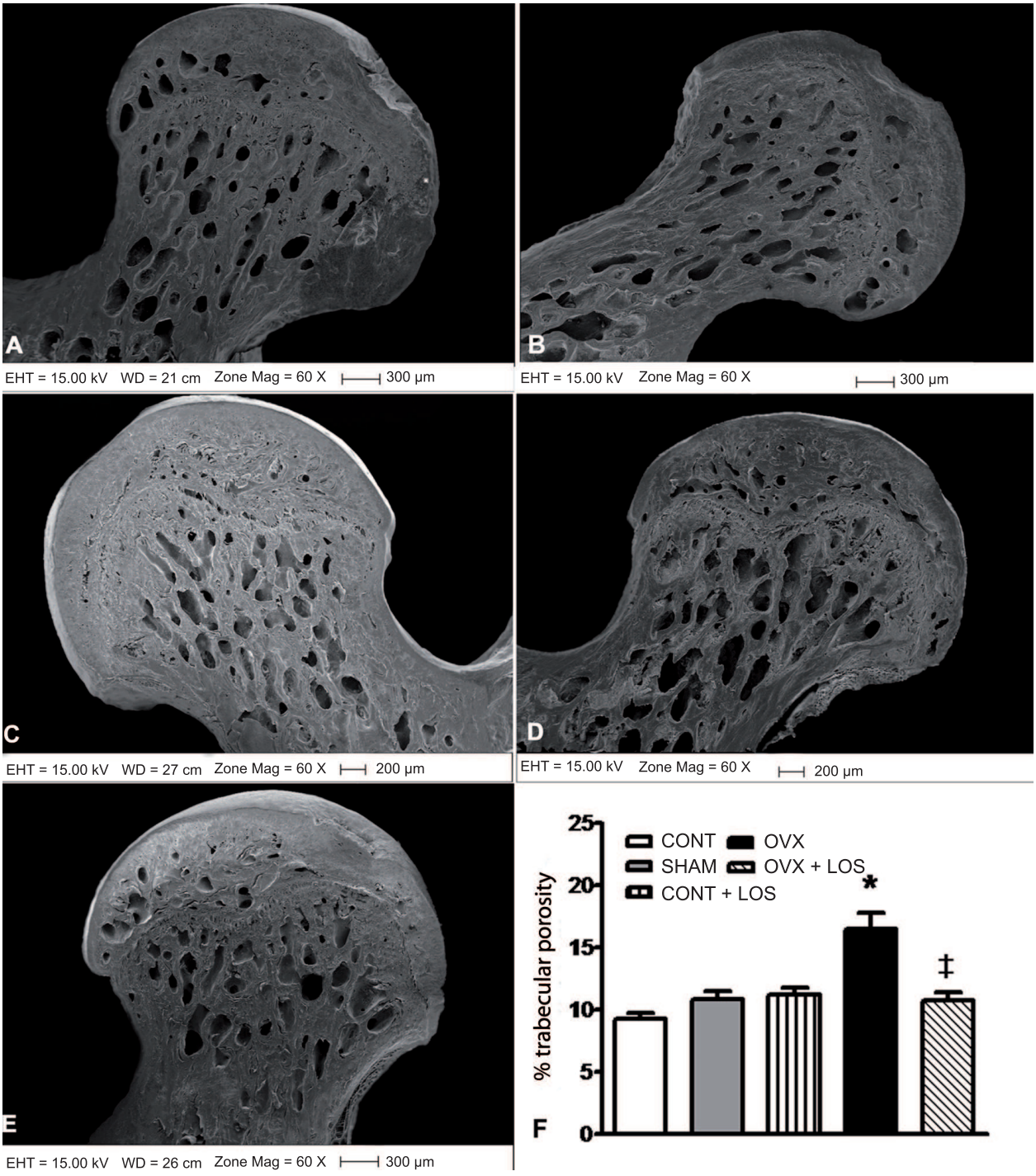

Fig. 4. Scanning electron microscopy showing trabecular bone aspects of femoral head. CONT (**A**), SHAM (**B**) and CONT-LOS (**C**) presented similar cancellous bone pattern with numerous connections but cancellous bone showing trabecular disconnection in OVX (**D**). Losartan treatment to ovariectomized animals (**E**) improved trabecular connection. Percentages of the trabecular porosity of bone from all groups are analyzed and presented in (**F**). * $p < 0.005$ *vs.* CONT group and ‡ $p < 0.005$ *vs.* OVX group

connection leads to an increase in total area of porosity compared to that in CONT group, but in OVX + LOS group (Fig. 4E) trabecular connection and porosity was compatible with the CONT groups. Similarly, trabecular connection and trabecular porosity of SHAM (Fig. 4B) and CONT + LOS (Fig. 4C) groups were not different that in CONT group (Fig. 4A).

