

Regional Sympathetic Blockade Attenuates Activation of Intestinal Macrophages and Reduces Gut Barrier Failure

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ABSTRACT

Background: Endotoxin-induced activation of monocytes may lead to extravasation of cells, excessive production of nitric oxide, and subsequent epithelial injury in the gut. Regional sympathetic blockade by means of thoracic epidural anesthesia has been implicated to protect the epithelial barrier. This study tested the hypothesis that thoracic epidural anesthesia decreases epithelial permeability by attenuating monocytic production of nitric oxide and nitrosative stress.

Methods: Rats were anesthetized, hemodynamically monitored, and mechanically ventilated. Endotoxemia was induced by an intravenous bolus injection of *Escherichia coli* lipopolysaccharide. Either lidocaine 2% or normal saline was injected as a bolus, followed by a continuous infusion *via* an epidural catheter. Three hundred minutes after injection of lipopolysaccharide or normal saline, gut epithelial permeability to fluorescein isothiocyanate-dextran (4 kDa), intestinal expression of inducible nitric oxide synthase by macrophages, and lipid peroxidation represented by 8-isoprostane tissue concentration were quantified.

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What We Already Know about This Topic

- Increased intestinal epithelial permeability associated with bacterial translocation may occur during systemic inflammation, hemorrhagic shock, and other pathophysiologic conditions. Thoracic epidural anesthesia has been shown to increase intestinal perfusion and to attenuate leukocyte adhesion.

What This Article Tells Us That Is New

- This study in normotensive rats exposed to endotoxemia shows that thoracic epidural anesthesia decreases epithelial permeability, monocytic extravasation, and inducible form of nitric oxide synthase expression in the gut.

Results: Thoracic epidural anesthesia significantly attenuated the endotoxin-induced increases in gut epithelial permeability (437 [293, 492] *vs.* 628 [532, 1,042] ng/ml, median [quartiles], $P = 0.03$), expression of nitric oxide synthase (2 [1,2] *vs.* 7 [5,8] cells per 384 μm^2 , $P = 0.003$), macrophage infiltration, and lipid peroxidation (22,460 \pm 11,476 *vs.* 37,840 \pm 17,551 pg/ml, mean \pm SD, $P = 0.05$).

Conclusions: Thoracic epidural anesthesia attenuates endotoxin-induced gut epithelial injury. This is likely due to a decrease in monocytic extravasation and intestinal nitrosative stress. As possible mechanisms, direct nerve-immune interplay, a reduction in plasma catecholamines, or a systemic lidocaine effect has to be considered.

INCREASED intestinal epithelial permeability has been reported in various pathophysiologic conditions, such as ileus, hemorrhagic shock, and systemic inflammation. Animal models of systemic inflammation have shown the association of increased intestinal permeability and bacterial translocation,^{1–3} which may contribute to multiple organ failure.⁴ Intravenous administration of lipopolysaccharide (LPS) is a widely used model of systemic inflammation. It is associated with activation of monocytes, which recognize the bacterial antigen *via* toll-like receptor 4 and cluster of differentiation 14.⁵ Subsequent immune cell adhesion to the vascular endothelium, extravasation to intestinal tissue, and excessive production of nitric oxide by the inducible form of nitric oxide synthase (iNOS) in macrophages⁶ may affect epithelial cells in the gut and induce gut barrier dysfunction.²

Regional sympathetic blockade by means of thoracic epidural anesthesia (TEA) has been shown to increase intestinal

perfusion,^{7–10} attenuate leukocyte adhesion,^{11,12} and reduce patient morbidity¹³ in systemic inflammation. Furthermore, a protective effect on the epithelial barrier has been suggested in rabbits subjected to hypoxia¹⁴ and endotoxemia.¹⁵ However, the mechanism involved in this latter phenomenon remains unclear.

The release of nitric oxide and inflammatory mediators by macrophages seems to be a crucial step in the dysregulation of tight junction proteins, which are responsible for the intestinal barrier function.^{16,17} We, therefore, tested the hypothesis that TEA decreases epithelial permeability by reducing macrophage-derived nitrosative stress. In a rodent model of normotensive endotoxemia, effects of TEA on intestinal epithelial permeability, iNOS expression by macrophages, nitric oxide metabolites, and lipid peroxidation in intestinal tissue were quantified.

Materials and Methods

Animals

We used male Sprague-Dawley rats (297 ± 30 g, mean ± SD), which were purchased (Charles River Laboratories International, Inc., Sulzfeld, Germany) and housed in our animal facility under standardized conditions. All animals were handled according to the National Research Council's guide on the care and use of laboratory animals¹⁸ and had free access to standard chow (Altromin Spezialfutter GmbH und Co.KG, Lage, Germany) and water *ad libitum*. All experiments were approved by the local animal care committee (Landesamt für Gesundheit und Soziales, Berlin, Germany).

Experimental Protocol

General anesthesia was provided by a subcutaneous injection of urethane (1.5 g/kg, Sigma-Aldrich-Chemie GmbH, Deisenhofen, Germany) and ketamine (50 mg/kg, Pharmacia & Upjohn GmbH, Erlangen, Germany). Ketamine was supplemented approximately every 2 h by 15–30 mg/kg. Under general anesthesia, epidural catheterization, tracheotomy, as well as vascular cannulation, were performed. Recording of hemodynamic values was started, and animals received an intravenous bolus injection of 5 ml of NaCl 0.9% solution (Boehringer Ingelheim KG Delta-Pharma, Pfullingen, Germany). Then, a continuous intravenous infusion of NaCl 0.9% (6 ml/h) and mechanical ventilation with an oxygen air mix (30/70%) were started. After a stabilization period of 15 min, blood was analyzed, and animals were randomly assigned to the following

groups (table 1): group LPS+/TEA– (n = 16) received an intravenous bolus injection of *Escherichia coli* LPS (50 mg/kg, serotype 026:B6, Sigma-Aldrich) and an epidural bolus injection (30 µl) of NaCl 0.9%, followed by a continuous infusion (30 µl/h, Genie Plus, Kent Scientific Corporation, Torrington, CT). In group LPS+/TEA+ (n = 12), endotoxin injection was paralleled by an epidural infusion of lidocaine 2% (B. Braun Melsungen AG, Melsungen, Germany) in analogous volumes to NaCl 0.9%. Group LPS–/TEA– (n = 9) received NaCl 0.9% instead of endotoxin and lidocaine. Three hundred minutes after injection of LPS or NaCl 0.9%, blood was analyzed and a loop of ileum was ligated for determination of epithelial permeability. Then blood and organs were harvested, shock frozen in liquid nitrogen, and stored at –80°C until quantification of nitric oxide metabolites and lipid peroxidation.

