Journal of Investigative Surgery, 19:19–30, 2006

ISSN: 0894-1939 print / 1521-0553 online
DOI: 10.1080/08941930500444396

Taylor & Francis
Taylor & Francis Group

Original Research

The Effect of Aminoguanidine on Blood and Tissue Lipid Peroxidation in Jaundiced Rats With Endotoxemia Induced With LPS

Zekai Ogetman, MD
Musa Dirlik, MD,
Mehmet Caglikulekci, MD
and Hakan Canbaz, MD
Department of General Surgery, Mersin University Medical School, Mersin, Turkey

Tugba Karabacak, MD
Department of Pathology, Mersin University Medical School, Mersin, Turkey

Faik Yaylak, MD
Department of General Surgery, Mersin University Medical School, Mersin, Turkey

Lulufer Tamer, MD
Department of Biochemistry, Mersin University Medical School, Mersin, Turkey

Arzu Kanik, PhD
Department of Biostatistics, Mersin University Medical School, Mersin, Turkey

Suha Aydin, MD
Department of General Surgery, Mersin University Medical School, Mersin, Turkey

Received 7 April 2005;
accepted 8 August 2005.

Address correspondence to Musa Dirlik, MD,Mersin University Faculty of Medicine General Surgery Department, Zeytinlibahçe Caddesi, Eski Otogar Yani, 33079, Mersin, Turkey. E-mail: musa_dirlik@yahoo.com

ABSTRACT Obstructive jaundice (OJ) is a severe condition that leads to several complications. One of the important problems in OJ is the increased incidence of endotoxemia, which is the result of bacterial translocation (BT) and defective host immune response. Lipid peroxidation (LP) is an important problem in OJ and sepsis in which nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) activity are increased and antioxidative activity is decreased. Formation of peroxynitrite ($ONOO^-$) anion leads to cellular damage and apoptosis. In this experimental study, we explore the effect of specific iNOS inhibitor aminoguanidine (AG) on blood and tissue (liver and renal) LP and iNOS levels in jaundiced rats with endotoxemia induced with lipopolysaccharide (LPS). Rats were randomized into six groups; group A, sham; group B, obstructive jaundice (OJ); group C, OJ + LPS; group D, OJ + AG; group E, OJ + LPS + AG; group F, OJ + AG + LPS. Serum malondialdehyde (MDA) and serum myeloperoxidase (MPO) activity and liver and renal tissue MDA, MPO, and Na^+/K^+-ATPase activity levels were detected in biochemical methods. Liver and renal tissue iNOS levels were examined immunohistopathologically. Serum and tissue MDA and MPO levels and tissue iNOS expression were increased significantly in groups B, C, and E, while tissue ATPase levels were decreased significantly in the same groups. In the group treated with AG (group D), serum and tissue MDA and MPO levels and tissue iNOS expression were decreased while tissue ATPase levels were increased significantly. In group F, if AG was administrated before LPS, we observed that serum and tissue MDA and MPO levels and tissue iNOS expression were decreased while tissue ATPase levels were increased significantly. Thus, our study showed that AG had a protective effect when it was administrated before LPS, but it

failed to prevent tissue iNOS expression and LP if there was established endotoxemia in OJ.

KEYWORDS aminoguanidine, inducible nitric oxide synthase, lipid peroxidation, lipopolysaccharide, obstructive jaundice

Obstructive jaundice (OJ) is an important clinical problem that may lead to serious complications. The systemic consequences of hyperbilirubinemia and OJ are wound breakdown, sepsis, coagulopathy, gastrointestinal hemorrhage, cardiovascular problems, immune depression, and hepatic and renal failure [1]. There is a very high incidence of morbidity and mortality in patients with OJ. One of the important problem in OJ is the increased incidence of endotoxemia that results from defective host immune response [1, 2]. Systemic endotoxemia developed in OJ is the result of the depression in the clearance of lipopolysaccharide (LPS) and other endotoxins in the portal circulation by Kuppfer cells in the liver. In OJ, hepatic Kuppfer cells' endocytosis is impaired and their endotoxin clearance ability is decreased. Portal endotoxemia, which is the result of impaired intestinal barrier, and systemic endotoxemia, which is developed as a result of impaired function of Kuppfer cells, lead to sepsis [1–4].

Lipid peroxidation (LP) is an important problem in OJ, in which free radical production is increased and antioxidative activity is reduced [5]. Nitric oxide (NO) formation and increased expression of inducible nitric oxide synthase (iNOS) take place [6]. Portal and systemic endotoxemia enhance the expression of iNOS and NO formation. The expression of iNOS can be induced by injection of LPS [7]. NO reacts with free oxygen radicals, and this leads to the formation of the most harmful peroxynitrite anion ($ONOO^-$), and this anion leads to LP, cellular damage, and apoptosis.

Peroxynitrite ($ONOO^-$), free oxygen radicals, and environment factors lead to DNA strand breakage, which triggers the activation of poly(ADP-ribose) synthase (PARS) [8, 9]. PARS is an energy-requiring enzyme that plays a role in the repair of strand breaks in DNA. Its activation results in a substantial depletion of NAD, thus leading to cell dysfunction [10].

Selective inhibition of iNOS, which is induced by bacterial LPS, is of value in the treatment of septic or endotoxemic states, as over produced NO has deleterious effects on tissue histology [11, 12]. This can be achieved by some specific iNOS inhibitors such as *N*-(1-iminoethyl)-L-lysine (L-NIL) and 1400W or by some selective inhibitors of iNOS, such as aminoguanidine (AG) [13–15]. In this experimental study, we tried to explore the effect of the specific iNOS inhibitor AG on serum and tissue (liver and kidney) LP and tissue iNOS levels in OJ and in jaundiced rats with endotoxemia induced with LPS. We investigated the role of oxidative injury and whether AG exerts any beneficial effect on lipid LP in OJ and in jaundiced rats with endotoxemia induced with LPS.