Discussion

In the present study we confirmed previous studies [1, 3, 7, 17, 19, 34, 38] that have shown that ovariectomy can cause biomechanical, histomorphologic and mineralization defects in bones such as the femur and lumbar vertebrae. Briefly, the values of ultimate tensile strength, Young's modulus, stiffness and energy absorption capacity of OVX + LOS animals was higher than those of OVX animals. Conversely, the strain value of OVX + LOS group was lower than the strain value of OVX group, which suggests that losartan treatment caused less compliant and stronger femurs reflected by decreasing strain and increasing Young's modulus. For the same reason, OVX group was more ductile than other groups considering the increased Young's modulus value. Three-point bending test results were also consistent with tensile parameters and showed recovery of bone strength with losartan treatment in ovariectomized rats.

Bone tissue properties such as ultimate stress, strain and elastic modulus are called intrinsic biomechanical properties [41, 42]. Accordingly, an effective treatment of bone fragility should improve the extrinsic biomechanical properties of bone, but at the same time not substantially impair the intrinsic properties. Additionally, an ideal drug to heal bone fragility would improve strength and decrease brittleness [41]. In the present study, our biomechanical results revealed that losartan and thus, AT1 receptor blockage recovered the structural properties of bone and did not impair the material properties.

Increased fracture risk and altered mechanical properties of bone are generally associated with the reduced BMD found in osteoporotic bone [6, 11, 12, 14, 22, 32, 40]. Moreover, it is well known that ovariectomy stimulates bone remodeling and bone loss in rats which manifests itself with reduced BMD [15]. Consistent with this findings, reduced bone mass in OVX rats and significant recovery after losartan treatment, led us to suggest that AT1 receptor blockage preserves bone mass. In the present study, the effect of the AT1 receptor blocker losartan on biomechanical properties of bone and their relevance to the amount of bone is demonstrated comprehensively. It has been reported that enhanced bone mineralization affects biomechanical properties of bone dramatically, which manifested itself by increased stiffness and decreased ultimate displacement [9, 41]. According to our results, losartan treatment increases BMD and stiffness along with a decrease in the strain in OVX rats. Conversely, losartan revealed a significant decrease in BMD in control rats, although it was not transmitted to mechanical parameters. This is due to the fact that basal activity of RAS is essential for bone metabolism and downregulation of RAS beyond basal level can deteriorate bone turnover.

Ang II type 1 (AT1) receptor blockage increases bone mass and biomechanical parameters, although the ratio of the trabecular porosity in bone decreases. Bone remodeling is mediated by the balanced activities of osteoclasts, which resorb existing bone, and of osteoblasts, which form new bone. Because of estrogen deficiency in OVX rats, ovariectomy resulted in the removal of whole bone structures. The studies by Erben et al. [13] showed that cancellous bone mass reduced both proximal tibia and vertebrae in OVX rats. Additionally, they revealed that the number of osteoclast increased in OVX rats [13]. Martel et al. [31] showed 77% decrease in the ratio of trabecular bone volume of tibia in OVX rat comparing with healthy rat. In our study, the significant increase in the ratio of porosity in OVX group compared to CONT group can be attributed to osteoporosis-dependent increase in the deformation of trabecular bone. However, the significant decrease in the ratio of porosity in the losartan treated OVX group suggests decreased bone resorption or promoted bone formation. These findings implicate a therapeutic role for RAS inhibition (specifically AT1) in osteoporosis-induced bone deformation.

