

Acta Medica Okayama

Volume 57, Issue 3

2003

Article 3

JUNE 2003

Effect of ornithine on the ileal histology, nitric oxide production and lipid peroxidation in LPS-induced endotoxemia.

Musa Dirlik*

Kansu Buyukafsar†

Ismail Cinel‡

Leyla Cinel**

Mehmet Caglikulekci††

Lulufer Tamer‡‡

Suha Aydin§

Ugur Oral¶

*Mersin University,

†Mersin University,

‡Mersin University,

**Mersin University,

††Mersin University,

‡‡Mersin University,

§Mersin University,

¶Mersin University,

Effect of ornithine on the ileal histology, nitric oxide production and lipid peroxidation in LPS-induced endotoxemia.*

Musa Dirlik, Kansu Buyukafsar, Ismail Cinel, Leyla Cinel, Mehmet Caglikulekci, Lulufer Tamer, Suha Aydin, and Ugur Oral

Abstract

Effect of ornithine which is known to inhibit L-arginine uptake via cationic amino acid transport system has been tested, and compared to aminoguanidine, an iNOS inhibitor in lipopolysaccharide (LPS)-induced endotoxemia in rats. Serum nitrite/nitrate and malondialdehyde (MDA) level have been measured, and ileal histology has also been examined. Endotoxin increased serum nitrite/nitrate and MDA levels from 15.7 \pm 2.4 micromol/ml and 2.1 \pm 0.2 nmol/ml to 23.1 \pm 1.0 micromol/ml and 5.2 \pm 0.3 nmol/ml (both $P < 0.05$), respectively. In addition, LPS caused ileal degeneration. L-ornithine (500 mg/kg) did not improve septic manifestations, i.e., serum nitrite/nitrate and MDA levels did not differ from those in endotoxemia. Neither does it have an improving action on ileal histology. However, higher dose of L-ornithine (2,500 mg/kg) lowered the increased level of nitrite/nitrate and MDA by LPS. Moreover, it restored ileal histology from grade 3 (median) to 0 (median) ($P < 0.05$). On the other hand, aminoguanidine (100 mg/kg) normalized serum nitrite/nitrate and MDA levels but not ileal histology in endotoxemic rats. In conclusion, high dose of L-ornithine could improve endotoxemic parameters in LPS-treated rats.

KEYWORDS: LPS, ornithine, aminoguanidine, endotoxemia, lipid peroxidation

*PMID: 12908009 [PubMed - indexed for MEDLINE]

Copyright (C) OKAYAMA UNIVERSITY MEDICAL SCHOOL

Original Article

Effect of Ornithine on the Ileal Histology, Nitric Oxide Production and Lipid Peroxidation in LPS-Induced EndotoxemiaMusa Dirlik^{a*}, Kansu Büyükaşar^b, İsmail Cinel^c, Leyla Cinel^d,
Mehmet Çağlıküleççi^a, Lülüfer Tamer^e, Süha Aydın^a, and Uğur Oral^c^aDepartment of Surgery, ^bDepartment of Pharmacology, ^cDepartment of Anesthesiology and Reanimation, ^dDepartment of Pathology, and ^eDepartment of Biochemistry of the Medical Faculty, Mersin University, Mersin, Turkey

Effect of ornithine which is known to inhibit L-arginine uptake via cationic amino acid transport system has been tested, and compared to aminoguanidine, an iNOS inhibitor in lipopolysaccharide (LPS)-induced endotoxemia in rats. Serum nitrite/nitrate and malondialdehyde (MDA) level have been measured, and ileal histology has also been examined. Endotoxin increased serum nitrite/nitrate and MDA levels from $15.7 \pm 2.4 \mu\text{mol/ml}$ and $2.1 \pm 0.2 \text{ nmol/ml}$ to $23.1 \pm 1.0 \mu\text{mol/ml}$ and $5.2 \pm 0.3 \text{ nmol/ml}$ (both $P < 0.05$), respectively. In addition, LPS caused ileal degeneration. L-ornithine (500 mg/kg) did not improve septic manifestations, *i.e.*, serum nitrite/nitrate and MDA levels did not differ from those in endotoxemia. Neither does it have an improving action on ileal histology. However, higher dose of L-ornithine (2,500 mg/kg) lowered the increased level of nitrite/nitrate and MDA by LPS. Moreover, it restored ileal histology from grade 3 (median) to 0 (median) ($P < 0.05$). On the other hand, aminoguanidine (100 mg/kg) normalized serum nitrite/nitrate and MDA levels but not ileal histology in endotoxemic rats. In conclusion, high dose of L-ornithine could improve endotoxemic parameters in LPS-treated rats.

