International Journal of Pediatric Otorhinolaryngology (2003) **67**, 957–963

International Journal of Pediatric Otorhinolaryngology

www.elsevier.com/locate/ijporl

Bronchoscopy induces intestinal mucosal barrier dysfunction: a possible role for nitric oxide☆

Ali Nayci[a,*], Sibel Atis[b], Derya Umit Talas[c], Ayse Polat[d], Ozlen Bagdatoglu[e], Gurbuz Polat[e]

[a] *Department of Pediatric Surgery, School of Medicine, Mersin University, Mersin, Turkey*
[b] *Department of Chest Diseases, School of Medicine, Mersin University, Mersin, Turkey*
[c] *Department of Otorhinolaryngology, School of Medicine, Mersin University, Mersin, Turkey*
[d] *Department of Pathology, School of Medicine, Mersin University, Mersin, Turkey*
[e] *Department of Biochemistry, School of Medicine, Mersin University, Mersin, Turkey*

Received 12 December 2002; received in revised form 23 April 2003; accepted 23 April 2003

KEYWORDS
Bronchoscopy;
Bacterial translocation;
Intestinal barrier dysfunction;
Mucosal damage;
Nitric oxide

Summary *Objective:* This study investigates the effect of bronchoscopy on intestinal mucosal barrier function and its association with intestinal nitric oxide production. *Methods:* 30 rats were used. The study group ($n = 15$) underwent rigid bronchoscopy. At 24 h following bronchoscopy, ileal nitrite/nitrate levels were evaluated. The ileum was also examined for mucosal damage, and graded according Chiu's histologic injury scale. *Results:* In the bronchoscopy group, the ileal nitrite/nitrate levels were significantly higher than those of controls (398.5 ± 85.1 and 44.5 ± 6.6 nmol/g tissue, respectively, $P = 0.001$). In the bronchoscopy group, the mucosal damage was significant, compared with those of controls (mean ranks, 22.8 and 8.2, $P < 0.0001$). The changes varied from denuded villi and dilated capillaries to significant architectural distortion, lamina propria disintegration, ulceration and hemorrhage. Significant correlation was found between ileal nitrite/nitrate levels and mucosal damage in the bronchoscopy group ($r_s = 0.56$, $P = 0.03$). *Conclusion:* This study suggests that bronchoscopy induces intestinal mucosal barrier dysfunction in association with excess intestinal nitric oxide production. These events may be involved in mechanisms responsible for bacterial translocation after bronchoscopy.

1. Introduction

Rigid tracheobroncoscopy for pediatric airway assessment is a commonly performed procedure. Bronchoscopy has been used successfully in pediatric patients for the management of tracheobronchial foreign bodies, dilatation of tracheal strictures, placement of airway stents, obtaining of bronchoalveolar lavage effluent, control of massive hemorrhage, removal of tenacious mucous

☆ This study was presented at the European Respiratory Society Congress, September 14–18, 2002, Stockholm (The study obtained the "Interventional Pulmonology Travel Award" for the best presentation for investigators aged 35 years old and under at the ERS Stockholm 2002 Congress).

*Corresponding author. Tel.: +90-324-325-4277; fax: +90-324-328-8742.
E-mail address: anayci@mersin.edu.tr (A. Nayci).

0165-5876/03/$ - see front matter
doi:10.1016/S0165-5876(03)00161-7

plugs and clots, and execution of lacer procedures. However, bronchoscopy has the potential to propagate infection. Studies have reported pneumonia, fever, or bacteremia following bronchoscopy [1–4]. Moreover, some reports have noted clinical features suggestive of sepsis, especially in immune deficient patients [5]. The tendency for bronchoscopic patients to develop infections is commonly related to carrying the indigenous microbial flora of the oropharynx and nasopharynx to distal regions, and impairment of the mucociliary function after bronchoscopic procedures [6]. Yet, very little attention has been focused on the indigenous microflora of the gastrointestinal tract of the bronchoscopy patient, as a possible source of bacteremia and systemic infection.