MATERIAL AND METHODS

The experiments described in this article were performed in adherence to National Institutes of Health guidelines on the use of experimental animals. Forty-eight male Wistar rats, weighing 150–220 g, were housed at constant temperature with 14/10-h periods of light and dark exposure, respectively. Animals were allowed access to standard rat feed and water ad libitum with an acclimatization period of at least 5 days prior to use in these experiments. Approval of our faculty's ethics committee was taken (date and number of approval: March 26, 2003, B.30.2.MEÜ.0.01.00.00./676). The experiments were performed in the Animal Experimental Laboratory of Mersin University Medical School.

We randomly assigned 48 rats into 6 groups:

Group A: sham group ($n = 8$).
Group B: OJ group ($n = 8$).
Group C: OJ + 10 mg kg^{-1} LPS intraperitoneally ($n = 8$).
Group D: OJ + 50 mg kg^{-1} AG intraperitoneally ($n = 8$).
Group E: OJ + 10 mg kg^{-1} LPS + 50 mg kg^{-1} AG intraperitoneally ($n = 8$).
Group F: OJ + 50 mg kg^{-1} AG + 10 mg kg^{-1} LPS intraperitoneally ($n = 8$).

Surgical Procedures

Bile Duct Ligation

Rats were anesthetized with intramuscular ketamine at 50 mg $kg^{-1}$and xylazine at 7 mg kg^{-1}. The chest and abdomen were shaved and each animal was fixed in a supine position on the operating table. The abdomen was cleaned with 1% polyvinyl iodine; when dry, the operating field was covered with a sterile drape and median laparotomy was performed.

Sham operation was performed on the rats in group A. The sham operation consisted of mobilization of the bile duct only. The rats in this group were sacrificed just after the procedure.

The experimental jaundice was created by ligation of the common bile duct by the technique described in detail by Lee [16].

At day 5, the rats in group B, C, D, E, and F were jaundiced. In group B, we anesthetized and sacrificed the rats at day 5 to reveal the prime changes on histopathology and LP in the jaundiced rats. In group C, at day 5, we injected 10 mg kg^{-1} LPS (*Escherichia coli* LPS serotype 055:B5, 100 mg, Sigma Chemical Co., St. Louis, MO) intraperitoneally into the jaundiced rats, and after 6 h we anesthetized and sacrificed the rats to reveal the prime changes on histopathology and LP in jaundiced rats with endotoxemia [17–19].

In group D, at day 5 we started 50 mg kg^{-1} AG (Sigma Chemical Co., St. Louis, MO) intraperitoneally into the jaundiced rats and repeated the same dose for 5 days, and the rats were anesthetized and sacrificed at day 10. In group E, at day 5 we injected 10 mg kg^{-1} LPS intraperitoneally into the jaundiced rats, and after 6 h, 50 mg kg^{-1} AG was administrated intraperitoneally; the same doses were repeated daily for 5 days and the rats were sacrificed at day 10. In group F, we administrated 50 mg kg^{-1} AG intraperitoneally at day 5 and repeated this daily for 5 days and at day 10, 10 mg kg^{-1} LPS was injected intraperitoneally; then after 6 h we sacrificed the rats.

The rats in groups D, E, and F were sacrificed at day 10 in order to find out and demonstrate clearly the histopathological and biochemical changes that developed when AG was administered in the jaundiced rats before or after the establishment of endotoxemia. These groups were designed to reveal the protective and therapeutical effect of the drug.

Table 1 shows the groups and interventions performed with motivation of dosage and timing of the administered substances (LPS and AG).

The rats were anesthetized with intramuscular ketamine (50 mg kg^{-1}), and the blood was taken by cardiac puncture for serum malondialdehyde (MDA) and serum myeloperoxidase (MPO) activity. In order to evaluate histopathological examination and liver and renal tissue inducible nitric oxide synthase (iNOS) expression, tissue samples were harvested through a midline incision. The liver and renal tissue iNOS expression was illustrated immunohistopathologically. The pathologist was blinded on to what group an animal belonged. In addition, liver and renal tissue MDA, MPO activity, and Na^+/K^+-ATPase activity levels were detected in biochemical methods.

Lipid Peroxides Assays

MDA Determination

The levels of serum and tissue lipid peroxidation products as thiobarbituric acid (TBA)–MDA adducts were measured spectrophotometrically by the method described by Yagi [20]. The results were expressed as nanomoles MDA formed per milliliter for serum and nanomoles of MDA per gram for tissue.

Determination of MPO

The determination of serum and tissue MPO activity depends on the fact that it reduces *o*-dianozidine. Reduced *o*-diazidine was measured at 410 nm by spectrophotometer [21]. The MPO activity was expressed as units per liter for serum and units per gram for tissue.

Determination of Na^+/K^+-ATPase Activities

ATPase activities were determined on the resulting tissue supernatants by measuring the rate of liberation of inorganic phosphate (Pi) from disodium ATP. Incubation media were made up as described previously [22, 23]. Protein content was determined

TABLE 1 The groups and interventions performed with motivation of dosage and timing of the administered substances (LPS and AG)

Intervention	*Group A (Sham)*	*Group B (OJ)*	*Group C (OJ + LPS)*	*Group D (OJ + AG)*	*Group E (OJ + LPS + AG)*	*Group F (OJ + AG + LPS)*
Sham operation, day 1	x	—	—	—	—	—
CBD Iligation, day 1		x	x	x	x	x
OJ, day 5	—	x	x	X	x	x
IPS (10 mg kg^{-1})						
Day 5	—	—	x	—	x	—
Day 6	—	—	—	—	—	—
Day 7	—	—	—	—	—	—
Day 8	—	—	—	—	—	—
Day 9	—	—	—	—	—	—
Day 10	—	—	—	—	—	x
AG (10 mg kg^{-1})						
Day 5	—	—	—	x	x[b]	x
Day 6	—	—	—	x	x	x
Day 7	—	—	—	x	x	x
Day 8	—	—	—	x	x	x
Day 9				x	x	x
Day 10				x	x	x
Sacrifice						
Day 1	x	—	—	—	—	—
Day 5	—	x	x[a]	—	—	—
Day 6	—	—	—	—	—	—
Day 7	—	—	—	—	—	—
Day 8	—	—	—	—	—	—
Day 9	—	—	—	—	—	—
Day 10	—	—	—	x	x	x[a]

[a]Six hours after the administration of LPS, the rats were sacrificed.
[b]AG was administered 6 hours after the administration of LPS.

according to the method described by Lowry [24], and bovine serum albumin was used as a standard. Specific activities were calculated as nanomoles Pi/per milligram protein/per hour.