Key words: LPS, ornithine, aminoguanidine, endotoxemia, lipid peroxidation

Bacterial lipopolysaccharide (LPS) or endotoxin is an initiator of the septic syndrome that is recognised as a major cause of multiorgan dysfunction syndrome [1]. Endotoxin triggers the release of numerous mediators including nitric oxide and reactive oxygen species (ROS) which activate a variety of pathological mechanisms that culminate in tissue dysfunction and organ failure [2].

It has been demonstrated that endotoxemia increases circulating level of nitrite/nitrate (NO_2^- , NO_3^-), stable products of NO in various animals as well as human

sepsis [3, 4]. Massive NO production which probably accounts for tissue damage in endotoxemia results from the induction of inducible nitric oxide synthase (iNOS) [5]. Therefore, inhibition of NO as well as ROS production seems to be vital in the treatment of septic syndrome.

NO is synthesized from a semiessential aminoacid, L-arginine and, its analogues have been used as inhibitors of NOS [6]. L-arginine is mainly transported into endothelial cells via cationic aminoacid transport system (y^+). L-lysine and L-ornithine are known to inhibit this system [7]. Although effect of L-lysine has been tested in ovine endotoxemia [8], there is no report with regard to ornithine, another chemically-related amino acid in LPS-induced endotoxemia in rats. For that reason, we

Received June 3, 2002; accepted January 7, 2003.

*Corresponding author. Phone: +90-324-337-4300; Fax: +90-324-337-4305
E-mail: musa_dirlik@yahoo.com (M. Dirlik)

have aimed to investigate if ornithine has any beneficial effects in rat endotoxemia by evaluating levels of serum nitrite/nitrate and malondialdehyde (MDA), indicators of NO and lipid peroxidation respectively. Further, we have examined any effects of the aminoacid on ileal histology.

Materials and Methods

The experiments in this study were conducted in adherence to the rules of the local ethic committee. Female Wistar rats weighing 170–200 g were housed at constant temperature with 12/12 h periods of light and dark exposure. The animals had free access to standard rat chow and water *ad libitum* during acclimation period of at least 5 days. After fasting over night in the morning at 10 o'clock the rats were randomly divided into 8 groups.

Group 1: Served as control, received intraperitoneal saline (1 ml/200 g, n = 6);

Group 2: Endotoxin (*Escherichia coli* lipopolysaccharide, 055:B5, 10 mg/kg, i.p., Sigma, St. Louis, MO, USA, n = 5),

Group 3: I.p. ornithine (500 mg/kg, Sigma, St. Louis, MO, USA, n = 4),

Group 4: I.p. ornithine (2,500 mg/kg, n = 5),

Group 5: I.p. aminoguanidine (100 mg/kg, n = 5)

Group 6: I.p. ornithine (500 mg/kg) 10 min before endotoxin injection (n = 6).

Group 7: I.p. ornithine (2,500 mg/kg) 10 min before endotoxin injection (n = 6).

Group 8: I.p. aminoguanidine (100 mg/kg) 10 min before endotoxin injection (n = 7).

Six hours later, rats anaesthetized with intramuscular ketamine (50 mg/kg) and the blood was taken by cardiac puncture for nitrite/nitrate levels. In order to evaluate the endotoxin-associated ileal injury, tissue samples were harvested through a midline incision, and fixed in 10% formaldehyde.

Detection of Serum Nitrite/Nitrate Level.