For immunohistochemical analysis of iNOS expression, five additional animals of each group were used. At the end of the experimental protocol, animals were transcardially perfused with 60 ml phosphate-buffered saline (PBS, pH = 7.4, Biochrom AG, Berlin, Germany) and 300 ml cold PBS containing paraformaldehyde 4% (fixative solution). Then three pieces of ileum (approximately 3 cm of length) were excised, postfixed at 4°C for 90 min in fixative solution, and cryoprotected overnight at 4°C in PBS containing sucrose 10%. The tissues were then embedded in Tissue-Tek® O.C.T.™ compound (Miles Laboratories Inc., Elkhart, IN) and frozen at –80°C until further processing. Then 8-µm-thick sections prepared on a cryostat were mounted onto gelatine-coated slides.

Instrumentation

Animals were tracheotomized and intubated using a polyethylene tube (PE 205, Portex Ltd., Hythe, United Kingdom). Epidural catheterization was performed as described before.¹⁰ Briefly, a polyethylene catheter of 200 mm length (PE 10, Portex) with a dead space of 5 µl was placed *via* a midline incision along the spinal processes of L2–S1. Subsequent suture and tape fixation prevented displacement of the catheter. After each experiment, the correct position of the catheter in the epidural space was confirmed by autopsy with a mean segmental level of T7. Former experiments had revealed a minimum spread of lidocaine to segments T4 to L1 associated with a plasma concentration of lidocaine of 0.33 µg/ml in endotoxemic rats.¹⁰ TEA in those experiments significantly reduced endotoxin-induced plasma concentration of adrenaline.¹⁰

Table 1. Experimental Groups

Group	Intravenous LPS Bolus	Epidural Infusion	n
LPS+/TEA–	50 mg/kg in 1 ml NaCl 0.9%	NaCl 0.9%, 30 µl bolus followed by 30 µl/h	16
LPS+/TEA+	50 mg/kg in 1 ml NaCl 0.9%	Lidocaine 2%, 30 µl bolus followed by 30 µl/h	12
LPS–/TEA–	1 ml NaCl 0.9%	NaCl 0.9%, 30 µl bolus followed by 30 µl/h	9

LPS = lipopolysaccharide; TEA = thoracic epidural anesthesia.

The right carotid artery and both external jugular veins were cannulated (PE 50, Portex) for monitoring of blood pressure and infusion of fluids or central venous pressure monitoring, respectively. Continuous hemodynamic monitoring was performed using pressure transducers (model p23 ID, Gould-Statham Instruments Inc., Hato Rey, Puerto Rico) connected to arterial and venous lines *via* three-way cocks and a recorder (Gould Pilot, Gould Instruments, Bal-lainvilliers, France). Mechanical ventilation with an oxygen/air mix (30/70%, Animal Respirator Advanced 4601-1, TSE Systems GmbH, Bad Homburg, Germany) was controlled by expired gas analysis (TSE Systems) to provide normo-ventilation. Arterial blood was analyzed for blood gases, acid base status, electrolytes (Rapidlab™ 348, Chiron Diag-nostics GmbH, Fernwald, Germany), and blood cell counts (Coulter® Ac-T™ Analyzer, Beckman Coulter, Inc., Miami, FL). Body temperature was kept at 36°C throughout the experiment using a warming pad.

Quantification of Intestinal Epithelial Permeability

Epithelial permeability was assessed using a ligated loop model as described elsewhere.¹⁹ Briefly, the ileum was incised and ligated at both ends of a 10-cm-long loop with the distal incision 5 cm proximal to the cecum. Before tying the ligature, feces were removed by cautiously flushing the segment with PBS, and a solution of 4-kDa fluorescein isothiocyanate-conjugated dextran (10 mg in 1 ml of PBS; Sigma-Aldrich) was injected into the intestinal segment *via* a flexible cannula. The gut loop was then wrapped in wet gauze and covered with a plastic film. After 30 min of incubation, blood was sampled from the portal vein and centrifuged to separate the plasma. The plasma was stored at -80°C until quantification of fluorescence with a spectrofluorometer (excitation at 480 nm, extinction at 520 nm, FluoroMax-2, ISA JobinYvon, Spex Instruments S.A., Inc., Edison, NJ). For quantification, plasma probes were diluted in PBS and compared with a standard dilution containing 0.5% bovine albumin (Sigma-Aldrich). PBS was used as blank.

Immunohistochemistry

For immunohistochemical detection of iNOS, tissue specimens were prepared as described previously.²⁰ Endogenous peroxidase was inhibited by incubating with 10% (vol/vol) H₂O₂ for 30 min. To prevent nonspecific binding, tissue sections were incubated for 60 min in PBS containing 0.3% Triton X-100, 1% bovine serum albumin, and 10% goat serum. Then, the tissue sections were incubated overnight at room temperature with a polyclonal rabbit anti-rat iNOS (Santa Cruz Biotechnology, Inc., Heidelberg, Germany, dilution 1:1,000) antiserum supplemented with 3% normal goat serum and 0.3% Triton X-100. Thereafter, the slides were washed in PBS for 6 × 10 min and exposed to the appropriated biotinylated secondary antibody goat anti-rabbit immunoglobulin (Vector Laboratories, Inc., Burlingame, CA) for 1–2 h. After washing in PBS for

3 × 10 min, tissue sections were then incubated with avidin-biotin-conjugated peroxidase for 45 min. Finally, the sections were washed and stained with 3',3'-diaminobenzidine tetrahydrochloride (Sigma-Aldrich) containing 0.01% H₂O₂ in 0.05 M Tris-buffered saline (pH 7.6) for 3–5 min. After the enzyme reaction, the sections were washed in tap water, counterstained with thionin, then dehydrated in alcohol, cleared in xylene, and mounted in dibutyl-phthalate-polystyrene-xylene (Merck KGaA, Darmstadt, Germany). Quantification of iNOS-immunoreactive cells was performed by an examiner blinded to the protocol as described previously.²¹ The mean number of iNOS-immunoreactive cells were counted in four sections per animal and 10 squares (384 μm² each) (objective ×20; eyepiece ×10). Immune cells were identified by the following morphologic criteria: (1) as polymorphonuclear leukocytes by large cell bodies and multisegmented nuclei; (2) as macrophages/monocytes by large cell bodies, vacuolated cytoplasm, and irregular-shaped nuclei; and (3) as lymphocytes by small cell bodies, large nuclei, and small amounts of cytoplasm.

