INHIBITION OF NITRIC OXIDE SYNTHESIS AMELIORATES BURN-INDUCED REMOTE ORGAN INJURY IN RATS: A LIGHT MICROSCOPIC STUDY

Özlem Güneysel, M.D.* / Berna K. Oktar, M.Sc. / Berrak Ç. Yeğen, M.D.** Cumhur Yeğen, M.D. ***/ Serap Arbak, Ph.D.*****

* *Department of Emergency Medicine, School of Medicine, Marmara University, Istanbul, Turkey.*

** *Department of Physiology, School of Medicine, Marmara University, Istanbul, Turkey.*

*** *Department of General Surgery, School of Medicine, Marmara University, Istanbul, Turkey.*

**** *Department of Histology and Embryology, School of Medicine, Marmara University, Istanbul, Turkey.*

ABSTRACT

Objective: To investigate the role of endogenous nitric oxide (NO) on remote organ injury in the early phase of burn trauma.

Methods: Wistar albino rats (200-300 g) were exposed to 90°C (burn) or 25°C (sham) water bath for 10 sec.

Results: Microscopic score in the stomach was increased in the burn group compared to sham. Hemorrhage areas, epithelial desquamation and glandular cell degeneration were observed in the gastric mucosa of the burn group. N^G-nitro-L-arginine methyl ester (L-NAME) treatment reduced the burn-induced damage score with an intact glandular architecture. In N^G-nitro-D-arginine methyl ester (D-NAME) pretreated groups, histologic scores were not different than burn group, with desquamated and degenerated surface epithelium. L-arginine (L-arg) plus L-NAME pretreatment partially reversed this effect with prominent gastric mucosal damage. In the liver, the microscopic score was increased in the burn group compared to sham. Hepatocyte vacuolar degeneration, sinusoidal congestion and increased number of Kupffer cells were observed in the burn group. Hepatic injury was slightly attenuated by L-NAME treatment whereas D-NAME or L-arg plus L-NAME pretreatment was ineffective.

Conclusion: In conclusion, inhibition of NO synthesis ameliorates gastric and hepatic damage, emphasizing the critical role of NO in the burn-induced remote organ injury.

Key Words: Burns, Nitric oxide, Remote organ injury, Myeloperoxidase activity

INTRODUCTION

Thermal trauma continues to be a serious clinical problem exposing patients to significant mortality and morbidity (1,2). Thermal injury may cause damage to multiple organs distant from the original burn wound and may lead to multiorgan failure (3). According to the clinical and experimental research findings, a local burn

(Accepted 27 March, 2002)

Marmara Medical Journal 2002;15(3):155-160

Correspondence to: Serap Arbak, M.D. - Department of Histology and Embryology
Marmara University, School of Medicine, Haydarpaşa, 81326, İstanbul, Turkey
e.mail address: arbaks@yahoo.com

insult produces oxidant-induced organ changes as evidenced by increased lipid peroxidation in lung, liver and gut (4). In fact, generalized tissue inflammation is present in uninjured organs within hours of injury, even in the absence of shock. It has been reported that circulating endotoxins become evident probably as a result of burn wound colonization and an early gut leak in experimental models of stress and injury (5,6). Endotoxin and other bacterial by-products are potent activators of the macrophage and neutrophil. This leads to the release of massive amounts of oxidants, arachidonic acid metabolites, proteases, which cause further local and systemic inflammation induced tissue damage (7).

Nitric oxide (NO), which was formerly named as endothelium-derived relaxing factor by Furchgott and Zawadzki (8) in 1980, is a local autocoid (9). NO is synthesized from a guanido group of the amino acid L-arginine by different NO synthase (NOS) enzymes. It is produced by all mammalian cells, including the endothelial cells, neurons of the central and enteric nervous system and cells of the immune system. The endogenous transcellular messenger NO, with its ubiquitous distribution and diverse mechanism of action, has been increasingly perceived to be important in a wide variety of physiological and pathophysiological pathways (10). It is thought to play a significant physiological role in the regulation of gastrointestinal motility, blood flow and mucosal protection (11). However, in addition to its important functions under normal conditions and in acute inflammation, there is strong evidence that during chronic inflammation too much NO may have detrimental effects (12). Clinical studies have shown that human burn injury is associated with an increase in NO production (13). It has been shown that inhibition of inducible form of NOS (iNOS) improves the intestinal barrier function after thermal injury in rats (14). It has also been shown that thermal injury induces intestinal mucosal iNOS, and inhibition of iNOS decreases intestinal permeability and bacterial translocation incidence to mesenteric lymph node concurrently (15).