In biological fluids NO is very rapidly deactivated by oxidation to nitrite and nitrate. After collecting the blood samples by cardiac puncture, we detected NO via nitrite/nitrate. Nitrate was reduced to nitrite by NADPH in the presence of nitrate reductase and the formed nitrite was put to react with sulphanilamide and N-(1-naphthyl)-ethylenediamine to give a red-violet diazo dye. The diazo dye measured on the basis of its absorbance in the visible range at 550 nm.

Lipid Peroxidation Assay. The levels of

serum lipid peroxidation products as thiobarbituric acid (TBA)-malondialdehyde (MDA) adducts were measured spectrophotometrically by the method described by Yagi [9]. The final results were expressed as nmol of MDA formed per milliliter of serum.

Histological Examination of ileal segments. The ileal specimens were fixed in 10% formaldehyde. Hematoxylin and eosin-stained slides were prepared by using standard methods. Intestinal mucosal damage was graded on a 6-tiered scale as defined by Chiu *et al.* [10] in a minimum of 20 separate locations on each sections. Briefly, mucosal damage was graded from 0 to 5 according to the following criteria:

Grade 0: Normal mucosal villi

Grade 1: Development of subepithelial space

Grade 2: Extension of the subepithelial space with moderate lifting of the epithelial layer from the lamina propria

Grade 3: Massive epithelial lifting down the side of the villi

Grade 4: Denuded villi with lamina propria and dilated capillaries exposed

Grade 5: Digestion and disintegration of lamina propria; hemorrhage and ulceration

Statistical Analysis. Values are given as mean \pm S.E.M., or median and quartiles. Statistical differences for serum nitrite/nitrate and MDA values were evaluated using one way of ANOVA followed Bonferroni post hoc test. Comparison for intestinal injury scores was analyzed using Kruskal-Wallis variance analysis followed by Dunn test. *P* values less than 0.05 were considered significant.