Quantification of Nitric Oxide Metabolites and Lipid Peroxidation

Tissue concentrations of nitric oxide metabolites and 8-isoprostane, a product of lipid peroxidation, were quantified in shock frozen tissue specimen stored at -80°C until further processing. For quantification of nitric oxide metabolites, tissue samples were homogenized in PBS and centrifuged at 10,000g for 20 min. The supernatant was ultrafiltered with 10 kDa cut-off filter units (Millipore GmbH, Schwalbach, Germany). Samples then were assayed for nitrate and nitrite content using a colorimetric assay (Cayman Chemical Company, Ann Arbor, MI). Absorption was measured at 540 nm (Spectra Rainbow Thermo Reader and Magellan Software V 2.22, Tecan Deutschland GmbH, Crailsheim, Germany). For quantification of 8-isoprostane, intestinal samples were homogenized in a buffer containing sodium hydrogen phosphate, EDTA, and indomethacin and centrifuged at 20,000g for 15 min. The supernatant was analyzed for 8-isoprostane concentration using a colorimetric assay (Cayman Chemi-cal). Absorption was measured at 405 nm.

Statistical Analysis

All tests were performed using Sigma Stat 3.10 (Systat Software GmbH, Erkrath, Germany). A sample size analysis using SD values from the literature¹⁹ generated a sample size of six animals. For randomization of animals we used a closed envelope protocol. The LPS-/TEA- group was excluded from the randomization procedure when interim analysis of data revealed that variability of epithelial permeability in endotoxemic animals was higher than assumed. Due to a time delay between the animal experiments and the quantification of epithelial permeability and due to drop-outs in the analysis of single samples, group sizes became inhomogeneous and differed between variables, respectively.

Retrospective sample size analysis yielded a sample size of 12 animals assuming an SD of 40%, a difference in mean values of 25%, a probability of error of 5%, and a power of 80%.

Normality of data was tested using the Kolmogorov–Smirnov analysis. Hemodynamic variables were analyzed using a Friedman repeated measures ANOVA on ranks with Tukey *post hoc* analysis. Hemodynamic data were depicted as mean and SEM for clear presentation. Other data were tested using a one-way ANOVA (with Student–Newman–Keuls as *post hoc* analysis) or a Kruskal–Wallis ANOVA on ranks (with Dunn test as *post hoc* analysis) as appropriate. Intragroup comparisons in laboratory data were performed using the Wilcoxon test, followed by the Bonferroni *post hoc* analysis. The comparison between arterial and portal venous values in epithelial permeability was performed using the Mann–Whitney test, followed by the Bonferroni *post hoc* analysis. To calculate exact *P* values, nonparametric data presented in the abstract were analyzed analogously. For all statistical tests, significance was assumed at *P* values less than 0.05.

Results

The amount of missing values was 1.59%. Missing values were not handled with a complete case analysis.

Hemodynamic Variables

Mean arterial pressure increased in all groups after bolus administration of NaCl 0.9% and transiently decreased with bolus injection of LPS in endotoxemic groups (fig. 1A). The combination of LPS administration and sympathetic blockade led to the most pronounced decrease in mean arterial pressure. Blood pressure was stable during the further course of the experiments in all groups.

Heart rate at baseline was higher in LPS–/TEA– animals without any changes during the experiment (fig. 1B). Heart rate increased similarly in both endotoxemic groups after the administration of LPS.

Central venous pressure remained stable throughout the experiments in all groups, without differences among groups (fig. 1C).

Laboratory Variables

All laboratory variables were comparable among groups at baseline (table 2). Arterial partial pressure of oxygen increased throughout the experiment and was higher in endotoxemic animals with sympathetic blockade compared to healthy controls after 300 min of endotoxemia (table 2). All animals were normoventilated at 300 min of endotoxemia, but pH decreased due to metabolic acidosis with lower base excess values in the endotoxemic groups (table 2). Sodium plasma concentration showed only small increases at 300 min of endotoxemia, whereas blood cell counts were lower in endotoxemic animals compared to healthy controls (table 2).