This study was carried out to assess the role of endogenous NO on remote organ injury following burn trauma, where the severity of burn-injury was evaluated with a histological approach.

MATERIALS AND METHODS

Animals

Wistar albino rats of both sexes (200-300 g) were fasted for 12 h, but were allowed free access to water before burn injury. Rats were kept in a room at a constant temperature of 22 ± 2°C with 12 h light and dark cycles in individual wire-bottomed cages and fed standard rat chow. This study was approved by Marmara University School of Medicine Animal Care and Use Committee.

Experimental Protocol

Rats were anesthetized with ketamine (100 mg/kg, ip) and chlorpromazine (0.75 mg/kg) and the dorsum of the rats was shaved, exposed to 90°C water bath for 10 sec, which resulted in a partial-thickness second-degree skin burn involving 30% of the total body surface area. Immediately after burn injury, subcutaneous administration of 10 ml/kg saline was performed for water resuscitation. The rats in sham group were treated identically except that they were dipped in a 25°C water bath for 10 sec.

In the burn groups, saline (1 ml/kg, intravenously, iv; n=8) or N^G-nitro-L-arginine methyl ester (L-NAME; Sigma Chemical, St. Louis, MO, USA; 10 mg/kg, iv bolus; n=8), a NOS blocker, or N^G-nitro-D-arginine methyl ester (D-NAME; Sigma Chemical, St. Louis, MO, USA; 10 mg/kg, iv bolus; n=8), an enantiomere of L-NAME, was administered 30 min before and 9 h after the burn injury. In another group of rats, L-arginine (L-arg; Sigma Chemical, St. Louis, MO, USA; n=8) was given as an exogenous NO precursor at 100mg/kg (iv; bolus) dose 5 min before L-NAME treatment (n=8). At the 24th hour following burn injury, the rats were decapitated and liver, lung and stomach samples were placed in 10 % (vol/vol) formaline solution and processed routinely by embedding in paraffin. Tissue sections (4-5 μm) were stained with Hematoxylin and Eosin and examined under a light microscope. (Olympus-BH-2) Histological assessments were made by an experienced histologist who was unaware of the treatment conditions. The severity of tissue damage was scored according to previously defined criteria (16), (Table I).

Table I: The criteria for histological scoring of tissues

Score	Lung	Liver	Stomach
0	Normal	Normal	Normal
1	Vascular congestion	Enlargement and vacuolization of hepatocyte	Desquamation of surface epithelial cells
2	Vascular congestion and interstitial edema	Vascular congestion and sinusoidal dilatation	Degeneration of both epithelial and pit-lining cells
3	Alveolar structural disturbance	Moderate enlargement in Kupffer cells	Mild degeneration of glandular cells
4	Massive alveolar structural disturbance and infiltration of inflammatory cells	Prominent enlargement in Kupffer cells	Severe degeneration of glandular cells and mucosal hemorrhage

Statistical analysis

Data are expressed as means ±SEM. Statistically significant differences among groups were identified using analysis of variance (ANOVA) followed by Tukey-Kramer test. Differences were considered to be significant at $p<0.05$.

RESULTS

In the saline-treated burn group, microscopic score in the stomach (3.17±0.17) was found to be significantly elevated compared to sham group (0.88±0.24; $p<0.001$) (Fig 1). Sham group gastric mucosa reflected a normal morphology (Fig. 2a), whereas distinct hemorrhage areas extending up to submucosa, epithelial desquamation and glandular cell degeneration were obviously observed in the gastric mucosae of the saline-treated burn group (Fig. 2b). Burn-induced damage score was reduced by L-NAME treatment (Fig. 1; $p<0.01$). The sections of L-Name group represented an intact glandular architecture with desquamated surface epithelium (Fig. 2c). In D-NAME pretreated groups, histologic scores (2.38±0.24) were not different than saline-treated burn group. On the other hand, L-arg plus L-NAME pretreatment partially reversed this effect with prominent gastric mucosal damage (2.25±0.15; $p<0.05$).