During the last decade, translocation of bacteria has been increasingly recognized as one of the potential causes of bacteremia and systemic infections, especially, in the absence of an identifiable focus of infection. Bacterial translocation is defined as the passage of viable indigenous bacteria, or their endotoxins across the intestinal mucosal barrier to sterile body sites such as mesenteric lymph nodes (MLNs), bloodstream, spleen, and the liver. The passage of bacteria through mucosa and survival in extra-intestinal sites depend on several factors, including bacterial overgrowth, immune deficiency status, and/or intestinal mucosal barrier dysfunction [7].

The mechanisms by which mucosal barrier function is altered and bacteria cross the intestinal wall has not been fully elucidated. Studies indicate that intestinal injury, mucosal barrier dysfunction and bacterial translocation are associated with increased nitric oxide (NO) production [8–11]. There is growing evidence that endogenous NO regulates mucosal barrier integrity under physiological conditions and counters the increase in mucosal permeability associated with acute pathophysiological states. NO is produced from L-arginine by nitric oxide synthase (NOS) in all mammalian cells. There are three isoforms of this enzyme in the intestine, consisting of two constitutive, the calcium-dependent forms (cNOS, endothelial and neuronal), and one inducible, the calcium-independent form (iNOS). The cNOS is present in small amounts, and regulates gastrointestinal blood flow. iNOS, has been shown to be upregulated in response to inflammatory stimuli and implicated in the breakdown of epithelial integrity [12].

In a preceding animal study, we have shown that enteric bacteria could penetrate through the mucosal barrier to extraintestinal sites, including MLNs, spleen, liver, and lung after bronchoscopy [13]. However, the mechanisms of bacterial translocation following bronchoscopy remains unclear. This study investigates the histological changes of the ileum following bronchoscopy and its relationship with intestinal NO production.

2. Materials and methods

2.1. Experimental design

Wistar-albino rats weighing 200–220 g were used. The rats had free access to standard laboratory diet and water. The animals were maintained according to the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals.

The experimental protocol was approved by the Animal Care and Use Committee, University of Mersin, Turkey. The rats were divided into two groups. Fifteen rats were used in the study group and underwent rigid bronchoscopy. Fifteen rats served as the control group. The animals were anesthetized with intramuscular 50 mg/kg ketamine hydrochloride (Ketalar; Eczacibasi-WL, Istanbul, Turkey).

Basically, we developed a bronchoscope that was a rigid, straight and hollow metallic tube. A 14-gauge angiocath (Braun, Melsungen, Germany) was prepared as the rigid bronchoscope. The length was 70-mm and the diameter was 2.2 mm. The tip of the cannula was rasped and smoothened. Four paired holes were opened on the distal third of the cannula. The bronchoscopy was inserted transorally into the trachea. Once the trachea was entered, the tip of the bronchoscope was gently slid along the right and left lateral wall of the trachea. This maneuver provided to easily enter either the right and left main stem bronchi. Excessive secretions were thoroughly aspirated. This was accomplished by inserting a long metal cannula through the lumen of the bronchoscope. Bronchoscopy lasted for 30 min in each rat. The control group underwent an identical procedure, except for the bronchoscopic instrumentation. After 24 h, samples from the ileum, 2-cm to the ileocecal valve were obtained. The segment was excised into two equal strips of 2×5 mm. One specimen was fixed in 10% neutral formalin for histologic examination. The remaining half was stored at $-20\,^{\circ}C$ until processing for NO production.