Shimizu et al. [37] succeeded in prevention of osteoporosis both by deletion and blockage of the AT1 receptor with olmesartan, which is consistent with our findings. Moreover, Ang II administration to the animals subjected to OVX suppressed bone mass level dramatically, which was suggested to act through AT1 but not AT2 receptor. Additionally, Hatton et al. [21] found angiotensin-dependent stimulation of bone resorption in co-cultures of osteoclasts with osteoblastic

cells and they showed that both Ang I and Ang II are potent stimulators of osteoclastic bone resorption. On the other hand, Ang II administration to ovariectomized animals aggravates the deformation further [37], which is consistent with our findings that suggests detrimental effects of AT1 over-stimulation on bone tissue. Moreover, our results clearly supported that AT1 blockage increased bone mass, bone strength and decreased percentage of the trabecular porosity in bone. Taken together, there are several lines of evidence that emphasize the importance of AT1 in bone metabolism and it was suggested to become a novel therapeutic target for patients in bone diseases such as osteoporosis and bone fracture [2].

The most impressive point of our data is that losartan treatment 12 weeks after ovariectomy has shown a therapeutic effect on osteoporotic rat's femur including BMD and biomechanical properties. Despite these striking findings, the studies that have presented contradictory or unchanged results cannot be excluded [5, 24, 28]. The main reason of these controversial results is probably due to application of the blocker prior to osteoporosis development or to other critical differences in experimental procedure. Li et al. [28] had started treatment just after the ovariectomy induction, although losartan was administered after establishment of reduction in bone mass in our study. So, we can speculate that AT1 receptor blockage may correct long term alterations that emerged following development of osteoporotic state and enhanced bone loss. This is due to the fact that overactivation of the Ang II signaling pathway may only be accomplished at the late stage, where generation of osteoporotic changes reaches a critical level. Bone turnover rate could be another factor underlying this delay between ovariectomy and apparent bone loss. Estrogen has been reported to downregulate Ang II production and AT1 expression significantly and thus modulates its growth-promoting and therapeutic effects in vascular smooth muscle cells [29, 39]. Therefore, bone loss and altered bone mechanics of OVX rats can be attributed to upregulated Ang II and/or enhanced AT1 expression. Thus, it is tempting to speculate that losartan had therapeutic effects on osteoporotic bone in OVX rats due to recovery of those upregulated RAS elements.

In conclusion, losartan increased femur strength according to tensile and three-point bending tests in osteoporosis. Losartan-induced increase in bone mass and trabecular connections could make the bone stronger in osteoporotic rats. Therefore, as an AT1 blocker, losartan may be used for treatment and/or recovery of detrimental effects of osteoporosis.

Disclosure of financial conflicts of interest:
None.

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References:

1. Bagi CM, Mecham M, Weiss J, Miller SC: Comparative morphometric changes in rat cortical bone following ovariectomy and/or immobilization. Bone, 1993, 14, 877–883.
2. Bandow K, Nishikawa Y, Ohnishi T, Kakimoto K, Soejima K, Iwabuchi S, Kuroe K et al.: Low-intensity pulsed ultrasound (LIPUS) induces RANKL, MCP-1, and MIP-1beta expression in osteoblasts through the angiotensin II type 1 receptor. J Cell Physiol, 2007, 211, 392–398.
3. Bonnet N, Benhamou CL, Malaval L, Goncalves C, Vico L, Eder V, Pichon C et al.: Low dose beta-blocker prevents ovariectomy-induced bone loss in rats without affecting heart functions. J Cell Physiol, 2008, 217, 819–827.
4. Boyle P, Leon ME, Autier P: Epidemiology of osteoporosis. J Epidemiol Biostat, 2001, 6, 185–192.
5. Broulik PD, Tesar V, Zima T, Jirsa M: Impact of antihypertensive therapy on the skeleton: effects of enalapril and AT1 receptor antagonist losartan in female rats. Physiol Res, 2001, 50, 353–358.
6. Burr DB: Osteoporosis and fracture risk: bone matrix quality. J Musculoskelet Neuronal Interact, 2002, 2, 525–526.
7. Comelekoglu U, Bagis S, Yalin S, Ogenler O, Yildiz A, Sahin NO, Oguz I et al.: Biomechanical evaluation in osteoporosis: ovariectomized rat model. Clin Rheumatol, 2007, 26, 380–384.
8. Cummings SR, Black DM, Rubin SM: Lifetime risks of hip, Colles', or vertebral fracture and coronary heart disease among white postmenopausal women. Arch Intern Med, 1989, 149, 2445–2448.
9. Currey JD: Physical characteristics affecting the tensile failure properties of compact bone. J Biomech, 1990, 23, 837–844.
10. Duarte PM, Goncalves PF, Sallum AW, Sallum EA, Casati MZ, Humberto Nociti F, Jr.: Effect of an estrogen-deficient state and its therapy on bone loss resulting from an experimental periodontitis in rats. J Periodontal Res, 2004, 39, 107–110.
11. Eastell R: Treatment of postmenopausal osteoporosis. N Engl J Med, 1998, 338, 736–746.