In the liver, the microscopic score was also found to be increased in the burn group (2.63±0.24) compared to sham group (1.5±0.20; $p<0.01$) (Fig. 3), while normal liver morphology was reflected in the sections of the sham group (Fig. 4a). Hepatocyte vacuolar degeneration, sinusoidal congestion and increased number of Kupffer cells suggested a critical degree of liver damage in the saline-treated burn group (Fig. 4b). Hepatic injury was slightly attenuated by L-NAME treatment (2.0 ± 0.0; $p<0.05$) (Figs. 3,4c), whereas D-NAME (2.33±0.33) or L-arg plus L-NAME (2.26±0.15) pretreatment was ineffective. In the lung, there was no significant difference in the microscopic scores between the sham (3.0±0.1) and saline-treated burn (2.67±0.3) groups. L-NAME-, D-NAME or L-arg plus L-NAME-treatments had no significant effects on lung tissue that was not affected by the burn insult (1.83±0.44; 2.5±0.5; 3.0±0.0, respectively).

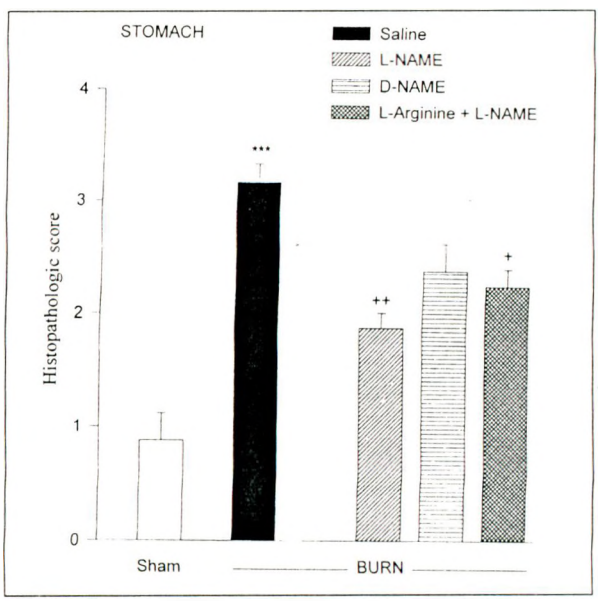

Fig. 1: Histopathologic scores in the stomachs of burn groups with different treatment regimens. ***$p<0.001$, compared to sham group; +$p<0.05$ and ++$p<0.01$, compared to saline-treated group.

Thus, in all experimental groups only slightly injured lung histology was observed (Data not shown).

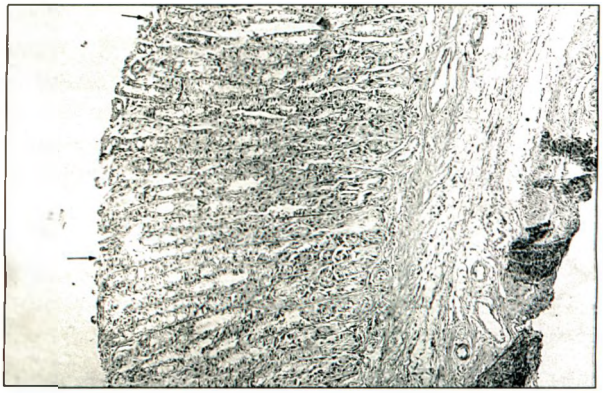

Fig.2 (a) : Sham group micrograph demonstrates normal morphology of stomach mucosa with slight surface epithelial degeneration (→), H&E X33;

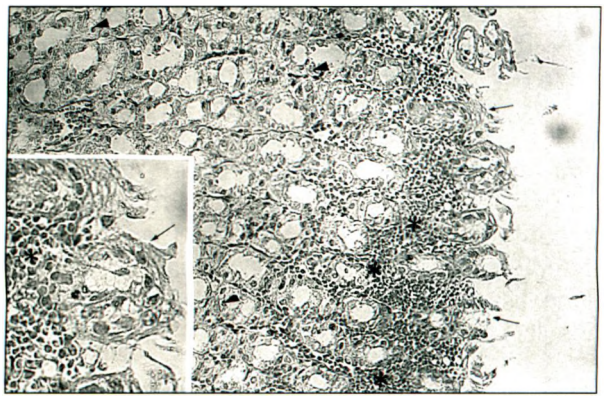

Fig.2 (b) : Saline-treated burn group micrograph indicates surface epithelial desquamation (→), glandular cell degeneration (➢) and widespread hemorrhagic areas (*), H&E X66. Inset: superficial epithelial desquamation (→) and hemorrhagic areas (*), H&E X132;