2.2. Analysis of intestinal NO production

The stable NO oxidation products, nitrite (NO_2^-) and nitrate (NO_3^-), were determined in ileum using a

procedure based on the Griess reaction (Nitric oxide colorimetric assay, 1-756-281, Roche Diagnostics GmbH, Mannheim, Germany). Samples of ileum were homogenized in 2 × lysis buffer (1 × lysis buffer: 1% Triton X-100, 20 mM Tris/HCl, pH 8.0, 137 mM NaCl, 10% glycerol, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 15 μg leupeptin per ml). The tissue extract was cleared by centrifugation and the supernatant diluted 1:1 with water. Equal volumes of the homogenate and potassium phosphate buffer were placed in an ultrafilter and centrifuged at 4000 rpm for 45 min. The ultrafiltrate was collected and used in the test. The nitrate present in the sample was enzymatically reduced to nitrite by NADPH and FAD, in the presence of nitrate reductase, incubated for 30 min at room temperature. *N*-1-(naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide in 5% H_3PO_4 and test solutions were mixed at a ratio of 1:1:2 (v/v), incubated for 5 min at room temperature in dimmed light and measured at 540 nm. Sodium nitrite of 1.00 mM was used as standard. The value was expressed as the nanomol/gram tissue (nmol/g tissue) amount of nitrite plus nitrate [14].

2.3. Processing of histological samples

Ileal samples were immersed in 10% formaldehyde solution. The samples were embedded in paraffin wax, serially sectioned and stained with hematoxylin/eosin. Intestinal morphologic characteristics were assessed and graded under light microscopy according to the histologic injury scale defined by Chiu et al. [15]. Mucosal damage was graded from 0 to 5 according to the following criteria: Grade 0, normal mucosal villi; Grade 1, development of subepithelial Gruenhagen's space at the apex of the villus, often with capillary congestion; 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 sides of villi, possibly with a few denuded tips; Grade 4, denuded villi with lamina propria and dilated capillaries, and increased cellularity of lamina propria; Grade 5, digestion and disintegration of the lamina propria, hemorrhage, and ulceration.

2.4. Statistics

Descriptive and data analyses were performed with the statistical package for the social science (SPSS 9.05). Data were represented as mean ± standard error of mean (SEM). Mann–Whitney *U* test was used for comparison of nitrite/nitrate levels and histopathologic grading between the groups. *Spearman correlation analysis* was performed to evaluate the relationship between nitrite/nitrate levels and histological changes. *P* values less than 0.05 were considered to be statistically significant.

3. Results

All animals survived the bronchoscopic procedure. NO production, as reflected in supernatant ileal nitrite/nitrate levels, was significantly higher in the bronchoscopy group compared with controls (Fig. 1). Ileal NO production of normal control rats averaged 44.5 ± 6.6 nmol/g tissue. However, 24 h after bronchoscopy, ileal NO production significantly increased to 398.5 ± 85.1 nmol/g tissue in the study group ($P = 0.001$).

Increased NO production following bronchoscopy was paralleled by significant intestinal mucosal damage. Histological examination of control animals revealed normal appearing ileum (mean rank = 8.2) (Fig. 2a). However, at 24 h after bronchoscopy, there were gross histopathological changes observed in the ileum (mean rank = 22.8) ($P < 0.0001$). The changes varied from edema, shortened/denuded villi and dilated capillaries to significant architectural distortion, lamina propria disintegration, ulceration and hemorrhage (Fig. 2b). The histologic appearance in animals within the study group was highly variable.

In addition, there was a significant positive correlation between ileal nitrite/nitrate levels and mucosal damage in the bronchoscopy group ($P = 0.03$). The linear regression correlation coefficients between them were $r_s = 0.56$ (Fig. 3).

4. Discussion

In the present study we found that bronchoscopy induces intestinal mucosal damage in association with excess intestinal NO production.

