BASIC RESEARCH

MESENTERIC MICROCIRCULATORY DYSFUNCTIONS AND TRANSLOCATION OF INDIGENOUS BACTERIA IN A RAT MODEL OF STRANGULATED SMALL BOWEL OBSTRUCTION

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PRUPOSE: Bacterial translocation has been shown to occur in critically ill patients after extensive trauma, shock, sepsis, or thermal injury. The present study investigates mesenteric microcirculatory dysfunctions, the bacterial translocation phenomenon, and hemodynamic/metabolic disturbances in a rat model of intestinal obstruction and ischemia.

METHODS: Anesthetized (pentobarbital 50 mg/kg, i.p.) male Wistar rats (250-350 g) were submitted to intestinal obstruction or laparotomy without intestinal obstruction (Sham) and were evaluated 24 hours later. Bacterial translocation was assessed by bacterial culture of the mesenteric lymph nodes (MLN), liver, spleen, and blood. Leukocyte-endothelial interactions in the mesenteric microcirculation were assessed by intravital microscopy, and P-selectin and intercellular adhesion molecule (ICAM)-1 expressions were quantified by immunohistochemistry. Hematocrit, blood gases, lactate, glucose, white blood cells, serum urea, creatinine, bilirubin, and hepatic enzymes were measured.

RESULTS: About 86% of intestinal obstruction rats presented positive cultures for *E. coli* in samples of the mesenteric lymph nodes, liver, and spleen, and 57% had positive hemocultures. In comparison to the Sham rats, intestinal obstruction induced neutrophilia and increased the number of rolling (~2-fold), adherent (~5-fold), and migrated leukocytes (~11-fold); this increase was accompanied by an increased expression of P-selectin (~2-fold) and intercellular adhesion molecule-1 (~2-fold) in the mesenteric microcirculation. Intestinal obstruction rats exhibited decreased PaCO2, alkalosis, hyperlactatemia, and hyperglycemia, and increased blood potassium, hepatic enzyme activity, serum urea, creatinine, and bilirubin. A high mortality rate was observed after intestinal obstruction (83% at 72 h *vs.* 0% in Sham rats).

CONCLUSION: Intestinal obstruction and ischemia in rats is a relevant model for the *in vivo* study of mesenteric microcirculatory dysfunction and the occurrence of bacterial translocation. This model parallels the events implicated in multiple organ dysfunction (MOD) and death.

KEYWORDS: Mesenteric microcirculation; Bacterial translocation; Intestinal obstruction; Leukocyte-endothelium interactions; Adhesion molecules.

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INTRODUCTION

Since Berg and Garlington[1] observed the presence of indigenous intestinal bacteria in the mesenteric lymph nodes of mice and the term "bacterial translocation" was created, several studies have supported the hypothesis of the "gut origin of sepsis." This hypothesis states that bacteria that normally inhabit the intestinal lumen cross the epithelial barrier and act as a source of sepsis in distant places.[2,3] The

concept of bacterial translocation (BT) leading to systemic sepsis has been supported by several experimental studies[4-8] and some clinical studies,[9-12] such as when the host is immunocompromised or critically ill. Conditions associated with splanchnic hypoperfusion, such as hemorrhagic shock and intestinal ischemia, result in the gut becoming a cytokine-generating organ, which is followed by intestinal mucosal injury and loss of gut-barrier function.[13,14]

The present study investigates microcirculatory alterations and bacterial translocation in a rat model of intestinal obstruction and ischemia, as well as the metabolic, hemodynamic, and inflammatory responses associated with sepsis and MOD development.

MATERIALS AND METHODS

Animal Model

The experimental protocols were approved by the Animal Subject Committee of the Heart Institute (InCor) of the Faculdade de Medicina da Universidade de São Paulo. All experiments adhered to the ethical principles in animal research adopted by the Brazilian College of Animal Experimentation. Male Wistar rats about 2 months of age that weighed 250-350 g at the beginning of the experiments were used. The animals were maintained at 23ºC ± 2ºC under a cycle of 12 h light/12 h darkness and allowed access to food and water *ad libitum*. The animals were randomized into the *Sham group*, in which animals were subjected only to laparotomy and the ileum was manipulated but not ligated, and the *Intestinal obstruction and ischemia (IO) group*, in which (after the induction of anesthesia) the animals were subjected to intestinal obstruction and ligature of mesenteric vessels.

Anesthesia and Monitoring

Rats were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg). The carotid artery and jugular vein were cannulated with a polyethylene (PE-10) catheter to monitor arterial pressure and collect blood samples.