Histological Evaluation

Immunohistochemical Assay

The extracted liver and renal tissue were fixed in 10% phosphate-buffered formalin and subsequently embedded in paraffin. Five-micrometer-thick sections were prepared from paraffin blocks of liver and renal tissue and were used to quantify the iNOS expression. İmmunohistochemistry for iNOS, a synthetic peptide derived from the extreme C-terminus of the human iNOS protein (Zymed Laboratories, San Francisco, CA, dilution of 1:1000), was performed by using a combination of the streptavidin–biotin peroxidase method and microwave antigen retrieval according to manufacturers' methods.

Morphometric Determination

iNOS protein was observed in the cytoplasm of parenchymal cells of liver and epithelial cells of kidney. iNOS protein expression in the groups was assessed according to the intensity of staining: score 0: <2%, absent; score 1: 2–10%, weak; score 2: 10–50%, intermediate; score 3: >50%, strong.

Statistical Analysis

Biochemical values were described as mean ± standard error of mean (SEM) values. Statistical

TABLE 2 Biochemical values of each group described as mean ± standard error of mean (SEM) values

	Group A (Sham)	*Group B (OJ)*	*Group C (OJ + LPS)*	*Group D (OJ + AG)*	*Group E (OJ + LPS + AG)*	*Group F (OJ + AG + LPS)*
Serum MDA	5.5 ± 0.3	14.0 ± 0.6	52.0 ± 1.8	12.1 ± 0.5	16.6 ± 0.6	12.4 ± 0.3
Serum MPO	0.5 ± 0.1	2.8 ± 0.1	4.0 ± 0.3	1.3 ± 0.1	2.5 ± 0.1	1.5 ± 0.1
Liver MDA	7.4 ± 0.3	16.4 ± 0.9	27.2 ± 3.1	13.9 ± 0.9	15.9 ± 1.3	12.9 ± 1.3
Renal MDA	6.5 ± 0.4	14.9 ± 0.8	19.2 ± 0.5	18.0 ± 1.3	13.5 ± 0.5	12.6 ± 1.0
Liver MPO	1.1 ± 0.1	2.7 ± 0.2	3.9 ± 0.1	2.2 ± 0.2	2.4 ± 0.1	1.7 ± 0.2
Renal MPO	1.4 ± 0.1	2.3 ± 0.2	3.2 ± 0.2	2.3 ± 0.4	2.7 ± 0.2	2.0 ± 0.2
Liver ATPase	72.3 ± 0.7	60.4 ± 0.9	31.5 ± 1.4	57.3 ± 1.7	38.3 ± 2.6	44.8 ± 2.4
Renal ATPase	78.8 ± 1.5	48.8 ± 3.7	34.3 ± 1.4	50.8 ± 1.4	42.3 ± 2.4	45.7 ± 1.5

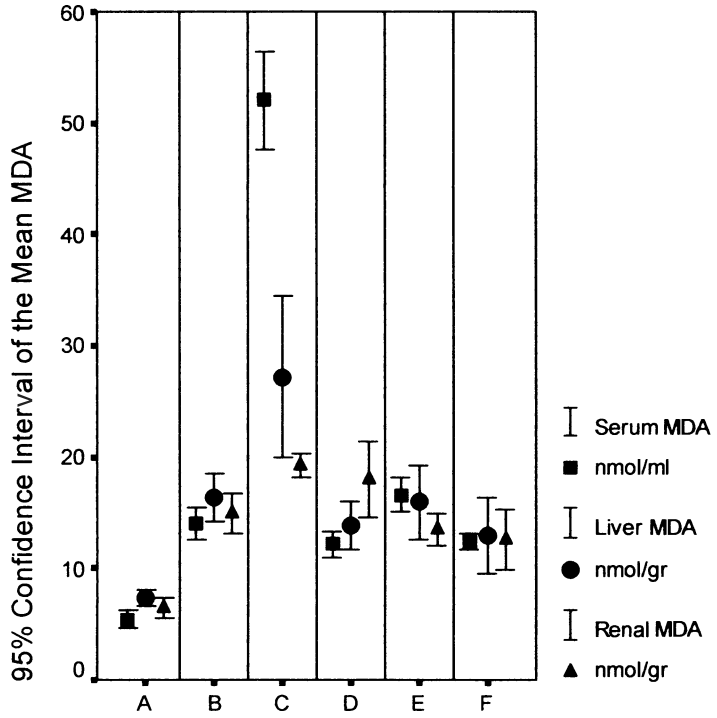
differences for serum and tissue MDA, serum and tissue MPO activity, and tissue ATPase activity values were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test. Histopatological examinations were presented as median and 25–75% percentiles in parenthesis. Comparisons of iNOS staining scores were analyzed using the Kruskal–Wallis test followed by Dunn's test; *p* values less than .05 were considered statistical significant. Box-plot graphs were used to show medians of liver and renal iNOS scores.

RESULTS

Biochemical Examination

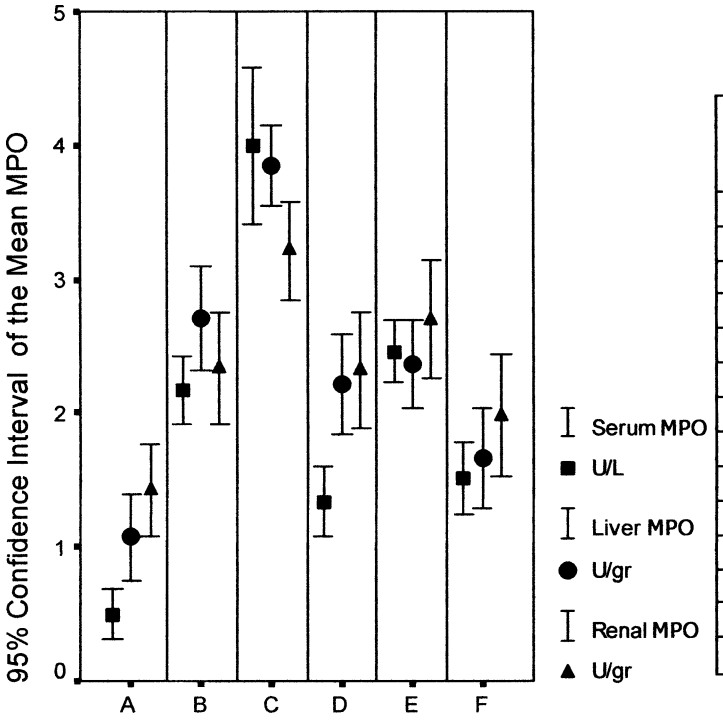
Biochemical values of each group are presented in Table 2.