Results

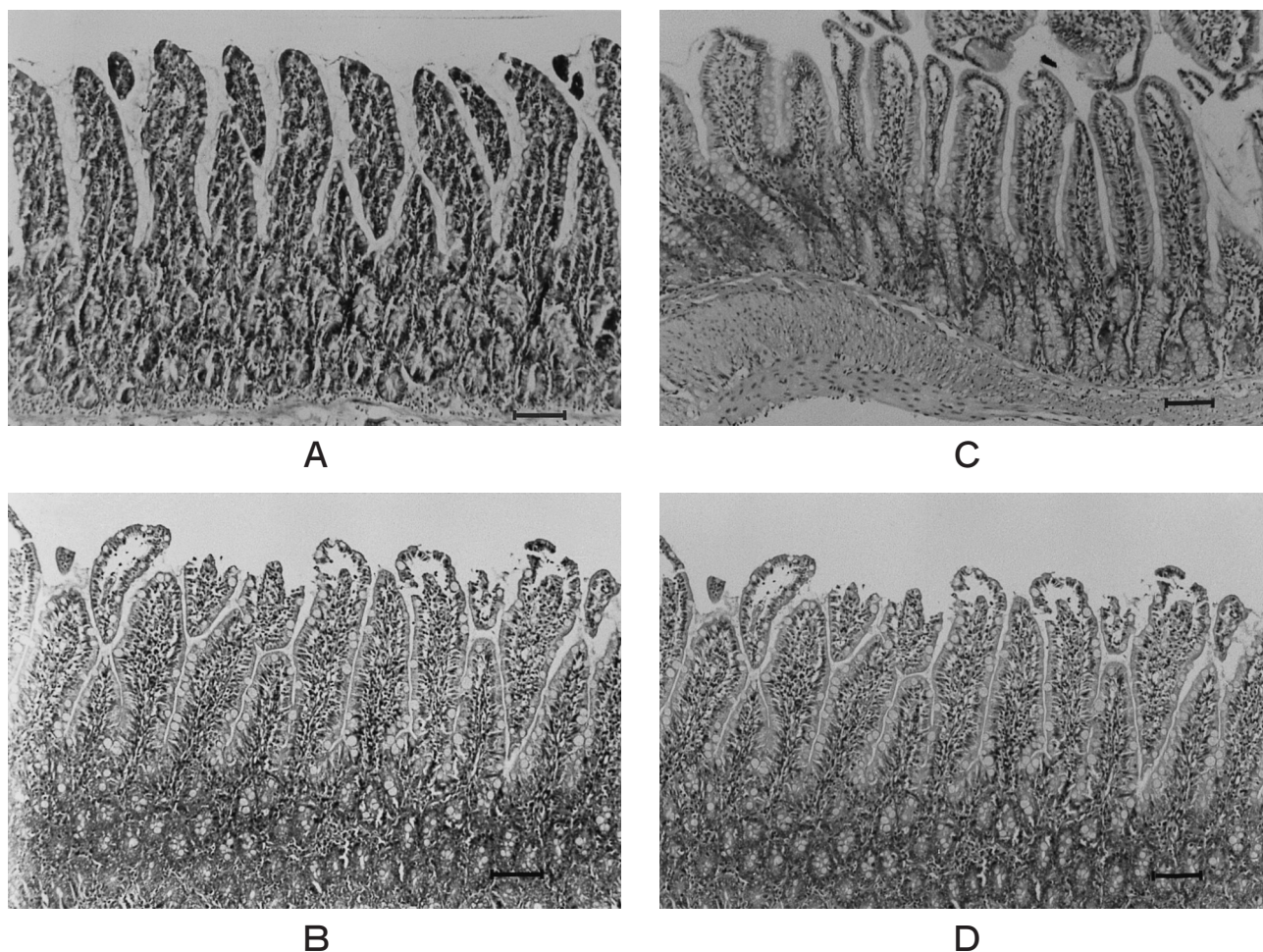
Serum Nitrite/Nitrate Levels. No animals died during the experimentation. As shown in Table 1, serum nitrite/nitrate level was found to be increased in endotoxemic group ($15.72 \pm 2.43 \mu\text{mol/l}$ vs. $23.10 \pm 1.0 \mu\text{mol/l}$, $P < 0.05$). While treatment of ornithine (500 mg/kg) failed to prevent the ability of LPS to elevate serum nitrite/nitrate level, higher dose of ornithine (2,500 mg/kg) markedly decreased that level ($P < 0.05$). Furthermore, aminoguanidine (100 mg/kg) significantly lowered nitrite/nitrate level ($P < 0.05$).

Lipid Peroxidation. In LPS-injected group the level of serum malondialdehyde (MDA) was $5.20 \pm 0.30 \text{ nmol/ml}$, whereas it was $2.12 \pm 0.21 \text{ nmol/ml}$ in

Table I Serum nitrite/nitrate and malondialdehyde (MDA) levels in all groups.

Group	Nitrite + Nitrate (mmol/ml)	MDA (nmol/ml)
Control (n = 6)	15.70 ± 2.40	2.12 ± 0.21
LPS (n = 8)	23.10 ± 1.04*	5.20 ± 0.30*
Ornithine (500 mg/kg, n = 4)	8.67 ± 2.62	1.24 ± 0.11
Ornithine (2,500 mg/kg, n = 5)	8.10 ± 0.43	1.16 ± 0.05
Aminoguanidine (100 mg/kg, n = 5)	14.20 ± 0.37	1.04 ± 0.24
LPS + Ornithine (500 mg/kg, n = 6)	21.00 ± 2.90	4.10 ± 0.73
LPS + Ornithine (2,500 mg/kg, n = 6)	17.50 ± 0.45**	3.05 ± 0.13**
LPS + Aminoguanidine (100 mg/kg, n = 7)	13.10 ± 0.49**	1.50 ± 0.13**

Data are expressed as mean ± S.E.M. *, Different from control; **, Different from LPS-treated group. Statistical differences were evaluated using one way of ANOVA followed Bonferroni post hoc test.