In the intestine there is a homeostasis between intraluminal bacteria, endotoxins, and the mucosal barrier. During the last decade, evidence has accumulated suggesting that loss of the intestinal mucosal barrier play a role in the development of bacteremia and systemic infections [9]. Consequently, a large number of studies have been carried out investigating potential mechanisms by which diverse insults could impair intestinal mucosal barrier function and promote bacterial translocation. For instance, lipopolysaccharide administration to experimental animals has been shown to cause derangement in intestinal barrier

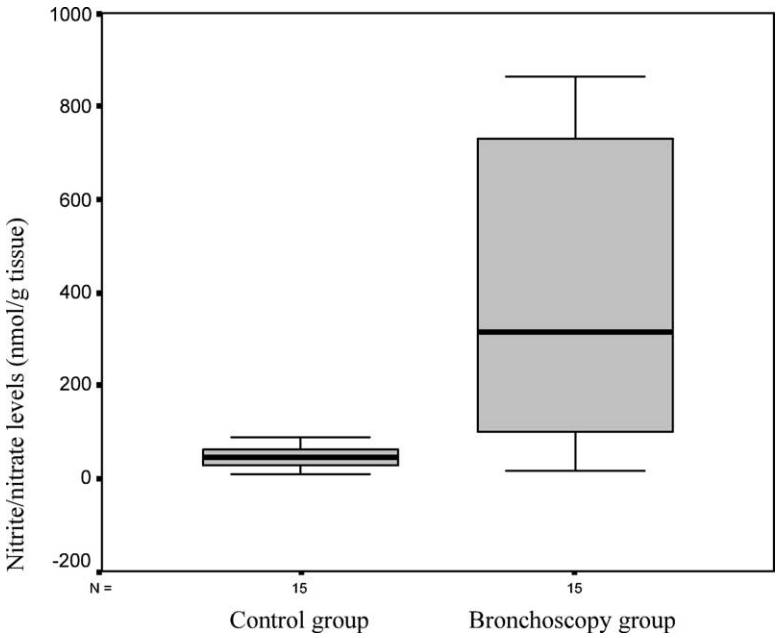


Fig. 1 Changes of intestinal nitrite (NO_2^-) and nitrate (NO_3^-) concentrations, 24-h following bronchoscopy ($P = 0.001$).

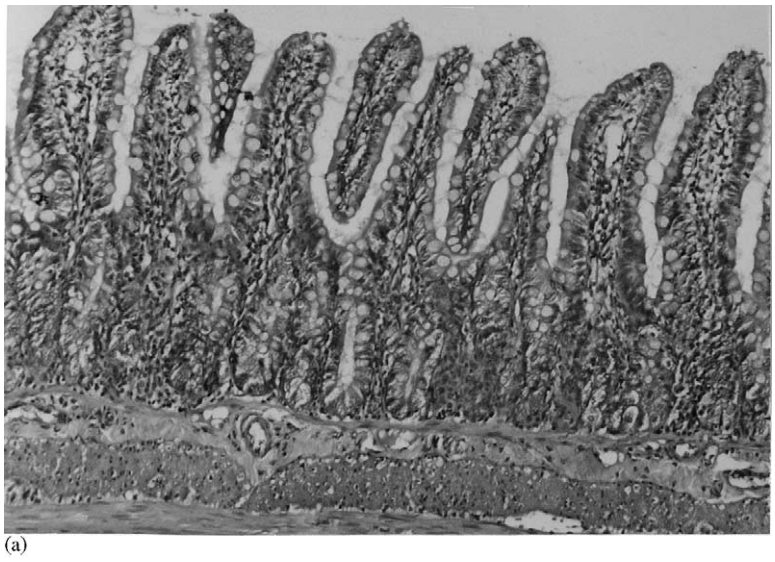
function and intestinal permeability increase, manifested by an increased incidence of bacterial translocation from the intestinal lumen to MLNs and other organs [16,17]. Thermal injury has been shown to cause mucosal barrier dysfunction, resulting in intestinal permeability increase, and translocation of bacteria and endotoxins [10].