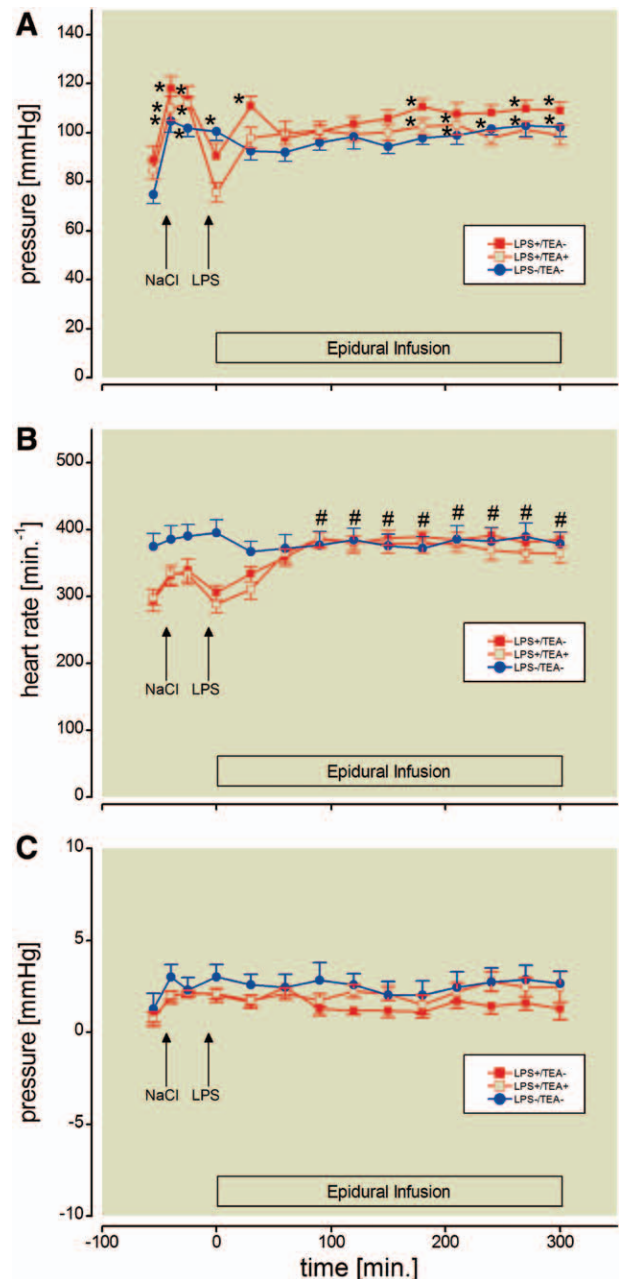


Fig. 1. Hemodynamic variables. A, Mean arterial pressure. B, Heart rate. C, Central venous pressure. LPS = lipopolysaccharide; TEA = thoracic epidural anesthesia. Data are mean and standard error. * *P* < 0.05 versus baseline, # *P* < 0.05 versus baseline in LPS+/TEA– and LPS+/TEA+ groups only.

Epithelial Permeability

Plasma concentration of fluorescent dye after permeation from the gut lumen was determined in arterial and portal venous blood (fig. 2). Concentration of dye was significantly increased only in arterial and venous blood of endotoxemic animals without sympathetic blockade. TEA significantly attenuated the increase of dye concentration in portal venous blood (fig. 2).

Table 2. Laboratory Variables

	Baseline	300 min Endotoxemia
P _{O₂} , mmHg		
LPS+/TEA-	227 (166, 260)	256 (198, 282)
LPS+/TEA+	243 (220, 263)	281 (267, 289) †
LPS-/TEA-	216 (170, 256)	209 (164, 252)
P _{CO₂} , mmHg		
LPS+/TEA-	41 (39, 46)	37 (36, 40) *
LPS+/TEA+	43 (42, 44)	43 (36, 48)
LPS-/TEA-	40 (39, 45)	42 (35, 44)
pH		
LPS+/TEA-	7.40 (7.36, 7.42)	7.32 (7.31, 7.35) ‡
LPS+/TEA+	7.38 (7.33, 7.39)	7.29 (7.26, 7.31) *
LPS-/TEA-	7.39 (7.37, 7.43)	7.34 (7.30, 7.37) ‡
Base excess, mM/l		
LPS+/TEA-	0.6 (-0.5, 1.0)	-6.3 (-7.6, -4.2) *
LPS+/TEA+	0.2 (-0.9, 0.7)	-7.2 (-7.9, -5.8) *
LPS-/TEA-	0.4 (-0.8, 1.1)	-4.2 (-5.7, -3.3) ‡
K ⁺ , mM/l		
LPS+/TEA-	4.6 (4.4, 5.2)	4.4 (4.0, 4.9)
LPS+/TEA+	4.8 (4.5, 5.1)	4.9 (4.7, 5.4)
LPS-/TEA-	5.0 (4.7, 5.0)	4.4 (4.3, 5.3)
Na ⁺ , mM/l		
LPS+/TEA-	139 (138, 141)	142 (141, 142) *
LPS+/TEA+	139 (137, 140)	142 (141, 143) *
LPS-/TEA-	138 (137, 139)	141 (140, 141) *
Hematocrit, %		
LPS+/TEA-	45 (43, 47)	35 (33, 39) *†
LPS+/TEA+	45 (42, 49)	35 (30, 37) *†
LPS-/TEA-	47 (44, 49)	40 (38, 42) *
Leukocytes × 10 ³ /μl		
LPS+/TEA-	10.5 (8.5, 12.1)	8.7 (7.7, 10.5) †
LPS+/TEA+	9.9 (9.2, 13.0)	7.7 (6.6, 8.9) *†
LPS-/TEA-	16.3 (11.1, 17.8)	15.0 (13.7, 17.2)
Erythrocytes × 10 ⁶ /μl		
LPS+/TEA-	6.7 (6.5, 7.2)	5.8 (5.4, 6.4) *
LPS+/TEA+	6.6 (6.1, 7.0)	5.7 (5.0, 6.1) *†
LPS-/TEA-	7.2 (6.8, 8.0)	6.5 (6.2, 6.7)
Platelets × 10 ³ /μl		
LPS+/TEA-	961 (887, 1,020)	672 (598, 714) *†
LPS+/TEA+	914 (824, 988)	611 (515, 645) *†
LPS-/TEA-	973 (891, 1,104)	894 (810, 990)

* $P < 0.05$ vs. baseline. † $P < 0.05$ vs. LPS-/TEA-. ‡ $P < 0.05$ vs. LPS+/TEA+.

LPS = lipopolysaccharide; P_{CO₂} = partial pressure of carbon dioxide, TEA = thoracic epidural anesthesia, data are median [quartiles].

Intestinal Expression of iNOS

Expression of iNOS in intestinal tissue was increased in all animals receiving endotoxin (fig. 3, A and B) compared to healthy controls (fig. 3C). However, the expression was

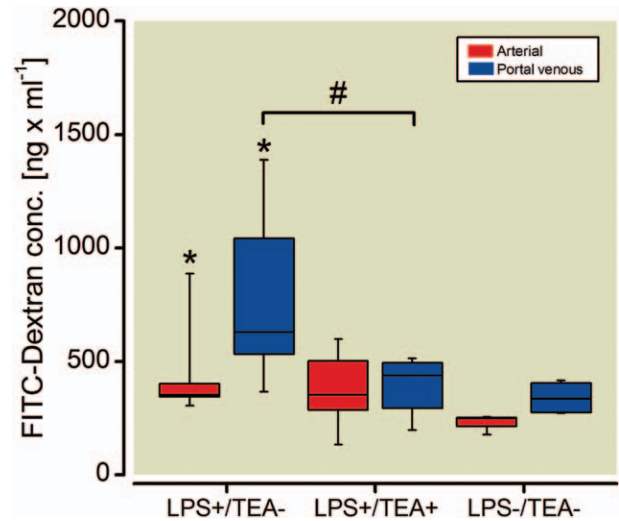


Fig. 2. Plasma concentration of fluorescent dye in carotid artery and portal vein after 300 min of endotoxemia. FITC = fluorescein isothiocyanate; LPS = lipopolysaccharide; TEA = thoracic epidural anesthesia; Box plots show median, quartiles, and range. * $P < 0.05$ versus LPS-/TEA-, # $P < 0.05$.

significantly higher in endotoxemic animals without TEA (fig. 3A) compared to animals with TEA (fig. 3B)

Histomorphologic Identification of Macrophages in Intestinal Tissue

Cells expressing iNOS could be identified as macrophages according to the above described histomorphologic criteria (for macrophages: by large cell bodies, vacuolated cytoplasm, and irregular-shaped nuclei). The number of macrophages equaled the number of cells expressing iNOS. As a consequence, quantification of macrophage infiltration was analogous to quantification of iNOS expression (graph not displayed, fig. 3).