The descriptive statistics of serum, liver, and renal MDA; serum, liver, and renal MPO; and liver, and renal ATPase are presented in Figures 1, 2, and 3 respectively. The significance levels of pairwise comparisons of groups according to Tukey's post hoc test are presented in each figure.



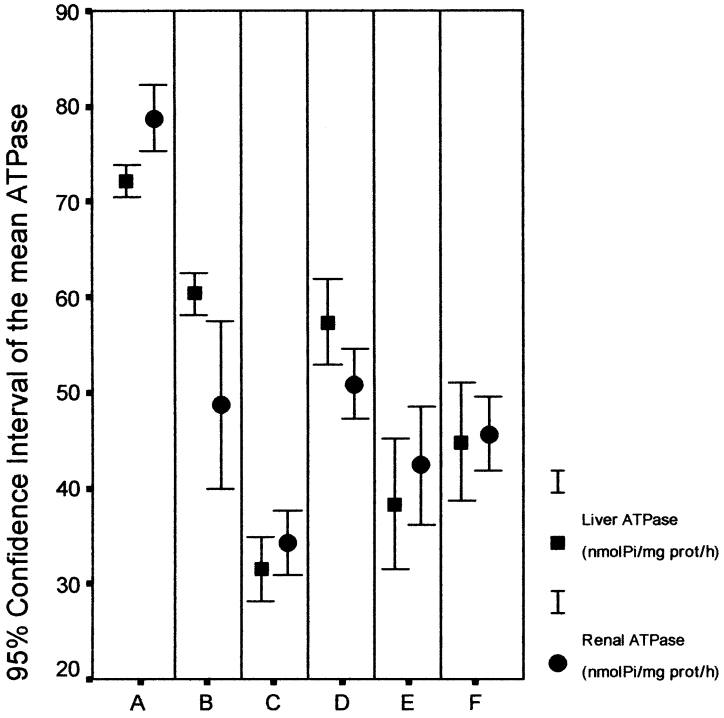
Pairwise Groups	P values for the mean MDA		
	Serum	Liver	Renal
A-B	0.0001*	0.0030*	0.0001*
A-C	0.0001*	0.0001*	0.0001*
A-D	0.0001*	0.0950	0.0001*
A-E	0.0001*	0.0120*	0.0001*
A-F	0.0001*	0.2100	0.0001*
B-C	0.0001*	0.0001*	0.0020*
C-D	0.0001*	0.0001*	0.8450
C-E	0.0001*	0.0001*	0.0001*
C-F	0.0001*	0.0001*	0.0001*
D-E	0.047*	0.9670	0.0070*
D-F	1.000	0.9990	0.0010*
*significant (p<0.05)			

FIGURE 1 The descriptive statistics of serum, liver, and renal MDA and the significance levels of pairwise comparisons of groups according to Tukey's post hoc test.



The Pairwise Groups	P values for the mean MPO		
	Serum	Liver	Renal
A-B	0.0001*	0.0001*	0.0030*
A-C	0.0001*	0.0001*	0.0001*
A-D	0.0030*	0.0001*	0.0080*
A-E	0.0001*	0.0001*	0.0001*
A-F	0.0001*	0.0680	0.2050
B-C	0.0001*	0.0001*	0.0040*
B-D	0.0030*	0.1790	1.0000
B-F	0.0320*	0.0001*	0.6760
C-D	0.0001*	0.0001*	0.0070*
C-E	0.0001*	0.0001*	0.2860
C-F	0.0001*	0.0001*	0.0001*
D-E	0.0001*	0.9830	0.6640
E-F	0.0020*	0.0330*	0.0790
*significant (p<0.05)			

FIGURE 2 The descriptive statistics of serum, liver, and renal MPO and the significance levels of pairwise comparisons of groups according to Tukey's post hoc test.



Pairwise Groups	P values for Liver ATPase	P values for Renal ATPase
A-B	0.0001*	0.0001*
A-C	0.0001*	0.0001*
A-D	0.0001*	0.0001*
A-E	0.0001*	0.0001*
A-F	0.0001*	0.0001*
B-C	0.0001*	0.0001*
B-E	0.0001*	0.3780
B-F	0.0001*	0.9310
C-D	0.0001*	0.0001*
C-F	0.0001*	0.0140*
D-E	0.0001*	0.1690
D-F	0.0001*	0.6760
*significant (p<0.05)		

FIGURE 3 The descriptive statistics of liver and renal ATPase and the significance levels of pairwise comparisons of groups according to Tukey's post hoc test.

TABLE 3 Immunohistochemical iNOS expression values of each group described as median, with interquartile range in brackets

	Group A (Sham)	*Group B (OJ)*	*Group C (OJ + LPS)*	*Group D (OJ + AG)*	*Group E (OJ + LPS + AG)*	*Group F (OJ + AG + LPS)*
Liver iNOS	0 [0 to 1]	2 [2 to 2]	3.5 [3 to 4]	1 [1 to 1]	3 [3 to 4]	1.5 [1 to 2]
Renal iNOS	0 [0 to 1]	2 [2 to 2.5]	3 [3 to 4]	1 [1 to 1]	3 [3 to 4]	1 [1 to 2]

When the serum, liver, and renal MDA of the groups were compared, there was a significant difference between groups A and B, between groups A and C, between groups A and E, between groups B and C, between groups C and E, and between groups C and F ($p < .05$) (Figure 1).