Most studies investigating a possible relationship between mucosal barrier function and bacterial translocation have focused on histological changes in the small intestine [7]. In the small intestine, a single-cell epithelial layer lines the crypts and covers the villi. Changes in mucosal architecture are easily measured by light microscopy. Villus height, mucosal thickness, and crypt depth are the parameters most frequently measured and have been applied in numerous animal and patient studies. Injury, ranging from reversible permeability changes to structural mucosal damage is commonly cited as representing alterations in intestinal-barrier function [18]. In many studies, they are in effect being used as a quantitative index of injury or dysfunction of the intestinal mucosal barrier, and the alterations in these parameters might indicate an increased propensity to bacterial translocation [10,18]. In our data, histologic examination showed mucosal damage manifested as denudation of the villus epithelium, reduction in villus height, epithelial necrosis, and infiltration of lymphocytes and polymorphonuclear leucocytes, after bronchoscopy. These characteristics were more evident in rats with bacterial translocation. Bronchoscopy may have a remote deleterious effect on intestinal epithelium barrier function.

The physiologic events leading to mucosal damage and breakdown of the mucosal barrier is poorly understood. Recently, a correlation between exaggerated NO production induced by endotoxin or endogenous inflammatory mediators and the loss of mucosal barrier function has been documented [9–11]. The studies have shown that endotoxin-induced mucosal damage, loss of barrier function and bacterial translocation were associated with increased iNOS activity and NO production [8,11]. Moreover, intestinal NO production has been suggested as a quantitative biomarker of intestinal bacterial translocation [10].

The upregulation of NO biosynthesis has been proven to play a pivotal role in the increase of mucosal permeability [10,17]. Chen et al. demonstrated that both the time course change of the intestinal iNOS expression and the intestinal permeability following thermal injury showed a similar fluctuation with a peak on postburn day 2 and decreased thereafter [10]. In our data, the positive correlation between intestinal NO production and mucosal damage further demonstrates their close relationship in vivo.

The present study revealed excess intestinal NO production following bronchoscopy. The exact mechanisms by which NO production causes mucosal damage following bronchoscopy cannot be


(a)

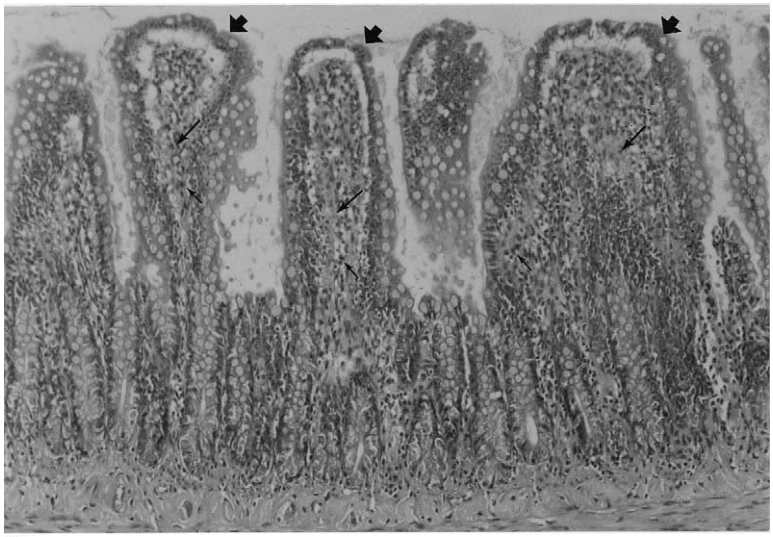

(b)

Fig. 2 (a) Ileum from the control animal with normal intestinal architecture (HEx40). (b) Histopathological manifestations of ileum from study group, 24-h following bronchoscopy. Shortened villi, epithelial lifting (bold arrow), inflammatory cell infiltration (small arrow) and prominent edema (large arrow) in lamina propria.