Blood and Tissue Concentrations of Nitric Oxide Metabolites

Concentrations of nitric oxide metabolites were significantly increased in blood and all investigated tissues of endotoxemic animals without sympathetic blockade. Values were lower in TEA animals without reaching statistical significance (table 3).

Intestinal Lipid Peroxidation

In endotoxemic animals without TEA, tissue concentration of intestinal 8-isoprostane was significantly higher compared to both other groups. TEA animals showed values comparable to healthy animals (fig. 4).

Discussion

This experimental animal study investigated the effects of regional sympathetic blockade on endotoxin-induced epithelial permeability and intestinal inflammation. We demonstrated in endotoxemic rats that TEA decreased gut

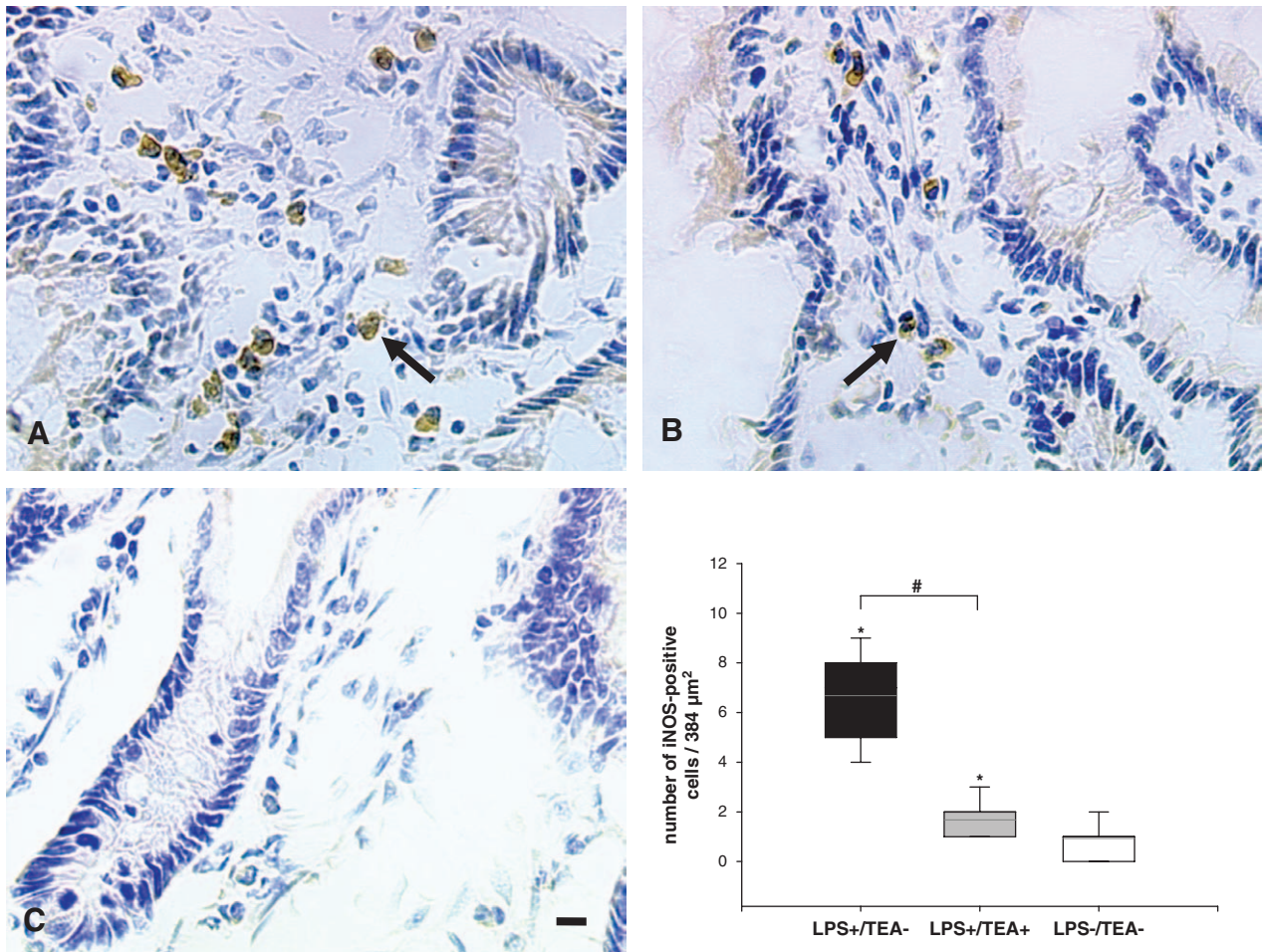


Fig. 3. Immunohistochemical staining and quantification of inducible nitric oxide synthase in the gut (brown color) and identification of macrophages in intestinal tissue. iNOS = inducible nitric oxide synthase; LPS = lipopolysaccharide; TEA = thoracic epidural anesthesia. A, Group LPS+/TEA-. B, Group LPS+/TEA+. C, Group LPS-/TEA-. Arrows show examples of macrophages expressing iNOS. Scale bar = 20 μm. Data in the graph are median, quartiles and range. * $P < 0.05$ versus LPS-/TEA-, # $P < 0.05$.

epithelial permeability, monocytic iNOS expression, and nitrosative stress.

Hemodynamic Variables

Bolus injection of endotoxin led to a short transient decrease in mean arterial pressure and a lasting increase in heart rate (fig. 1, A and B). Hypotension associated with bolus injection of endotoxin has been discussed to be mediated by a sudden release of nitric oxide and prostacyclin from vascular endothelium.²² After this short period of hypotension, endotoxemic animals showed mean arterial pressure values comparable to healthy control animals, consistent with a model of normotensive endotoxemia. Heart rate baseline values were higher in healthy animals compared to both endotoxemic groups, although all animals did not differ in treatment at that time point. Heart rate did not change in healthy animals, whereas it increased in animals after endotoxin injection. Tachycardia is the heart's response to systemic inflammation and has been demonstrated to go along with increases in plasma catecholamines in former experiments.¹⁰

Epidural bolus infusion of lidocaine had a synergistic hypotensive effect, which can be well explained by blockade of the sympathetic innervation of resistance vessels. However, mean arterial pressure stabilized quickly also in TEA animals without intergroup differences in heart rate, implying that the heart was outside the sympathetic block. This is in line with the results of former experiments on the epidural spread of lidocaine in the same rat model of TEA.¹⁰ Furthermore, stable mean arterial pressure and comparable central venous pressure values (fig. 1C) between groups demonstrate adequate volume therapy in all animals.