12. Egermann M, Goldhahn J, Schneider E: Animal models for fracture treatment in osteoporosis. Osteoporos Int, 2005, 16, Suppl 2, S129–S138.
13. Erben RG, Bromm S, Stangassinger M: Short-term prophylaxis against estrogen depletion-induced bone loss with calcitriol does not provide long-term beneficial effects on cancellous bone mass or structure in ovariectomized rats. Osteoporos Int, 1998, 8, 82–91.
14. Faulkner KG: Bone matters: are density increases necessary to reduce fracture risk? J Bone Miner Res, 2000, 15, 183–187.
15. Flieger J, Karachalios T, Khaldi L, Raptou P, Lyritis G: Mechanical stimulation in the form of vibration prevents postmenopausal bone loss in ovariectomized rats. Calcif Tissue Int, 1998, 63, 510–514.
16. Folwarczna J, Zych M, Trzeciak HI: Effects of curcumin on the skeletal system in rats. Pharmacol Rep, 2010, 62, 900–909.
17. Fox J, Miller MA, Newman MK, Metcalfe AF, Turner CH, Recker RR, Smith SY: Daily treatment of aged ovariectomized rats with human parathyroid hormone (1-84) for 12 months reverses bone loss and enhances trabecular and cortical bone strength. Calcif Tissue Int, 2006, 79, 262–272.
18. Hagiwara H, Hiruma Y, Inoue A, Yamaguchi A, Hirose S: Deceleration by angiotensin II of the differentiation and bone formation of rat calvarial osteoblastic cells. J Endocrinol, 1998, 156, 543–550.
19. Han SM, Szarzanowicz TE, Ziv I: Effect of ovariectomy and calcium deficiency on the ultrasound velocity, mineral density and strength in the rat femur. Clin Biomech (Bristol, Avon), 1998, 13, 480–484.
20. Hart KJ, Shaw JM, Vajda E, Hegsted M, Miller SC: Swim-trained rats have greater bone mass, density, strength, and dynamics. J Appl Physiol, 2001, 91, 1663–1668.
21. Hatton R, Stimpel M, Chambers TJ: Angiotensin II is generated from angiotensin I by bone cells and stimulates osteoclastic bone resorption in vitro. J Endocrinol, 1997, 152, 5–10.
22. Hayirlioglu A, Gokaslan H, Andac N: The effect of bilateral oophorectomy on bone mineral density. Rheumatol Int, 2006, 26, 1073–1077.
23. Hiruma Y, Inoue A, Hirose S, Hagiwara H: Angiotensin II stimulates the proliferation of osteoblast-rich populations of cells from rat calvariae. Biochem Biophys Res Commun, 1997, 230, 176–178.
24. Izu Y, Mizoguchi F, Kawamata A, Hayata T, Nakamoto T, Nakashima K, Inagami T et al.: Angiotensin II type 2 receptor blockade increases bone mass. J Biol Chem, 2009, 284, 4857–4864.
25. Kaczmarczyk-Sedlak I, Zych M, Rotko K, Sedlak L: Effects of thalidomide on the development of bone damage caused by prednisolone in rats. Pharmacol Rep, 2012, 64, 386–395.
26. Lamparter S, Kling L, Schrader M, Ziegler R, Pfeilschifter J: Effects of angiotensin II on bone cells in vitro. J Cell Physiol, 1998, 175, 89–98.
27. Lavoie JL, Sigmund CD: Minireview: overview of the renin-angiotensin system – an endocrine and paracrine system. Endocrinology, 2003, 144, 2179–2183.
28. Li YQ, Ji H, Shen Y, Ding LJ, Zhuang P, Yang YL, Huang QJ: Chronic treatment with angiotensin AT1 receptor antagonists reduced serum but not bone TGF-beta1 levels in ovariectomized rats. Can J Physiol Pharmacol, 2009, 87, 51–55.
29. Liu HW, Iwai M, Takeda-Matsubara Y, Wu L, Li JM, Okumura M, Cui TX et al.: Effect of estrogen and AT1 receptor blocker on neointima formation. Hypertension, 2002, 40, 451–457; discussion 48–50.