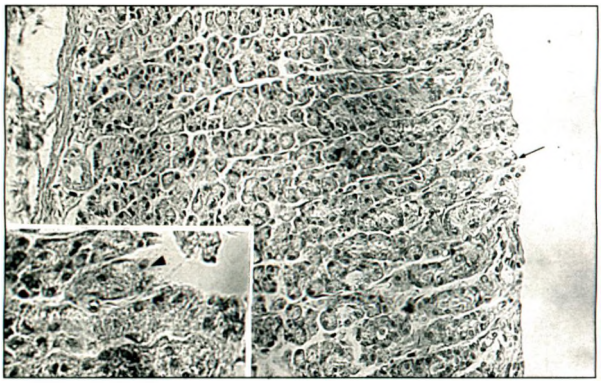

Fig.2 (c) : (c) L-NAME group micrograph: mild degree of gastric mucosal damage (→) and normal morphology of glandular structures. H&E X66. Inset: degenerated superficial epithelial cells (➢), H&E X132.

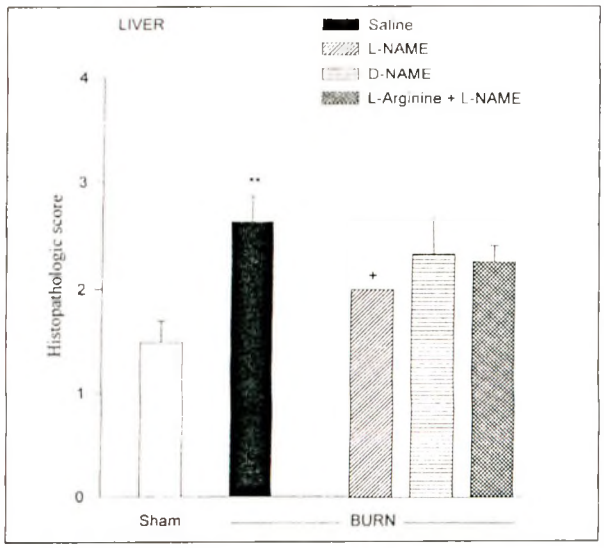

Fig.3: Histopathologic scores in the livers of burn groups with different treatment regimens. **p<0.01, compared to sham group; +p<0.05, compared to saline-treated group.

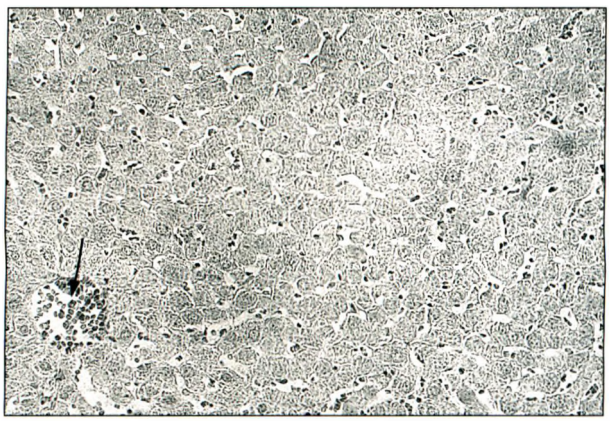

Fig.4 (a) : Sham group micrograph indicates mild degree of congestion (→) with normal liver lobular morphology. H&E X66;

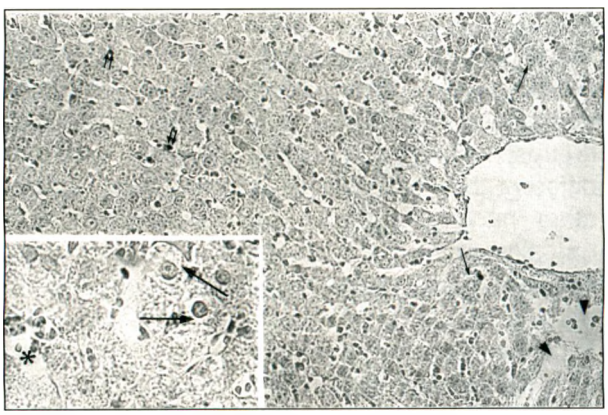

Fig.4 (b) : Saline-treated burn group liver micrograph demonstrates swollen hepatocytes (→), dilated sinusoids (➢), and prominent enlargement in Kupffer cells (→), H&E X66. Inset: hepatocytes with vacuolar degeneration (*), H&E X132;