Laboratory Variables

All animal groups presented with mild acidosis at the end of the experiment (table 2). This can be attributed to the infusion of normal saline, which is known to cause hyperchloremic acidosis with greater volumes infused.²³ Normal saline is one of the standard fluids used for volume replacement in animal experiments; nevertheless, using

Table 3. Blood and Tissue Concentration of Nitric Oxide Metabolites

	LPS+/TEA-	LPS+/TEA+	LPS-/TEA-
Portal venous blood, $\mu\text{M/l}$	108 (91,134) *	71 (58,102) *	14 (10,21)
Intestine, nM/g tissue	133 (96,207) *	104 (93,116) *	55 (41,66)
Liver, nM/g tissue	66 (59,79) *	45 (27,67)	26 (23,35)
Lung, nM/g tissue	111 (103,139) *	91 (76,103) *†	40 (36,47)
Heart, nM/g tissue	123 (97,130) *	81 (71,104) *†	44 (36,48)
Kidney, nM/g tissue	107 (95,120) *	94 (85,99) *	50 (38,63)

Data are median and quartiles.

* $P < 0.05$ vs. LPS-/TEA-; † $P < 0.05$ vs. LPS+/TEA-.

LPS = lipopolysaccharide; TEA = thoracic epidural anesthesia.

a balanced fluid acidosis might have been prevented. Furthermore, the use of a colloidal resuscitation fluid instead of normal saline would have significantly decreased the amount of resuscitation fluid. Base excess values were lower in endotoxemic animals (table 2) consistent with a well-described mitochondrial effect of endotoxin, leading to an increase in serum lactate.²⁴ Lowest pH values were found in TEA animals (table 2), which also had highest values in partial pressure of carbon dioxide, albeit in the normal range. The number of all blood cells decreased with the course of endotoxemia (table 2), which can be interpreted as adhesion of these cells to the vascular endothelium.²⁵⁻²⁷ In a similar rat model, the endotoxin-induced decrease in circulating leukocytes was associated with an increase in the number of adherent leukocytes to mesenteric venules within 2 h of endotoxemia.²⁸

Intestinal Epithelial Permeability

Injection of endotoxin induced a significant increase in epithelial permeability (fig 2). Endotoxin-induced epithelial permeability to intraluminal fluorescent dye was significantly decreased by TEA (fig. 2). This is in line with the

results of Kosugi *et al.*,¹⁵ who found less epithelial permeability and also demonstrated less histologic signs of tissue injury with TEA in endotoxemic rabbits. We were able to differentiate between the portal and the systemic circulation and found less intergroup differences in the systemic circulation, which may be due to hepatic clearance of the dye. Although not investigated in our study, bacterial translocation to the systemic circulation may have been affected by TEA, postulating a strong correlation of epithelial permeability and bacterial translocation in rats.²⁹ As a possible cellular mechanism for the increase in epithelial permeability during endotoxemia, excessive production of nitric oxide in monocytic cells⁶ with a subsequent damage of epithelial tight junction proteins has been described.^{16,30}

Intestinal Monocytic Cell Infiltration and Production of Nitric Oxide

Our experiments demonstrated that endotoxemia was associated with increased infiltration of iNOS-expressing macrophages in intestinal tissue (fig. 3). The inflammatory pathway after injection of *E. coli* LPS has been described in detail.^{6,31-33} It includes binding of LPS to a binding protein and initiating a mediator cascade *via* toll-like receptor 4 on monocytes. One consequence is the excessive production of the reactive nitrogen species nitric oxide by monocytic cells. The detection of an increased amount of nitric oxide metabolites in different tissues demonstrated the systemic inflammatory effect of LPS (table 3).

TEA was associated with less tissue infiltration of iNOS-expressing macrophages (fig. 3) and a smaller amount of nitric oxide metabolites in organs inside and outside the sympathetic block (table 3). The attenuation effect of TEA on nitric oxide production in different tissues, including the intestine, was less pronounced than the effect on intestinal iNOS expression and did not reach statistical significance. This could be due to other sources of nitric oxide production, such as endothelial cells, mast cells, and smooth muscle cells, which may not have been influenced by TEA. Nevertheless, an effect of TEA on nitric oxide production is visible in all organs.

Effects on macrophages in organs within the sympathetic block may be explained by a direct communication between

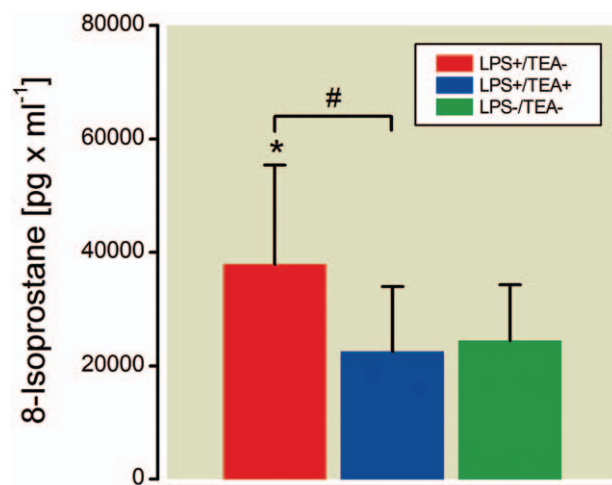


Fig. 4. Intestinal concentration of lipid peroxidation product 8-isoprostane. LPS = lipopolysaccharide; TEA = thoracic epidural anesthesia; Data are mean and SD. * $P < 0.05$ versus LPS-/TEA-, # $P < 0.05$.

sympathetic nerves and immune cells (nerve-immune interplay), which has been previously demonstrated for the parasympathetic system.^{34,35}

Organs outside the blocked region (as demonstrated for nitric oxide metabolites in heart and lung), however, can only be influenced by a systemic effect. A possible explanation is that TEA reduces tissue infiltration of nitric oxide-producing monocytic cells *via* a decrease in catecholamine plasma concentration. Catecholamines have been demonstrated to induce expression of adhesion molecules on endothelium and immune cells³⁶ and to increase iNOS expression.³⁷ A reduction of plasma concentration of adrenaline has been shown in this model of TEA,¹⁰ which makes this a likely mechanism. Furthermore, this would be a possible explanation for the beneficial effects of β -receptor blocking drugs in sepsis.³⁸ Another explanation, however, is a systemic effect of epidurally applied local anesthetic. Albeit the systemic concentration of lidocaine in this model was determined to be low (0.33 $\mu\text{g/ml}$),¹⁰ effects of lidocaine on leukocyte adhesion²⁸ and cytokine release³⁹ have been described by other authors.