**Fig. 1** Photomicrographs of small intestine segments (H & E × 200).

A, Control group showing normal histology; **B**, LPS only group showing massive epithelial lifting down the sides of villi with a few denuded tips; **C**, Ornithine (2,500 mg/kg) reduced the LPS induced intestinal injury ($P < 0.05$); **D**, Aminoguanidine (100 mg/kg) did not improve ileal damage by LPS administration. Bars indicate 100 μ m

control which is statistically different ($P < 0.001$). In ornithine alone injected groups, the levels of MDA were 1.24 ± 0.11 nmol/ml and 1.16 ± 0.05 at the doses of 500 mg/kg and 2,500 mg/kg, respectively. In the serum from the rats given LPS and 500 mg/kg ornithine MDA levels were 4.10 ± 0.73 nmol/ml which was not significantly different from LPS-injected group. However, at the dose of 2,500 mg/kg, ornithine significantly decreased MDA level from 5.20 ± 0.30 nmol/ml to 3.10 ± 0.13 ($P < 0.01$, Table 1). Similarly aminoguanidine lowered both nitrite/nitrate and MDA levels in endotoxemic rats (Table 1).

Light Microscopic Findings. Small intestine of the rats was assessed for tissue damage by histologic examination. As shown in Fig. 1B, endotoxemia caused significant tissue damage. These changes varied from denuded villi with exposed dilated capillaries to significant architectural distortion, lamina propria disintegration, ulceration and hemorrhage. The histopathologic scores of control and ornithine groups (500 mg/kg and 2,500 mg/kg) were significantly smaller than LPS group (Fig. 2). LPS plus ornithine (500 mg/kg) did not reduce the LPS induced intestinal injury. There was no statistical significant difference between the scores of LPS and LPS plus ornithine (500 mg/kg) group (Fig. 2). However, 2,500 mg/kg ornithine normalized the intestinal damage by LPS (Fig. 1C). The Chui scores of this group were

shown in Fig. 2. However, aminoguanidine (100 mg/kg) did not prevent LPS-induced ileal injury (Fig. 1D, Fig. 2).

Discussion

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 1,400 W or by some nonspecific inhibitors of iNOS namely aminoguanidine [13-15]. However, it has been reported that large doses of non-selective NOS inhibitors may increase mortality, and the mechanism underlying this effect is not clear but might include impaired tissue perfusion due to excessive vasoconstriction, excessive suppression of cardiac contractility or inhibition of 'physiological' NO, which might be exerting cytoprotective effects [11, 12].

On the other hand, uptake of L-arginine has been proposed to be rate limiting under certain conditions for NO production [16]. These conditions most probably involve septic and endotoxemic states since iNOS needs further L-arginine after consuming up the intracellular store of the aminoacid to generate NO with a massive amount. Inhibition of the cationic aminoacid transport

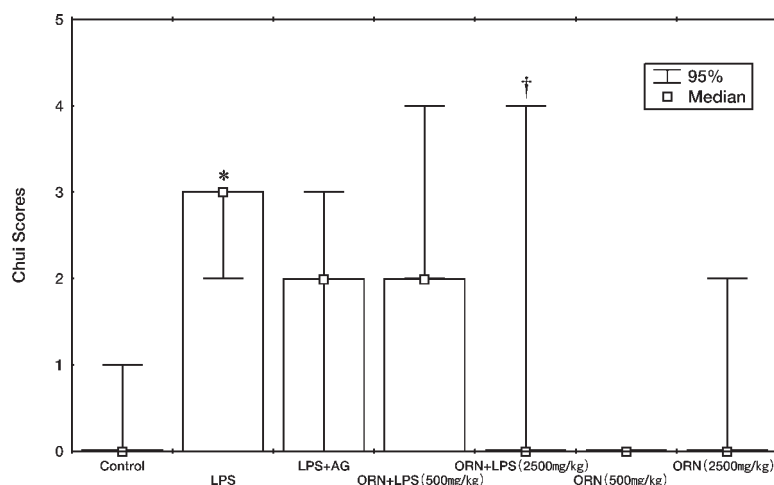


Fig. 2 Histopathologic scores of the bowel specimens obtained from the study groups. Treatment of L-ornithine (2,500 mg/kg) but not aminoguanidine (100 mg/kg) decreased ileal injury score by LPS administration. *, the difference from control; †, from LPS-administrated group. Comparison for intestinal injury scores was analyzed using Kruskal-Wallis variance analysis followed by Dunn test. Results are presented median and quartiles. The value of 95% indicates the quartiles and the rest is 5%. P values less than 0.05 were considered significant.