determined from this study. Excess NO production, however, may exert its detrimental effect by causing a maldistribution in regional blood flow [9]. Intestinal mucosa is highly sensitive to various systemic and regional factors associated with ischemia, and the tips of the intestinal villi may be severely hypoxic even after short-duration of hypoperfusion [19]. Based on the results of experimental studies, it appears that excess NO production can directly result in intestinal permeability increase and mucosal damage, and induce intestinal barrier dysfunction [10,11,20]. Another possible mechanism by which high levels of NO can lead to mucosal damage is through production of nitrosyl complex formation and intestinal lipid peroxidation in the rat small intestine [9,21]. Studies implicate NO oxidant products, such as peroxynitrite, as a major contributor to the intestinal injury [21]. The nitrosylation of the mitochondrial complex results in inhibition of cellular respiration and enterocyte apoptosis [22].

In conclusion, experimental rigid bronchoscopy model in the rat induces intestinal mucosal damage in association with increased NO production. These physiopathologic events may have been responsible for bacterial translocation from the intestine, following bronchoscopy. Further studies will be required to address the exact mechanisms by which NO impair intestinal barrier function.

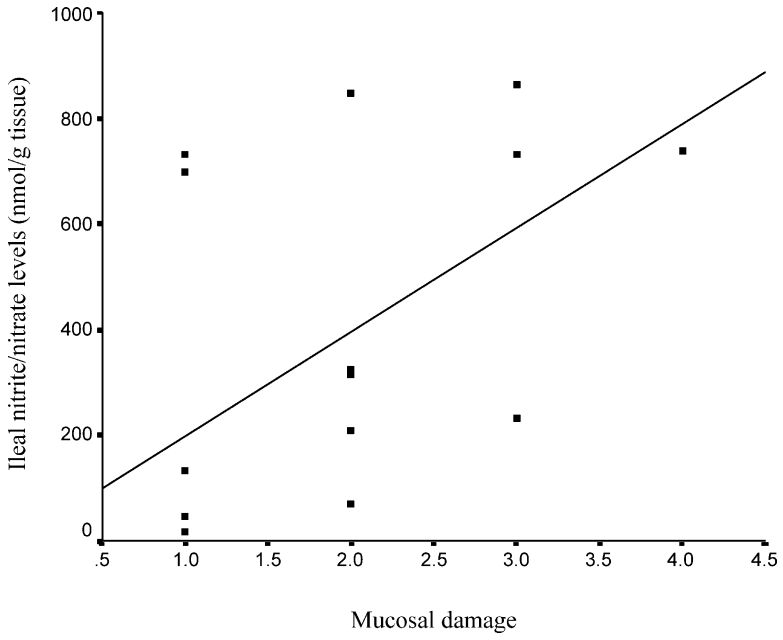


Fig. 3 Correlation analysis between individual pair data of intestinal nitrite/nitrate levels and intestinal mucosal damage demonstrates significant relationship in the bronchoscopy group ($r_s = 0.56$, $P = 0.03$).

References

[1] W. Pereira, D.M. Kovnat, M.A. Khan, J.R. Iacovino, M.L. Spivack, G.L. Snider, Fever and pneumonia after flexible fiberoptic bronchoscopy, Am. Rev. Respir. Dis. 112 (1975) 59–64.

[2] J.F. Ansley, N.L. Shapiro, M.J. Cunningham, Rigid bronchoscopy-induced bacteremia in the pediatric population, Arch. Otolaryngol. Head Neck Surg. 125 (1999) 774–776.

[3] M. Yigla, I. Oren, L. Bentur, A. Solomonov, N. Elias, R. Altshuler, et al., Incidence of bacteremia following fiberoptic bronchoscopy, Eur. Respir. J. 14 (1999) 789–791.

[4] E. Picard, S. Schwartz, S. Goldberg, T. Glick, Y. Villa, E. Kerem, A prospective study of fever and bacteremia after flexible fiberoptic bronchoscopy in children, Chest 117 (2000) 573–577.