In the comparison of serum, liver, and renal MPO of the groups, there was significant difference between groups A and B, groups A and C, groups A and D, groups A and E, groups B and C, groups C and D, and groups C and F ($p < .05$) (Figure 2).

When the liver and renal ATPase activity levels of the groups were compared, there was significant difference between groups A and B, groups A and C, groups A and D, groups A and E, groups A and F, groups B and C, groups C and D, and groups C and F ($p < .05$) (Figure 3).

Immunohistochemical iNOS Expression

The statistical analysis of liver and renal iNOS staining values of each group is presented in Table 3.

Liver

In the immunohistochemical examination of iNOS expression in the liver of rats (Figure 4) in the sham group (group A), very weak iNOS staining was detected in hepatocytes around the central venules of the lobules. In group B (OJ group), the number of iNOS stained cells were increased. In groups C (OJ + LPS group) and E (OJ + LPS + AG group), there was intense iNOS staining. However, in groups D (OJ + AG group), and F (OJ + AG + LPS group), iNOS staining was sparse.

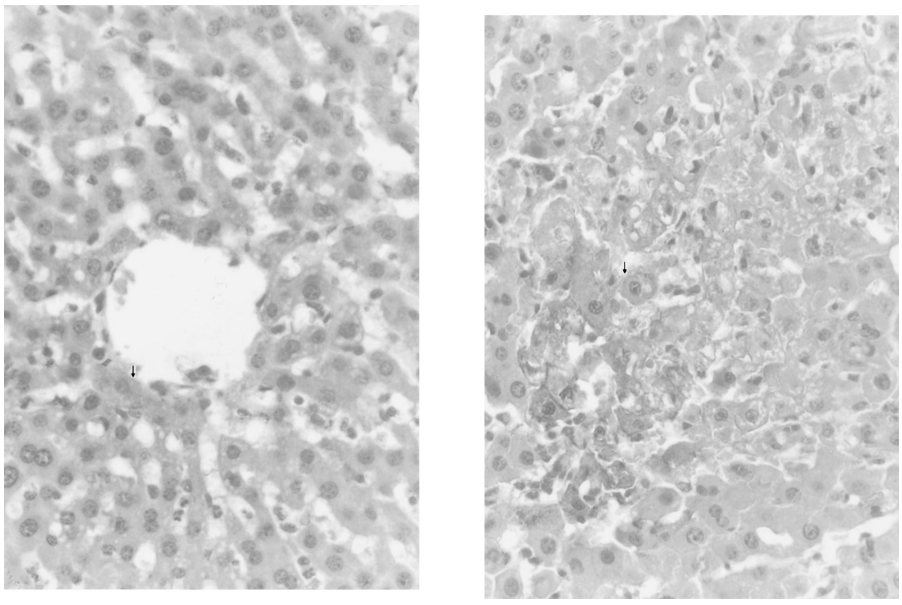
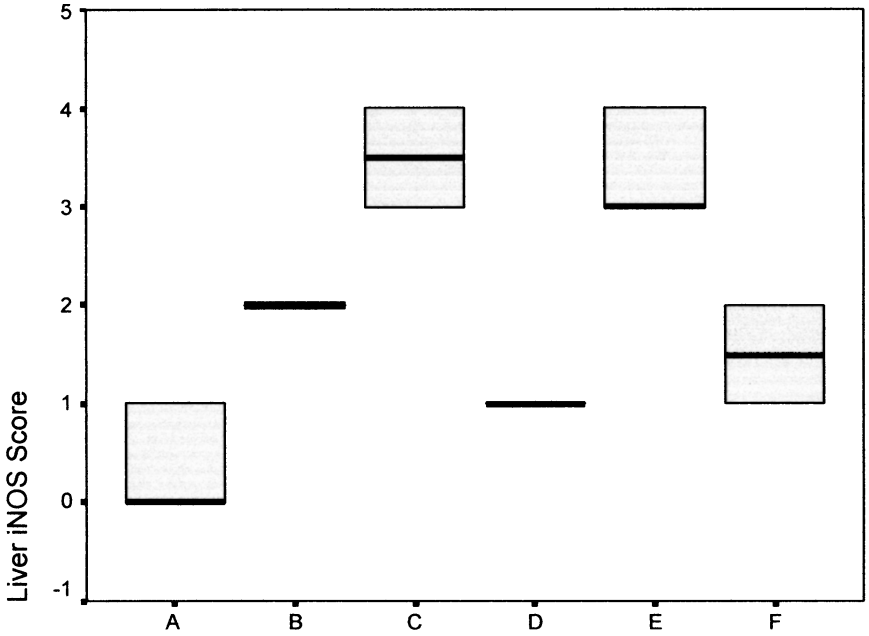


FIGURE 4 Representative sparse and intense iNOS staining in the immunohistochemical examination of liver tissue. ×400.



pairwise group	p value
A-B	0.0010*
A-C	0.0010*
A-E	0.0010*
A-F	0.0070*
B-C	0.0010*
B-D	0.0030*
B-E	0.0020*
C-D	0.0010*
C-F	0.0010*
D-E	0.0020*
D-F	0.0430*
E-F	0.0030*
* P <0,05	

FIGURE 5 The differences between medians of groups in the immunohistochemical examination of liver tissue with iNOS staining. Multiple comparisons between the groups for liver iNOS ($p = .05$).

There was a significant difference between groups A and B, groups A and C, groups A and E, groups A and F, groups B and C, groups B and D, groups B and E, groups C and D, groups C and F, groups D and E, groups D and F, and groups E and F ($p < .05$) (Figure 5).

The immunohistochemical examination with iNOS demonstrated that iNOS staining in group A (sham-operated control group), group D (OJ + AG), and group F (OJ + AG + LPS) was significantly lower than in group B (OJ), group C (OJ + LPS), and group E (OJ + LPS + AG).