system with other chemically-related aminoacids such as L-lysine or L-ornithine may take an advantage by restricting the extra supply of L-arginine in endotoxemic condition but not the physiological formation of NO by the constitutive NOSs because intracellular L-arginine store is sufficient for those enzyme-catalysed NO production. Indeed, it has been demonstrated that L-arginine uptake is inhibited by L-lysine, L-homoarginine and L-ornithine [17, 18]. It has been suggested that constitutive NO formation be unaffected in sepsis or related states as it is physiologically necessary for the regulation hemodynamic parameters [11, 19]. One attempt has been made in ovine endotoxemia with L-lysine. However, the amino acid failed to restore the refractory hypotension but did L-NAME, a non-specific NOS inhibitor [8]. Neither did L-lysine change serum nitrite/nitrate level. Conversely, in rat endotoxemic model L-lysine administration caused the inhibition of NO production by iNOS but not by constitutive NOS (cNOS) [20]. In the present study, however, we have tried another inhibitor aminoacid, ornithine for the γ^+ system. Smaller dose of ornithine had no improving effects on either ileal histology or serum nitrite/nitrate level as well as lipid peroxidation in endotoxemic condition. One reason for the failure seems to be due to the insufficient amount of L-ornithine given. Accordingly, we examined higher dose (2,500 mg/kg), and it restored endotoxin-induced ileal damage. Likewise, the dose dramatically lowered both nitrite/nitrate and MDA levels, supporting the restorative effects of the aminoacid. On the other hand L-ornithine significantly decreased LPS-induced nitrite accumulation in murine bone marrow derived macrophages [21], confirming the results of our study. Apart from the relationship of L-ornithine with L-arginine: NO pathway, the aminoacid is also a substrate for polyamine synthesis. It is not known whether polyamine synthesis is increased with ornithine treatment, and possible increased level of polyamines have any effects on the parameters in this study. It has been reported that NO can inhibit ornithine decarboxylase which is the initial and rate limiting step in polyamine synthesis that is necessary for cell growth in mammals [22].

On the other hand, marginally-specific iNOS inhibitor, aminoguanidine did not improve ileal damage, although it dramatically diminished nitrite/nitrate overproduction and MDA levels in the study. This may reflect that restorative effect of ornithine might not be parallel with the decreased amount of nitrite/nitrate and MDA, or it has

improving effect via different mode of action, or aminoguanidine itself has deleterious effect on the ileal histology although it substantially lowered both MDA and nitrite/nitrate levels.

In conclusion, L-arginine uptake which is the rate limiting for NO biosynthesis can be inhibited by high dose of L-ornithine. Therefore, it seems to be a beneficial agent which can reduce noxious NO formation that could mediate tissue damage in the rat endotoxemia.

Acknowledgments. This work has been supported by the Turkish Academy of Sciences, in the framework of the Young Scientist Award Program (K.B./TÜBA-GEBİP/2002-1-5).