Operative Technique

Under anesthesia and in aseptic conditions (shaved skin, sterile operative fields, and use of povidone-iodine), a median laparotomy (3 cm midline ventral abdominal skin incision and a similar incision in the abdominal muscles) was carried out. The cecum was exposed, and the ileum was ligated at 1.5 cm proximal to the ileocecal valve, followed by ligation of the mesenteric vessels that supply 7 – 10 cm of the ileal loop. The midline incision was closed in 2 layers with a 4-0 suture (Ethicon, Somerville, NJ, USA). After the surgical procedures, the animals were kept warm at 37ºC for 1 h and returned to their cages.

Hematocrit, Blood Gases, and Blood Lactate

Hematocrit, blood gases, and blood lactate analyses were performed on blood samples obtained from the carotid artery at baseline (0 h) and 24 h after intestinal obstruction and ischemia. Hematocrit was measured by microcapillary tube centrifugation. Arterial blood gases and lactate were analyzed by a gas analyzer (Radiometer ABL 555, Radiometer Medical, Copenhagen, Denmark).

White Blood Cell Counts and Blood Glucose Levels

White blood cell counts and blood glucose levels (Advantage glucose monitor, Lilly, São Paulo, SP) were measured in blood samples obtained from the cut tip of the tail at baseline (0 h) and 24 h after the surgical procedures. Total cell counts were determined using a hemocytometer. Differential cell counts were carried out on stained films under oil immersion microscopy. A total of 100 cells were counted and classified on the basis of normal morphological criteria.

Serum Biochemistry

Twenty-four hours after the surgical procedures, blood was collected from the abdominal aorta for analysis of urea, creatinine, bilirubin, and the activities of the enzymes alanine aminotransferase (ALT), alkaline phosphatase, aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactic dehydrogenase (LDH); all of these were measured using commercially available kits (Modular Analytics, Roche Diagnostics GmbH, Mannheim, Germany).

Microbiological Assay

Samples of mesenteric lymph nodes (MLN), liver, spleen, and blood from the abdominal aorta were obtained 24 h after the surgical procedures. The tissues were macerated and diluted with 1.0 mL (6.0 mL for liver) NaCl 0.9%. Aliquots of 100 µL were sown on Mac Conkey agar (Difco) and incubated for 24 h at 37ºC. Blood samples (1 mL) were inoculated into Hemocult® I (Laborclin, PR, Brazil) under sterile conditions for 24 to 48 h at 37ºC. Samples were then sown on Mac Conkey agar and incubated for 24 h at 37ºC.

Intravital Microscopy of the Mesenteric Microcirculation

Intravital microscopy of the mesenteric microcirculation was performed as previously described.[16,17] In brief, the animals were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg). After an abdominal midline incision, the distal ileum and its accompanying mesentery were exposed for *in vivo* microscopic examination of the microcirculation. The animals were maintained on a specially designed stage warmed by circulating water kept at 37ºC. The stage had a transparent platform on which the tissue to be transilluminated was placed. The mesentery was continuously perfused throughout the study period with a warmed (37ºC) Krebs-Henseleit solution (113 mmol/L NaCl, 4.7 mmol/L KCl, 2.5 mmol/L $CaCl_2.2H_2O$, 25 mmol/L $NaHCO_3$, 1.1 mmol/L $MgSO_4$, 1.1 mmol/L KH_2PO_4, 5 mmol/L glucose, pH 7.20-7.40) that was saturated with a mixture of gases (95% N_2 and 5% CO_2). This procedure kept the microcirculatory characteristics unchanged throughout the intravital microscopic analysis. The mesenteric microcirculation was assessed after 10 min of stabilization. Three to five postcapillary venules (diameter 15-25 μm) were selected for each animal. A charge-coupled device color camera (TK-C1380U, JVC Co, Tokyo, Japan) was incorporated into a triocular microscope (Axioplan 2, Carl Zeiss Co, München-Hallbergmoos, Germany) to facilitate the observation of the enlarged image (425x) on a microcomputer monitor (SyncMaster 753DFX, Samsung, Manaus, Brazil). Analyses of leukocyte-endothelium interactions were performed online using image-computer software (Axiovision 4.1, Carl Zeiss Co) with an incorporated modulus of interactive measurements and time laps. Images were stored, enabling off-line playback analysis. Rolling leukocytes were defined as white blood cells that moved at a significantly slower velocity than the erythrocytes in a given microvessel.[18] The number of rolling leukocytes was presented as the mean number of cells passing at a designated line perpendicular to the venular axis per 10 min. A given section of the vascular bed was tested only once. Three to five microvessels were selected in a single animal to avoid sampling variability. Individual leukocyte rolling velocity was calculated using the time required for steady rolling leukocytes to travel a defined distance in the microvessel. [19] Rolling velocity in each vessel was calculated as the average velocity of 10 leukocytes. Results are presented in micrometers per second. A leukocyte was considered to be adherent to the venular endothelium if it remained stationary for more than 30 s. [18,20] Adherent cells were counted during a 10 min period in a 100 μm segment of the vessel. The number of leukocytes accumulating in the connective tissue adjacent to the chosen postcapillary venule was measured in a standard area of 5,000 μm^2. Two to three different fields were evaluated for each microvessel, and three to five microvessels were selected for a single animal.