Although not focused in our experiments, an effect of TEA on the endothelial surface layer, glycocalyx, may explain parts of our results. Intravenous LPS has been shown to deteriorate the glycocalyx^{40,41} and to cause the release of nitric oxide and prostacyclin from the vascular endothelium, leading to increased adhesion of leukocytes.⁴² Protective effects of TEA on the glycocalyx, therefore, may be a challenging subject for further investigations.

Intestinal Lipid Peroxidation

Lipid peroxidation in intestinal tissue was only found to be increased in endotoxemic animals without TEA (fig. 4). In contrast, endotoxemia in combination with TEA was associated with 8-isoprostane values comparable to healthy animals (fig. 4). This suggests a protective effect of TEA on intestinal tissue. Because lipid peroxidation is catalyzed by free radicals,⁴³ which may result from nitrosative stress, this protective effect of TEA may be directly due to the attenuation of intestinal nitric oxide production. An additional mechanism could be a mitigating effect of TEA on activation and extravasation of granulocytes, with less release of peroxidases from these cells into intestinal tissue. Former experiments had demonstrated a decrease in hemorrhage-induced leukocyte adhesion with TEA.¹¹ Differentiation of white blood cells, however, was not possible.

Conclusion

Regional sympathetic blockade by means of thoracic epidural anesthesia decreased endotoxin-induced epithelial permeability of the gut. This is likely due to an inhibition of monocytic cell infiltration into intestinal tissue, with less production of nitric oxide and less nitrosative tissue injury as verified by reduced lipid peroxidation. As possible mechanisms, direct nerve-immune interplay, a reduction in plasma

catecholamines, or a systemic lidocaine effect has to be considered.

References

- Deitch EA, Berg R, Specian R: Endotoxin promotes the translocation of bacteria from the gut. *Arch Surg* 1987; 122: 185–90
- Unno N, Wang H, Menconi MJ, Tytgat SH, Larkin V, Smith M, Morin MJ, Chavez A, Hodin RA, Fink MP: Inhibition of inducible nitric oxide synthase ameliorates endotoxin-induced gut mucosal barrier dysfunction in rats. *Gastroenterology* 1997; 113:1246–57
- Moriez R, Salvador-Cartier C, Theodorou V, Fioramonti J, Eutamene H, Bueno L: Myosin light chain kinase is involved in lipopolysaccharide-induced disruption of colonic epithelial barrier and bacterial translocation in rats. *Am J Pathol* 2005; 167:1071–9
- Carrico CJ, Meakins JL, Marshall JC, Fry D, Maier RV: Multiple-organ-failure syndrome. *Arch Surg* 1986; 121:196–208
- Jerala R: Structural biology of the LPS recognition. *Int J Med Microbiol* 2007; 297:353–63
- Aktan F: iNOS-mediated nitric oxide production and its regulation. *Life Sci* 2004; 75:639–53
- Adolphs J, Schmidt DK, Korsukewitz I, Kamin B, Habazettl H, Schäfer M, Welte M: Effects of thoracic epidural anaesthesia on intestinal microvascular perfusion in a rodent model of normotensive endotoxaemia. *Intensive Care Med* 2004; 30:2094–101
- Daudel F, Freise H, Westphal M, Stubbe HD, Lauer S, Bone HG, Van Aken H, Sielenkämper AW: Continuous thoracic epidural anesthesia improves gut mucosal microcirculation in rats with sepsis. *Shock* 2007; 28:610–4
- Freise H, Lauer S, Anthonsen S, Hlouschek V, Minin E, Fischer LG, Lerch MM, Van Aken HK, Sielenkämper AW: Thoracic epidural analgesia augments ileal mucosal capillary perfusion and improves survival in severe acute pancreatitis in rats. *ANESTHESIOLOGY* 2006; 105:354–9
- Schäper J, Ahmed R, Perschel FH, Schäfer M, Habazettl H, Welte M: Thoracic epidural anesthesia attenuates endotoxin-induced impairment of gastrointestinal organ perfusion. *ANESTHESIOLOGY* 2010; 113:126–33
- Adolphs J, Schmidt DK, Mousa SA, Kamin B, Korsukewitz I, Habazettl H, Schäfer M, Welte M: Thoracic epidural anesthesia attenuates hemorrhage-induced impairment of intestinal perfusion in rats. *ANESTHESIOLOGY* 2003; 99:685–92
- Freise H, Daudel F, Grosserichter C, Lauer S, Hinkelmann J, Van Aken HK, Sielenkaemper AW, Westphal M, Fischer LG: Thoracic epidural anesthesia reverses sepsis-induced hepatic hyperperfusion and reduces leukocyte adhesion in septic rats. *Crit Care* 2009; 13:R116
- Tyagi A, Seelan S, Sethi AK, Mohta M: Role of thoracic epidural block in improving post-operative outcome for septic patients: A preliminary report. *Eur J Anaesthesiol* 2011; 28:291–7
- Ai K, Kotake Y, Satoh T, Serita R, Takeda J, Morisaki H: Epidural anesthesia retards intestinal acidosis and reduces portal vein endotoxin concentrations during progressive hypoxia in rabbits. *ANESTHESIOLOGY* 2001; 94:263–9
- Kosugi S, Morisaki H, Satoh T, Ai K, Yamamoto M, Soejima J, Serita R, Kotake Y, Ishizaka A, Takeda J: Epidural analgesia prevents endotoxin-induced gut mucosal injury in rabbits. *Anesth Analg* 2005; 101:265–72
- Han X, Fink MP, Delude RL: Proinflammatory cytokines cause NO^{*}-dependent and -independent changes in expression and localization of tight junction proteins in intestinal epithelial cells. *Shock* 2003; 19:229–37
- Han X, Fink MP, Yang R, Delude RL: Increased iNOS activity is essential for intestinal epithelial tight junction dysfunction in endotoxemic mice. *Shock* 2004; 21:261–70