[5] R.P. Smith, G.K. Sahetya, A.L. Baltch, J. O'Hern, D. Gort, Bacteremia associated with fiberoptic bronchoscopy, NY State J. Med. 83 (1983) 1045–1047.

[6] U.B. Prakash, Does the bronchoscope propagate infection?, Chest 104 (1993) 552–559.

[7] P. Naaber, I. Smidt, K. Tamme, A. Liigant, H. Tapfer, M. Mikelsaar, et al., Translocation of indigenous microflora in an experimental model of sepsis, J. Med. Microbiol. 49 (2000) 431–439.

[8] E.A. Deitch, J. Morrison, R. Berg, R.D. Specian, Effect of hemorrhagic shock on bacterial translocation, intestinal morphology, and intestinal permeability in conventional and antibiotic-decontaminated rats, Crit. Care Med. 18 (1990) 529–536.

[9] S. Mishima, D. Xu, Q. Lu, E.A. Deitch, The relationships among nitric oxide production, bacterial translocation, and intestinal injury after endotoxin challenge in vivo, J. Trauma 44 (1998) 175–182.

[10] L.W. Chen, C.M. Hsu, M.C. Cha, J.S. Chen, S.C. Chen, Changes in gut mucosal nitric oxide synthase (NOS) activity after thermal injury and its relation with barrier failure, Shock 11 (1999) 104–110.

[11] D.Z. Xu, Q. Lu, E.A. Deitch, Nitric oxide directly impairs intestinal barrier function, Shock 17 (2002) 139–145.

[12] E.P. Nadler, H.R. Ford, Regulation of bacterial translocation by nitric oxide, Pediatr. Surg. Int. 16 (2000) 165–168.

[13] A. Nayci, S. Atis, D. Talas, G. Ersoz, Rigid bronchoscopy induces bacterial translocation: an experimental study in rats, Eur. Respir. J. (2003) 5, in press.

[14] L.C. Green, D.A. Wagner, J. Glogowski, P.L. Skipper, J.S. Wishnok, S.R. Tannenbaum, Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids, Anal. Biochem. 126 (1982) 131–138.

[15] C.J. Chiu, A.H. McArdle, R.A. Brown, H.J. Scott, F.N. Gurd, Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal, Arch. Surg. 101 (1970) 478–483.

[16] N. Unno, H. Wang, M.J. Menconi, S. Tytgat, V. Larkin, M. Smith, et al., Inhibition of inducible nitric oxide synthase ameliorates endotoxin-induced gut mucosal barrier dysfunction in rats, Gastroenterology 113 (1997) 1246.

[17] N. Unno, M.J. Menconi, M. Smith, M.P. Fink, Nitric oxide mediates interferon-gamma-induced hyperpermeability in cultured human intestinal epithelial cells, Crit. Care Med. 23 (1995) 1170–1176.

[18] J. MacFie, Enteral versus parenteral nutrition: the significance of bacterial translocation and gut-barrier function, Nutrition 16 (2000) 606–611.

[19] E. Eleftheriadis, K. Kotzampassi, K. Papanotas, N. Heliadis, K. Sarris, Gut ischemia, oxidative stress, and bacterial translocation in elevated abdominal pressure in rats, World J. Surg. 20 (1996) 11–16.

[20] K. Chen, M. Inoue, A. Okada, Expression of inducible nitric oxide synthase mRNA in rat digestive tissues after endotoxin and its role in intestinal mucosal injury, Biochem. Biophys. Res. Commun. 224 (1996) 703–708.

[21] W. Chamulitrat, N.V. Skrepnik, J.J. Spitzer, Nitrosyl complex formation during endotoxin-induced injury in the rat small intestine, Shock 5 (1996) 59–65.

[22] S. Hortelano, B. Dallaporta, Nitric oxide induces apoptosis via triggering mitochondrial permeability transition, FEBS Lett. 410 (1997) 373–377.