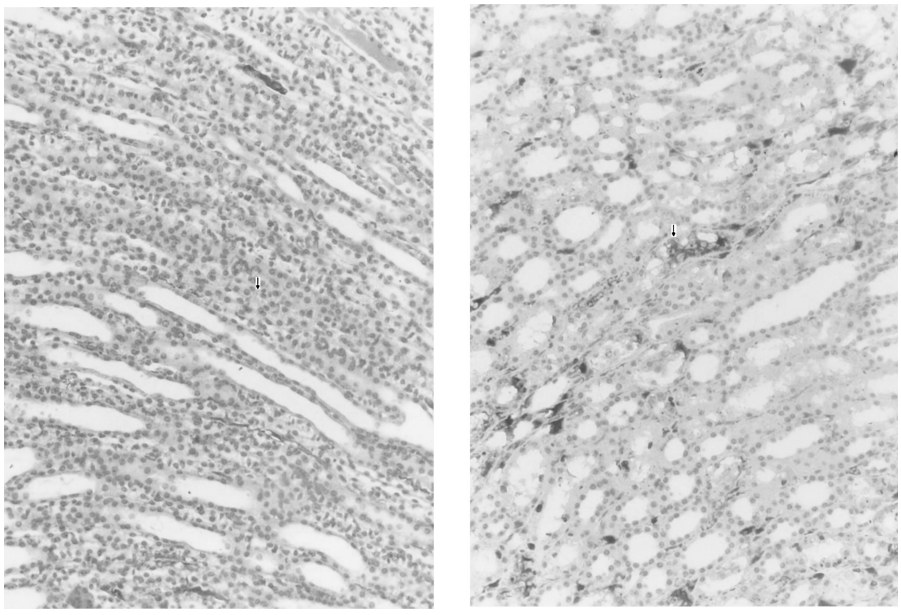
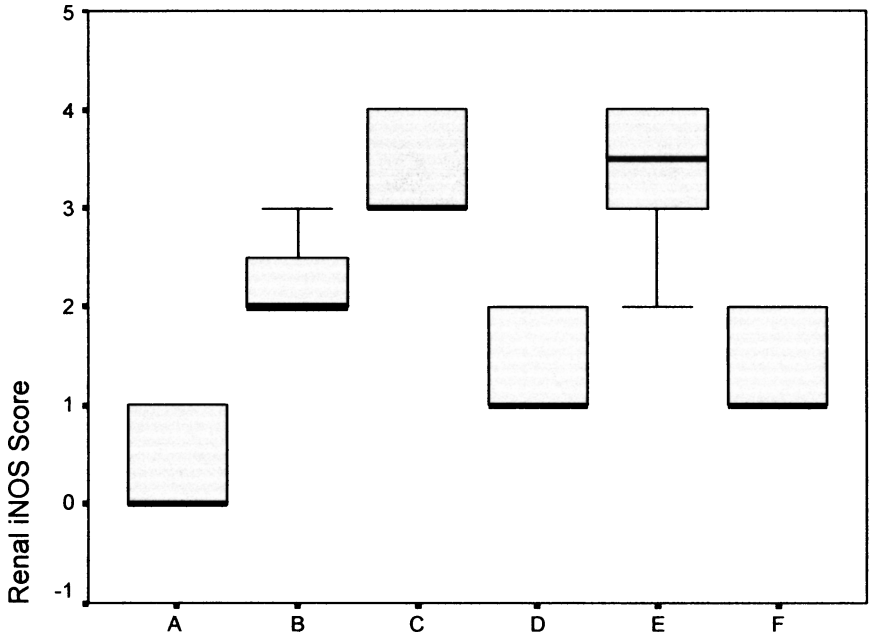


FIGURE 6 Representative sparse and intense iNOS staining in the immunohistochemical examination of renal tissue. ×200.



pairwise group	p value
A-B	0.0001*
A-C	0.0001*
A-D	0.0110*
A-E	0.0010*
A-F	0.0110*
B-C	0.0020*
B-D	0.0090*
B-E	0.0170*
B-F	0.0090*
C-D	0.0010*
C-F	0.0010*
D-E	0.0050*
E-F	0.0050*
* P < 0,05	

FIGURE 7 The differences between medians of groups in the immunohistochemical examination of renal tissue with iNOS staining. Multiple comparisons between the groups for renal iNOS ($p = .05$).

Renal

In the immunohistochemical examination of renal iNOS expression (Figure 6), it was noted in the tubular epithelium. There was severe immunostaining in the tubular epithelium, in both the cortical and medullar region, and prominent in the outer medulla.

In sham group (group A), very weak iNOS staining was detected in a few tubular epithelium samples. In groups B (OJ group), C (OJ + LPS group), and E (OJ + LPS + AG group) the numbers of iNOS stained cells were increased. In groups D (OJ + AG group) and F (OJ + AG + LPS group), iNOS staining was sparse.

There was a significant difference between groups A and B, groups A and C, groups A and D, groups A and E, groups A and F, groups B and C, groups B and D, groups B and E, groups B and F, groups C and D, groups C and F, groups D and E, and groups E and F ($p < .05$) (Figure 7).

The tubular epithelium showed statistically significant and more intense staining in the cases of group C (OJ + LPS) and group E (OJ + LPS + AG) when compared to group D (OJ + AG) and group F (OJ + AG + LPS group).

DISCUSSION

Sepsis is a severe and major complication related to OJ, and it is also the reason of high morbidity and mortality [25]. The neutralization and elimination of the intestinal endotoxin related to the lack of bile in the intestinal lumen are reduced in OJ [26]. At the same time, impaired gut barrier function leads to bacterial translocation [27]. There is also increased sensitivity to LPS and endotoxins in OJ. The activation of polynuclear leukocytes, monocytes, and macrophages in OJ leads to release of many mediators, which contribute to the pathophysiology of the systemic inflammatory response syndrome, sepsis, and multiple organ failure [28–30]. On the other hand, these mediators bind to the respective cell surface receptors and activate tyrosine kinase and nuclear factor kappa B (NFκB). This leads to transcription of iNOS protein in different cells and organs and to overproduction of NO [31, 32]. For this reason, iNOS was expressed in hepatocytes and renal tubular epithelium in our study.