18. Commission on Life Sciences NRC: Guide for the Care and Use of Laboratory Animals, 7th edition. Washington, D.C., National Academies Press, 1996
19. Wang W, Smail N, Wang P, Chaudry IH: Increased gut permeability after hemorrhage is associated with upregulation of local and systemic IL-6. *J Surg Res* 1998; 79:39–46
20. Mousa SA, Bopaiah CP, Richter JF, Yamdeu RS, Schäfer M: Inhibition of inflammatory pain by CRF at peripheral, spinal and supraspinal sites: Involvement of areas coexpressing CRF receptors and opioid peptides. *Neuropsychopharmacology* 2007; 32:2530–42
21. Mousa SA, Machelska H, Schäfer M, Stein C: Immunohistochemical localization of endomorphin-1 and endomorphin-2 in immune cells and spinal cord in a model of inflammatory pain. *J Neuroimmunol* 2002; 126:5–15
22. Höcherl K, Schmidt C, Kurt B, Bucher M: Activation of the PGI(2)/IP system contributes to the development of circulatory failure in a rat model of endotoxic shock. *Hypertension* 2008; 52:330–5
23. Scheingraber S, Rehm M, Sehmisch C, Finsterer U: Rapid saline infusion produces hyperchloremic acidosis in patients undergoing gynecologic surgery. *ANESTHESIOLOGY* 1999; 90:1265–70
24. Ince C: The microcirculation is the motor of sepsis. *Crit Care* 2005; 9 Suppl 4:S13–9
25. Andonegui G, Kerfoot SM, McNagny K, Ebbert KV, Patel KD, Kubes P: Platelets express functional Toll-like receptor-4. *Blood* 2005; 106:2417–23
26. Eichelbrönnner O, Sielenkämper A, Cepinskas G, Sibbald WJ, Chin-Yee IH: Endotoxin promotes adhesion of human erythrocytes to human vascular endothelial cells under conditions of flow. *Crit Care Med* 2000; 28:1865–70
27. McCuskey RS, Urbaschek R, Urbaschek B: The microcirculation during endotoxemia. *Cardiovasc Res* 1996; 32:752–63
28. Schmidt W, Schmidt H, Bauer H, Gebhard MM, Martin E: Influence of lidocaine on endotoxin-induced leukocyte-endothelial cell adhesion and macromolecular leakage *in vivo*. *ANESTHESIOLOGY* 1997; 87:617–24
29. Swank GM, Deitch EA: Role of the gut in multiple organ failure: Bacterial translocation and permeability changes. *World J Surg* 1996; 20:411–7
30. Salzman AL, Menconi MJ, Unno N, Ezzell RM, Casey DM, Gonzalez PK, Fink MP: Nitric oxide dilates tight junctions and depletes ATP in cultured Caco-2BBE intestinal epithelial monolayers. *Am J Physiol* 1995; 268(2 Pt 1):G361–73
31. Liu S, Gallo DJ, Green AM, Williams DL, Gong X, Shapiro RA, Gambotto AA, Humphris EL, Vodovotz Y, Billiar TR: Role of toll-like receptors in changes in gene expression and NF-kappa B activation in mouse hepatocytes stimulated with lipopolysaccharide. *Infect Immun* 2002; 70:3433–42
32. Schröder NW, Opitz B, Lamping N, Michelsen KS, Zähringer U, Göbel UB, Schumann RR: Involvement of lipopolysaccharide binding protein, CD14, and Toll-like receptors in the initiation of innate immune responses by *Treponema glycolipids*. *J Immunol* 2000; 165:2683–93
33. Stuehr DJ, Marletta MA: Mammalian nitrate biosynthesis: Mouse macrophages produce nitrite and nitrate in response to *Escherichia coli* lipopolysaccharide. *Proc Natl Acad Sci USA* 1985; 82:7738–42
34. Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, Wang H, Abumrad N, Eaton JW, Tracey KJ: Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 2000; 405:458–62
35. Guarini S, Altavilla D, Cainazzo MM, Giuliani D, Bigiani A, Marini H, Squadrito G, Minutoli L, Bertolini A, Marini R, Adamo EB, Venuti FS, Squadrito F: Efferent vagal fibre stimulation blunts nuclear factor-kappaB activation and protects against hypovolemic hemorrhagic shock. *Circulation* 2003; 107:1189–94
36. Rainer TH, Lam N, Cocks RA: Adrenaline upregulates monocyte L-selectin *in vitro*. *Resuscitation* 1999; 43:47–55
37. Chi DS, Qui M, Krishnaswamy G, Li C, Stone W: Regulation of nitric oxide production from macrophages by lipopolysaccharide and catecholamines. *Nitric Oxide* 2003; 8:127–32
38. de Montmollin E, Aboab J, Mansart A, Annane D: Bench-to bedside review: Beta-adrenergic modulation in sepsis. *Crit Care* 2009; 13:230
39. Taniguchi T, Shibata K, Yamamoto K, Mizukoshi Y, Kobayashi T: Effects of lidocaine administration on hemodynamics and cytokine responses to endotoxemia in rabbits. *Crit Care Med* 2000; 28:755–9
40. Hofmann-Kiefer KF, Kemming GI, Chappell D, Flondor M, Kisch-Wedel H, Hauser A, Pallivathukal S, Conzen P, Rehm M: Serum heparan sulfate levels are elevated in endotoxemia. *Eur J Med Res* 2009; 14:526–31
41. Marechal X, Favory R, Joulin O, Montaigne D, Hassoun S, Decoster B, Zerimech F, Neviere R: Endothelial glycocalyx damage during endotoxemia coincides with microcirculatory dysfunction and vascular oxidative stress. *Shock* 2008; 29:572–6
42. Becker BF, Chappell D, Jacob M: Endothelial glycocalyx and coronary vascular permeability: The fringe benefit. *Basic Res Cardiol* 2010; 105:687–701
43. Roberts LJ 2nd, Morrow JD: Isoprostanes. Novel markers of endogenous lipid peroxidation and potential mediators of oxidant injury. *Ann N Y Acad Sci* 1994; 744:237–42