Immunohistochemistry for Adhesion Molecules

Twenty-four hours after their surgical procedures, the animals were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg) and exsanguinated by abdominal aorta puncture. The mesentery was removed, immersed in hexane, and frozen in liquid nitrogen. Serial 8 μm cryostat sections were placed onto glass slides previously coated with organosilane (Sigma Chemical Co, St. Louis, MO, USA). For the immunodetection of intercellular adhesion molecule (ICAM)-1 and P-selectin on the mesenteric microvessels, samples were fixed in acetone and exposed to 3% hydrogen peroxide. SuperBlock buffer (Pierce Biotechnology, Rockford, IL, USA) was used to block nonspecific sites. Tissue sections were incubated overnight at 4ºC with a biotin-conjugated mouse monoclonal antibody anti-rat ICAM-1 (CD54) (Seikagaku Co, Tokyo, Japan) that was diluted 1:100 in phosphate buffered saline (PBS) containing 0.3% Tween 20. After washing the slides with PBS, sections were incubated with streptoavidin (R & D Systems Inc, Minneapolis, MN, USA) that was diluted 1:500 in PBS for 1 h at room temperature, developed with 3.3'-diaminobenzidine (DAB) (Sigma Chemical Co, St Louis, MO, USA), and counterstained with hematoxylin. A biotin-conjugated mouse monoclonal antibody anti-human P-selectin (R&D Systems Inc, Minneapolis, MN, USA) diluted 1:100 in PBS was used for the immunodetection of P-selectin on mesenteric microvessels. After fixation in acetone, tissue samples were incubated overnight with the antibody at 4ºC and rinsed in PBS. The samples were then treated with streptoavidin at room temperature for 1 h, developed with DAB, and counterstained with hematoxylin. Analyses were performed with Image-Pro Plus, version 4.1 (Media Cybernetics, Silver Spring, Md). Results are presented as mean optical density.

Statistical Analysis

Data are presented as means ± SEM and analyzed by Student's *t* test. The incidence of BT was evaluated by Chi-square analysis, and quantification of colony formation units/g tissue was evaluated by Mann-Whitney test. *P* values less than 0.05 were considered significant.

Table 1 - Analysis of arterial blood gases, lactate, hematocrit, electrolytes and glucose levels

Groups	Sham		IO	
	Baseline	24 h	Baseline	24 h
PaO_2 (mmHg)	77 ± 2	69 ± 3	92 ± 2	91 ± 14
$PaCO_2$ (mmHg)	48 ± 1	45 ± 1	44 ± 0	34 ± 7*
SO_2 (%)	94 ± 1	93 ± 1	97 ± 0	95 ± 3
pH	7.35 ± 0.01	7.40 ± 0.01	7.36 ± 0.01	7.49 ± 0.05*
HCO_3^- (mmol/L)	25.9 ± 0.8	27.0 ± 0.4	24.1 ± 0.3	24.4 ± 2.9
Lactate (mmol/L)	2.4 ± 0.2	2.4 ± 0.2	2.3 ± 0.2	4.8 ± 0.3§
Hematocrit (%)	43 ± 1	40 ± 1	42 ± 1	45 ± 2
Sodium (mmol/L)	137 ± 5	143 ± 1	139 ± 3	136 ± 4
Potassium (mmol/L)	4.0 ± 0.2	3.6 ± 0.2	4.5 ± 0.3	5.0 ± 0.3*
Glucose (mg/dL)	91 ± 4	96 ± 3	96 ± 2	196 ± 15§

IO, intestinal obstruction and ischemia; Sham, laparotomy without IO. Data are presented as mean ± SEM for 5 to 7 animals.
* $P< 0.05$; § $P< 0.001$ *vs.* corresponding value in Sham group