INOS is not expressed under normal conditions, but cytokines and endotoxin induce its expression [6, 33]. In OJ and endotoxemia, activated iNOS (independently of Ca) leads to excessive production of

NO [34, 35]. Recently, oxidative stress and formation of peroxynitrite anion ($ONOO^-$) are recognized as an important initiator of apoptosis [36]. Increased LP and formation of peroxynitrite anion lead to oxidative damage in lungs, kidneys, and liver tissue [35].

AG is a nucleophylic hydrazine that selectively inhibits iNOS expression. It inhibits the inducible form of NOS 30–40 times more than endothelial NOS (eNOS) and the constitutional form of NOS (cNOS).

In our study, we observed that serum, liver, and renal MDA and MPO levels were increased in group B. We found decreased liver and renal ATPase levels. This data show that there is increased LP in the rats with OJ. In the immunohistochemical examination of iNOS expression in group B, we observed that liver and renal iNOS expression was increased significantly (Figures 4 and 5). Since AG is a selective iNOS inhibitor, we have administered it to the jaundiced rats (group D) to see whether it exerts any beneficial effect on LP and iNOS expression. We observed that serum, liver, and renal MDA and serum, liver, and renal MPO levels were decreased and liver and renal ATPase activity were increased in group D (OJ + AG). These results show that LP and oxidative stress are prevented with the protective effect of AG. Since this effect of AG depends on its selective iNOS inhibitor effect, we examined iNOS expression immunohistochemically in group D. We observed that iNOS staining in group D (OJ + AG) was lower than in group B (OJ). The level of iNOS staining in this group was almost near the level of group A (sham-operated control group). This shows that defective host immune response in OJ enhances sensitivity of the host to endotoxemia, which leads to increased tissue iNOS expression, and AG decreases this expression. These results are very important because in patients with OJ, host immune response can be repaired by AG and establishment of endotoxemia can be prevented.

In the second part of our investigation, we administered LPS to the rats with OJ. We observed that LP and oxidative stress were exaggerated. We observed that serum, liver, and renal MDA and MPO levels in group C were much more higher than serum, liver, and renal MDA and MPO levels in groups B and A. We found decreased liver and renal ATPase levels in group C. In the immunohistochemical examination of iNOS expression, we found that liver and renal iNOS expression were increased significantly in group C as compared to sham and OJ groups (Figures 4 and 5). This can be explained by the fact that the increased sensitivity to LPS and endotoxin in OJ can lead to exaggerated iNOS expression, LP, and oxidative stress, which may lead to organ dysfunction.

In order to evaluate the protective and therapeutical effect of AG, we administered it to the jaundiced rats in which endotoxemia is induced with LPS before (OJ + LPS + AG group, group E) or after administration of AG (OJ + AG + LPS group, group F), to see whether it exerts any beneficial effect on LP and iNOS expression. We observed that serum, liver, and renal MDA and serum, liver, and renal MPO levels were decreased and liver and renal ATPase activity were increased in group F (OJ + AG + LPS). These results show that LP and oxidative stress are prevented with the protective effect of AG. Since this effect of AG depends on its selective iNOS inhibitor effect, we examined iNOS expression immunohistochemically in groups E and F. We observed that iNOS staining was very strong in group E. It was almost equal to the level of iNOS staining in group C. We observed that iNOS staining in group F (OJ + AG + LPS) was lower than in group B (OJ) or group C (OJ + LPS). The level of iNOS staining in this group was near the level of group A (sham-operated control group). This data shows that the enhanced sensitivity of the host to LPS and other endotoxins in OJ leads to increased tissue iNOS expression, and AG decreased this expression if it was administrated before LPS, but it failed to diminish the deleterious effects of LPS if it was administrated after LPS. This shows that there is no therapeutical effect of AG if organ dysfunction in any kind of endotoxemia is established because of the second hit model. But if AG is administrated before LPS as in group F, establishment of organ dysfunction is prevented by maintaining the host immune defense to LPS and other endotoxins via reducing LP.

In this study, we aimed to investigate the deleterious effects of OJ and LPS on LP and on liver and renal tissue iNOS expression in jaundiced rats with

endotoxemia induced with LPS and the protective effect of AG in OJ to show whether it is effective when administered before or after LPS. Our study had indicated that AG performed a protective role in OJ by decreasing LP and by maintaining the host immune defense to LPS and other endotoxins when it was administered before LPS, but it failed to prevent the tissue iNOS expression and LP if there was established endotoxemia in OJ. AG can be used in clinic as a protective agent to protect patients with OJ from development of endotoxemia.