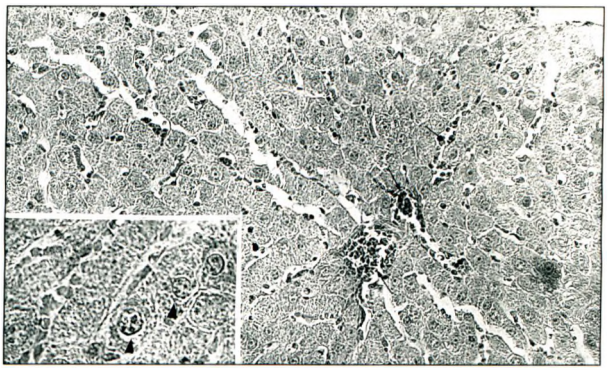

Fig.4 (c) : L-NAME group micrograph demonstrates a regular morphology of liver lobulus and sinusoids with congestion (→), H&E X66. Inset: hepatocyte vacuolization reflecting a mild degree of degeneration (➢), H&E X132.

DISCUSSION

In the present study, burn-induced remote organ damages were found to be severe in the gastric and hepatic tissues, whereas a slight degree of injury was noticed in the lung. Since a significant degree of reduction was observed in the severity of liver and stomach injuries through the inhibition of NOS and this reduction was cancelled by adding L-arginine as a precursor of NO, it is likely to conclude that endogenous NO has a significant exacerbatory role in the pathogenesis of burn-induced remote organ injury.

It has been concluded that both endogenous and exogenous NO reduce the sequelae of acute gastrointestinal inflammation. However, as the inflammatory response progresses from acute to a chronic state, the beneficial effect of NO may be lost (12). The transition from acute to chronic inflammation and the divergent effects of NO synthesis are best exemplified in a model of septic shock. In the first hour of sepsis, intestinal injury was greatly enhanced when NO synthesis was inhibited (17). However, as sepsis progressed, intestinal injury progressively worsened and NO synthesis inhibition with L-NAME no longer exacerbated the injury but significantly reduced intestinal injury in a dose-dependent manner (18). These conflicting results have been attributed to the fact that early in sepsis, endothelial NO maintains adequate perfusion of organs, and its inhibition leads to blood flow maldistribution and tissue injury. However, the overproduction of NO as sepsis progresses causes tissue injury, where the inhibition of NO under these conditions is beneficial (12). In the present study, the histologic analysis of the organs were made at the postburn 24 hours, when the local injury is already expected to be replaced by a systemic inflammatory response. Thus, inhibition of NO is likely to reduce the severity of remote organ injury.

Localized thermal injury is potentially important by affecting numerous systems and resulting in microcirculatory changes that cause multiorganal damage. It could disturb vital functions as well as causing late complications in the body. The local tissue trauma activates a number of systemic mediator cascades, such as complement activation, arachidonic acid release, and cytokine–interleukin-1 and tumor necrosis factor-production. In several experimental and clinical studies of septic shock, the data suggest that NO production is enhanced (19,20). Plasma nitrite and nitrate levels, the stable end products of NO in vivo, were shown to be elevated in burns (13,21). On the other hand, for patients who sustain minor burns, plasma levels of nitrate were decreased from those of normal controls (22). These data strongly suggest that it is not possible to comment on whether NO production in the vicinity of the wound is increased in small thermal burns, but clinically detectable multiple organ dysfunction is accompanied with increased NO production (22). Failure of gastrointestinal mucosa to act as a barrier against bacterial translocation has been proposed as a potential source of sepsis and subsequent multiple organ failure following burn insult. Chen et al. (14) demonstrated that S-methylisothiourea, a specific inhibitor of iNOS, improved the postburn barrier function by suppression of the intestinal mucosal iNOS activity, which resulted in decreased formation of peroxynitrite and subsequently decreased damage of mucosal tissue. Similarly, in acute gastric mucosal injury induced by ischemia-reperfusion, administration of L-NAME attenuated both the increase in NO level and gastric mucosal lesions (23). In accordance with these results, our findings also demonstrate that L-NAME treatment reduces hepatic and gastric damage due to local burn trauma, indicating a detrimental role of NO in the pathogenesis of burn-induced remote organ injury.