References

- O'Reilly M, Newcomb DE and Remick D: Endotoxin, sepsis and the primrose path. *Shock* (1999) 12: 411-420.
- van Dissel JT, van Langevelde P, Westendorp RG, Kwappenberg K and Frolich M: Anti-inflammatory cytokine profile and mortality in febril patients. *Lancet* (1998) 351: 950-953.
- Shieh P, Zhou M, Ornan DA, Chaudry IH and 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: 325-329.
- Doughty L, Carcillo JA, Kaplan S and Janosky J: Plasma nitrite and nitrate concentrations and multiple organ failure in pediatric sepsis. *Crit Care Med* (1998) 26: 157-162.
- Marin J and Rodriguez-Martinez MA: Role of vascular nitric oxide in physiological and pathological conditions. *Pharmacol Ther* (1997) 75: 111-134.
- Moncada S, Palmer RM and Higgs EA: Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacol Rev* (1991) 43: 109-142.
- Bogle RG, Baydoun AR, Pearson JD, Moncada S and Mann GE: L-arginine transport is increased in macrophages generating nitric oxide. *Biochem J* (1992) 284: 15-18.
- Allman, KG, Stoddart AP and Young JD: Effect of L-lysine on nitric oxide production in ovine endotoxaemia. *Br J Anaesth* (1998) 81: 188-192.
- Yagi K: Lipid peroxides and related radicals in clinical medicine; in *Radicals in Diagnostic Medicine*, Armstrong D ed, Plenum Press, New York (1994) pp 1-15.
- Chiu CJ, McArdle AH, Brown R, Scott HJ and Gurd FN: Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabol reappraisal. *Arch Surg* (1970) 101: 478-483.
- Vallance P, Rees D and Moncada S: Therapeutic potential of NOS inhibitors in septic shock; in *Handbook of experimental pharmacology*. Mayer B ed, Springer, New York (2000) pp 386-397.
- Vallance P and Moncada S: Role of endogenous nitric oxide in septic shock. *New Horiz* (1993) 1: 77-86.
- Lortie MJ, Ishizuka S, Schwartz D and Blantz RC: Bioactive products of arginine in sepsis: Tissue and plasma composition after LPS and iNOS blockade. *Am J Physiol Cell Physiol* (2000) 278: C1191-1199.
- Parkinson JF: Nitric oxide synthase inhibitors I: Substrate analogs and heme ligands; in *Handbook of Experimental Pharmacology*. Mayer B ed, Springer, New York (2000) pp 111-135.
- Cinel I, Büyükaşar K, Cinel L, Polat A, Atıcı Ş, Tamer L and Oral U:

- The role of poly(adp-ribose) synthetase inhibition in preventing endotoxemia-induced intestinal epithelial apoptosis. *Pharmacol Res* (2002) 46: 119-127.
16. Bogle RG, Coade SB, Moncada S, Pearson JD and Mann GE: Bradykinin and ATP stimulate L-arginine uptake and nitric oxide release in vascular endothelial cells. *Biochem Biophys Res Commun* (1991) 180: 926-932.
 17. Bogle RG, Moncada S, Pearson JD and Mann GE: Identification of inhibitors of nitric oxide synthase that do not interact with the endothelial cell L-arginine transporter. *Br J Pharmacol* (1992) 105: 768-770.
 18. Wu F, Cholewa B and Mattson DL: Characterization of L-arginine transporters in rat renal inner medullary collecting duct. *Am J Physiol Regul Integr Comp Physiol* (2000) 278: R1506-1512.
 19. Tracey WR, Tse J and Carter G: Lipopolysaccharide-induced changes in plasma nitrite and nitrate concentrations in rats and mice: Pharmacological evaluation of nitric oxide synthase inhibitors. *J Pharmacol Exp Ther* (1995) 272: 1011-1015.
 20. Liaudet Y, Gnaegi A, Rosselet A, Markert M, Boulat O, Perret C and Feihl F: Effect of L-lysine on nitric oxide overproduction in endotoxic shock. *Br J Pharmacol* (1997) 122: 742-748.
 21. Kierner AK and Vollmar AM: Induction of L-arginine transport is inhibited by atrial natriuretic peptide: A peptide hormone as a novel regulator of inducible nitric-oxide synthase substrate availability. *Mol Pharmacol* (2001) 60: 421-426.
 22. Bauer PM, Buga GM, Fukuto JM, Pegg AE and Ignarro LJ: Nitric oxide inhibits ornithine decarboxylase via S-nitrosylation of cysteine 360 in the active site of the enzyme. *J Biol Chem* (2001) 276: 34458-34464.