REFERENCES

1. Grey JD, Krukowski ZH, Matheson NA. Surgical mortality and morbidity in one hundred and twenty patients with obstructive jaundice. *Br J Surg*. 1998;75:216–219.
2. Regan MC, Keane RM, Little D. Postoperative immunological function and jaundice. *Br J Surg*. 1994;81:271–273.
3. Plusa S, Webster N, Primrose J. Obstructive jaundice causes reduced expression of polymorphonuclear leucocyte adhesion molecules and a depressed response to bacterial wall products in vitro. *Gut*. 1996;38:784–787.
4. Sheen-Chen SM, Chau P, Harris HW. Obstructive jaundice alters Kupffer cell function independent of bacterial translocation. *J Surg Res*. 1998;80:205–209.
5. Jolobe OM. Role of endotoxin and nitric oxide in the pathogenesis of renal failure in obstructive jaundice. *Br J Surg*. 1997;84:1747–1752.
6. Arriero MM, Lopez-Farre A, Fryeiro O, Rodriguez–Feo JA, Velasco S, Garcia-Duran M, Fortes J, DeLa Pinta JC, Munoz LE, Celdran A. Expression of inducible nitric oxide synthase in the liver of bile duct-ligated Wistar rats with modulation by lymhomononuclear cells. *Surgery*. 2001;129(3):255–266.
7. Shimizu Y, Miyazaki M, Ito H. Enhanced endothelial cell injury by activated neutrophils in patients with obstructive jaundice. *J Hepatol*. 1997;27:803–809.
8. Casini A, Ceni E, Salzano R, Biondi P, Parola M, Galli A, Foschi M, Caliguiri A, Pinzani M. Neutrophil-derived superoxide anion induces lipid peroxidation and stimulates collagen synthases in human hepatic steallate cells: Role of nitric oxide. *Hepatology*. 1997;25:361–367.
9. Szabo C, Cuzzocrea S, Zingarelli B, O'Connor M, Salzman AL. Endothelial dysfunction in a rat model of endotoxic shock. Importance of the activation of poly (ADP-ribose) synthetase by peroxynitrite. *J Clin Invest*. 1997;100(3):723–735.
10. Szabo C, Dawson WL. Role of poly (ADP-ribose) synthetase in inflammation and ischemia-reperfusion. *TIPS*. 1998;19:287–298.
11. Vallance P, Rees D, Moncada S. Therapeutic potential of NOS inhibitors in septic shock. In: Mayer B, ed. *Handbook of Experimental Pharmacology*. New York: Springer; 2000;386–397.
12. Vallance P, Moncada S. Role of endogenous nitric oxide in septic schock. *New Horiz*. 1993;1:77–86.
13. Lortie MJ, Ishizuka S, Schwartz D, Blantz RC. Bioactive products of arginine in sepsis: Tissue and plasma composition after LPS and iNOS blockade. *Am J Physiol*. 2000;278:1191–1199.
14. Parkinson JF. Nitric oxide synthase inhibitors I: substrate analogs and heme ligands In: Mayer B, ed. *Handbook of Experimental Pharmacology.* New York: Springer; 2000;111–135.
15. Cinel I, Büyükafşar K, Cinel L, Polat A, Atıcı Ş, Tamer L, Oral U. The role of poly(ADP-ribose) synthetase inhibition in preventing endotoxemia-induced intestinal epithelial apoptosis. *Pharm Res.* 2002;46:119–127.
16. Lee E. The effect of obstructive jaundice on the migration of reticulo-endothelial cells and fibroblasts into early experimental granulomata. *Br J Surg.* 1972;59(11):875–877.
17. Taner AS, Cinel I, Ozer L, Onde U, Taner D, Koksoy C. Poly (ADP-ribose) synthetase inhibition reduces bacterial translocation in rats after endotoxin challenge. *Shock*. 2001;16:159–162.
18. Takao Y, Mikawa K, Nishina K, Obara H. Attenuation of acute lung injury with propofol in endotoxemia. *Anesth Analg.* 2005;100:810–816.
19. Hayashi Y, Abe M, Murai A, Shimizu N, Okamoto I, Katsuragi T, Tanaka K. Comparison of effects of nitric oxide synthase (NOS) inhibitors on plasma nitrite/nitrate levels and tissue NOS activity in septic organs. *Microbiol. Immunol.* 2005;49:139–147.
20. Yagi K. Lipid peroxides and related radicals in clinical medicine. In: Armstrong D, ed. *Free Radicals in Diagnostic Medicine*. New York: Plenum; Press, 1994;1–15.
21. Golowich SP, Kaplan SD. *Methods in enzymology.* Vol. II. New York: Academic Press; 1955;769.
22. Reading HW, Isbir T. Action of lithium on ATPase in transmitter release from rat iris. *Biochem Pharmacol* 1979;28:3471–3474.
23. Reading HW, Isbir T. The role of cation-activated ATPases in transmitter release from the rat iris. *Q J Exp Physiol.* 1988;65:105–116.
24. Lowry OH, Roscbrough NJ, Farr AI, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1954;193:265–275.
25. Reynolds JV, Murchan P, Redmond HP, Watson RW, Leonard N, Hill A, Clarke P, Marks P, Keane FB, Tanner WA. Failure of macrophage activation in experimental obstructive jaundice: Association with bacterial translocation. *Br J Surg.* 1995;82:534–538.
26. Stocum MM, Sitting KM, Specian RD, Deitch EA. Absence of intestinal bile promotes bacterial translocation. *Am Surg.* 1992;58:305–310.
27. Ding JW, Andersson R, Soltestz V, Willen R, Bengmark S. Obstructive jaundice impairs reticuloendothelial function and promotes bacterial translocation in the rat. *J Surg Res.* 1994;57:238–245.
28. Bemelmans MHA, Gouma DJ, Greve JW, Buurman WA. Cytokines tumour necrosis factor and IL-6 in experimental

biliary obstruction in mice. *Hepatology.* 1992;15:1132–1136.
29. Kennedy JA, Clements WD, Kirk SJ, McCaigue MD, Campbell GR, Erwin PJ, Halliday MI, Rowlands BJ. Characterization of the Kupffer cell response to exogenous endotoxin in a rodent model of obstructive jaundice. *Br J Surg.* 1999;86(5):628–633.
30. O'Neil S, Hunt J, Filksins J, Gamelli R. Obstructive jaundice in rats results in exaggerated hepatic production of tumor necrosis factor-alpha and systemic and tissue tumor necrosis factor-alpha levels after endotoxin. *Surgery.* 1997;122:281–286.
31. Shoemaker W. Cellular effectors of the septic process. In: *Textbook of Critical Care.* 4th ed. Philadelphia: Saunders; 2000:523–542.
32. Casini A, Ceni E, Salzano R, Biondi P, Parola M, Galli A, Foschi M, Caliguiri A, Pinzani M. Neutrophil-derived superoxide anion induces lipid peroxidation and stimulates collagen synthases in human hepatic steallate cells: Role of nitric oxide. *Hepatology.* 1997;25:361–367.
33. Chesrown SE, Monnier J, Visner G, Nick HS. Regulation of inducible nitric oxide synthase mRNA levels by LPS, IFN-gamma, TGF-beta, and IL-10 in murine macrophage cell lines and rat peritoneal macrophages. *Biochem Biophys Res Commun.* 1994;15:126–134.
34. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacol Rev.* 1999;43:109–142.
35. Shieh P, Zhou M, Ornan DA, Chaudry IH, Wang P. Upregulation of inducible nitric oxide synthase and nitric oxide occurs later than the onset of the hyperdynamic response during sepsis. *Shock.* 2000:13(4);325–329.
36. Buttke JM, Sandstrom PA. Oxidative stress as a mediator of apoptosis. *Immunol Today.* 1994;15:7–10.