In conclusion, the result of the present study indicates that burn trauma resulted in remote organ injury in the liver and stomach, where

failure of gut barrier function due to burn injury has direct effects through bacterial translocation. It is well known that burn injury accompanied with smoke-inhalation has severe complications on lung morphology. However, in the present burn model, which lacks smoke-inhalation, lung tissue was not affected much as compared to other remote organs. Our results also indicate that L-NAME treatment, through the inhibition of NO synthesis, ameliorated gastric and hepatic damage, emphasizing the critical role of NO in the burn-induced remote organ injury.

Acknowledgement: This study was supported by a grant from Marmara University Research Fund.

REFERENCES

1. Moncrief JA. Effect of various fluid regimens and pharmacologic agents on the circulatory hemodynamics of the immediate postburn period. Ann Surg 1966;164:723-752.
2. Martyn JAJ, Snider MT, Szyfelbein SK, Burke JF, Laver MB. Right ventricular dysfunction in acute thermal injury. Ann Surg 1980;191:330-335.
3. Bonate PL. Pathophysiology and pharmacokinetics following burn injury. Clin Pharmacokinet 1990;18:118-130.
4. Demling RH, LaLonde C. Systemic lipid peroxidation and inflammation induced by thermal injury persists into the post resuscitation period. J Trauma 1990;30:69-71.
5. Jones II WG, Minei JP, Barber AE, Fahey III TJ, Shires III GT, Shires GT. Splancnic vasoconstriction and bacterial translocation after thermal injury. Am J Physiol 1991;261:H1190-H1196.
6. Morris SE, Navaratnam N, Townsend CM, Herndon DN. A comparison of the effect of thermal injury and smoke inhalation on bacterial translocation. J Trauma 1990;30:639-643.
7. Youn Y-K, LaLonde C, Demling R. The role of mediators in the response to thermal injury. World J Surg 1992;16:30-36.
8. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288:373-376.
9. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: Physiology, pathophysiology and pharmacology. Pharmacol Rev 1991;43:109-142.
10. Nussler AK, Billiar TR. Inflammation, immunoregulation, and inducible nitric oxide synthase. J Leukocyte Biol 1993;54:171-178.
11. Lloyd KC. Gut hormones in gastric function. Ballières Clin Endocrinol Metab 1994;8:111-136.
12. Alican İ, Kubes P. A critical role for NO in intestinal barrier function and dysfunction. Am J Physiol 1996;33:G225-G237.
13. Preiser JC, Reper P, Vlasselaer D, et al. Nitric oxide production is increased in patients after burn injury. J Trauma 1996;40:368-371.
14. Chen LW, Hsu CM, Wang JS, Chen JS, Chen SC. Specific inhibition of iNOS decreases the intestinal mucosal peroxynitrite level and improves the barrier function after thermal injury. Burns 1998;24:699-705.
15. Chen LW, Hsu CM, Cha MC, Chen JS, Chen SC. Changes in gut mucosal nitric oxide synthase (NOS) activity after thermal injury and its relation with barrier failure. Shock 1999;11:104-110.
16. Demling R, LaLonde C, Knox J, Youn Y, Zhu D, Daryani R. Fluid resuscitation with deferoxamine prevents systemic burn-induced oxidant injury. J Trauma 1991; 31: 538-544.
17. Hutcheson IR, Whittle BJR, Boughton-Smith NK. Role of nitric oxide in maintaining vascular integrity in endotoxin-induced acute intestinal damage in the rat. Br J Pharmacol 1990;101:815-820.
18. Boughton-Smith NK, Evans SM, Laszlo F, Whittle BJR, Moncada S. Br J Pharmacol 1993;110:1189-1195.
19. Ochoa JB, Udekwu AO, Billiar TR, et al. Nitrogen oxide levels in patients after trauma and during sepsis. Ann Surg 1991;214:621-626.
20. Gomez-Jimenez J, Salgado A, Mourelle M, et al. L-Arginine: nitric oxide pathway endotoxaemia and human septic shock. Crit Care Med 1995;23:253-258.
21. Gamelli RL, George M, Sharp-Pucci M, Dries DJ, Radisavljevic Z. Burn-induced nitric-oxide release in humans. J Trauma 1995;39:869-877.
22. Harper R, Parkhouse N, Green C, Martin R. Nitric oxide production in burns: plasma nitrate levels are not increased in patients with minor thermal injuries. J Trauma 1997;43:467-474.
23. Wada K, Kamisaki Y, Ohkura T, et al. Direct measurement of nitric oxide release in gastric mucosa during ischemia-reperfusion in rats. Am J Physiol 1998;274:G465-G471.