30. Lynn H, Kwok T, Wong SY, Woo J, Leung PC: Angiotensin converting enzyme inhibitor use is associated with higher bone mineral density in elderly Chinese. Bone, 2006, 38, 584–588.
31. Martel C, Picard S, Richard V, Belanger A, Labrie C, Labrie F: Prevention of bone loss by EM-800 and raloxifene in the ovariectomized rat. J Steroid Biochem Mol Biol, 2000, 74, 45–56.
32. Meunier PJ: Evidence-based medicine and osteoporosis: a comparison of fracture risk reduction data from osteoporosis randomised clinical trials. Int J Clin Pract, 1999, 53, 122–129.
33. Osterman T, Lauren L, Kuurtamo P, Hannuniemi R, Isaksson P, Kippo K, Peng Z et al.: The effect of orally administered clodronate on bone mineral density and bone geometry in ovariectomized rats. J Pharmacol Exp Ther, 1998, 284, 312–316.
34. Otomo H, Sakai A, Ikeda S, Tanaka S, Ito M, Phipps RJ, Nakamura T: Regulation of mineral-to-matrix ratio of lumbar trabecular bone in ovariectomized rats treated with risedronate in combination with or without vitamin K2. J Bone Miner Metab, 2004, 22, 404–414.
35. Sakai K, Agassandian K, Morimoto S, Sinnayah P, Cassell MD, Davisson RL, Sigmund CD: Local production of angiotensin II in the subfornical organ causes elevated drinking. J Clin Invest, 2007, 117, 1088–1095.
36. Sastry TP, Chandrsekaran A, Sundaraseelan J, Ramasastry M, Sreedhar R: Comparative study of some physicochemical characteristics of osteoporotic and normal human femur heads. Clin Biochem, 2007, 40, 907–912.
37. Shimizu H, Nakagami H, Osako MK, Hanayama R, Kunugiza Y, Kizawa T, Tomita T et al.: Angiotensin II accelerates osteoporosis by activating osteoclasts. Faseb J, 2008, 22, 2465–2475.
38. Shiraishi A, Miyabe S, Nakano T, Umakoshi Y, Ito M, Mihara M: The combination therapy with alfacalcidol and risedronate improves the mechanical property in lumbar spine by affecting the material properties in an ovariectomized rat model of osteoporosis. BMC Musculoskelet Disord, 2009, 10, 66.
39. Takeda-Matsubara Y, Nakagami H, Iwai M, Cui TX, Shiuchi T, Akishita M, Nahmias C et al.: Estrogen activates phosphatases and antagonizes growth-promoting effect of angiotensin II. Hypertension, 2002, 39, 41–45.
40. Taylor BC, Schreiner PJ, Stone KL, Fink HA, Cummings SR, Nevitt MC, Bowman PJ et al.: Long-term prediction of incident hip fracture risk in elderly white women: study of osteoporotic fractures. J Am Geriatr Soc, 2004, 52, 1479–1486.
41. Turner CH: Biomechanics of bone: determinants of skeletal fragility and bone quality. Osteoporos Int, 2002, 13, 97–104.

42. Turner CH, Rho J, Takano Y, Tsui TY, Pharr GM: The elastic properties of trabecular and cortical bone tissues are similar: results from two microscopic measurement techniques. J Biomech, 1999, 32, 437–441.
43. Wiens M, Etminan M, Gill SS, Takkouche B: Effects of antihypertensive drug treatments on fracture outcomes: a meta-analysis of observational studies. J Intern Med, 2006, 260, 350–362.
44. Yalin S, Sagir O, Comelekoglu U, Berkoz M, Eroglu P: Strontium ranelate treatment improves oxidative damage in osteoporotic rat model. Pharmacol Rep, 2012, 64, 396–402.

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