RESULTS

Clinic and Hemodynamic

Both Sham and IO rats presented a reduction in body weight 24 h after the surgical procedures. Loss of weight was greater in the IO group (-18±1 g *vs.* -7±1 g in Sham, $P<0.001$). There were no differences in mean arterial pressure between the groups 120 min after surgery (115±3 mmHg in Sham *vs.* 113±4 mmHg in IO, $P>0.05$). Total blood leukocyte counts were similar in the Sham and IO rats at baseline (13,571±730 cells/mm^3 in Sham *vs.* 13,050±1,238 cells/mm^3 in IO, $P>0.05$) and 24 h after surgery (12,201±1,223 cells/mm^3 in Sham *vs.* 16,614±1,997 cells/mm^3 in IO, $P>0.05$). Neutrophil/lymphocyte ratios were similar in both groups at baseline (0.33±0.04 in Sham *vs.* 0.32±0.03 in IO, $P>0.05$) but increased in the IO group at 24 h (4.30±0.54 vs. 0.18±0.01 in Sham, $P< 0.001$). In the IO group (n = 12), 58% of the animals died at 48 h and 83% at 72 h after surgery, whereas no animal in the Sham group (n = 12) died within the same period ($P<0.001$).

Arterial Blood Gases, Lactate, Hematocrit, Electrolytes, and Glucose Levels

All animals presented similar arterial oxygenation 24 h after surgery, as depicted by the values for PaO_2 and SO_2 in Table 1. Relative to blood gases and pH levels, the IO rats presented decreased $PaCO_2$ and alkalosis. However, HCO_3^- levels did not change. No differences were observed in hematocrit and sodium concentration. Blood potassium was increased in the IO group 24 h after surgery ($P < 0.05$). The IO rats also showed significant increases compared to Sham rats in blood lactate and blood glucose levels 24 h after surgery ($P < 0.001$).

Serum Biochemistry

Twenty-four hours after intestinal obstruction and ischemia, the serum activity of AST and ALP and the levels of creatinine, urea, and total bilirubin were significantly increased in the IO group compared to the Sham group ($P < 0.05$). Results are shown in Table 2.

Table 2 - Serum biochemistry

	Sham	IO
Urea (mg/dL)	44.0 ± 1.7	111.0 ± 26.2*
Creatinine (mg/dL)	0.25 ± 0.02	0.63 ± 0.09§
ALT (IU/L)	43 ± 6	84 ± 17
AST (IU/L)	124 ± 8	302 ± 71*
ALP (IU/L)	130 ± 7	274 ± 78§
LDH (IU/L)	1,904 ± 395	3,067 ± 908
Bilirubin (mg/dL)		
Indirect	0.10 ± 0.00	0.26 ± 0.06*
Direct	0.10 ± 0.00	0.06 ± 0.02
Total	0.20 ± 0.00	0.30 ± 0.06

IO, intestinal obstruction and ischemia; Sham, laparotomy without IO; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase. Data are presented as mean ± SEM for 5 animals in each group
* $P< 0.05$ and § $P< 0.01$ *vs.* Sham group

Microbiological Assays

The results, summarized in Table 3, showed that samples

Table 3 - Microbiological assays

Group	MLN		Liver		Spleen		Blood
	+/n	CFU/g	+/n	CFU/g	+/n	CFU/g	+/n
Sham	1/7	57	0/7	NG	0/7	NG	0/7
IO	6/7*	2,939±1,751§	6/7§	953±525§	6/7§	4,616±1,973§	4/7

IO, intestinal obstruction and ischemia; Sham, laparotomy without IO; +/n, number of animals with positive cultures for *E. coli* / total number of animals; CFU/g, colony formation units /g tissue (mean value ± SEM, n=7 animals in each group); NG, no growth.
* $P< 0.05$ and § $P< 0.01$ *vs.* Sham group

of MLN, liver, and spleen were positive for the presence of *E. coli* in 86% of the IO rats, and 57% of these animals had positive hemoculture for *E. coli*. The presence of *E. coli* was observed in a sample of MLN from one Sham rat only.

Leukocyte-Endothelial Interactions

For observation of mesenteric microcirculation, single and unbranched postcapillary venules were selected; their diameters ranged from 15 to 25 µm in all groups. Rolling leukocyte velocity and the number of rolling, adherent, and migrated leukocytes are presented in Figure 2. Twenty-four hours after surgery, rolling velocity was lower in IO rats (~13 µm/s) than in Sham rats (~18 µm/s, $P<0.001$). Relative to Sham-operated rats, which exhibited 131±4 rolling cells/10 min, 3±0 adherent cells/100 µm venule length, and 1±0 migrated cells/5,000 µm², IO rats presented a 2-fold increase in the number of rolling leukocytes, a 5-fold increase in the number of adherent leukocytes, and an 11-fold increase in the number of migrated leukocytes (Figure 1).

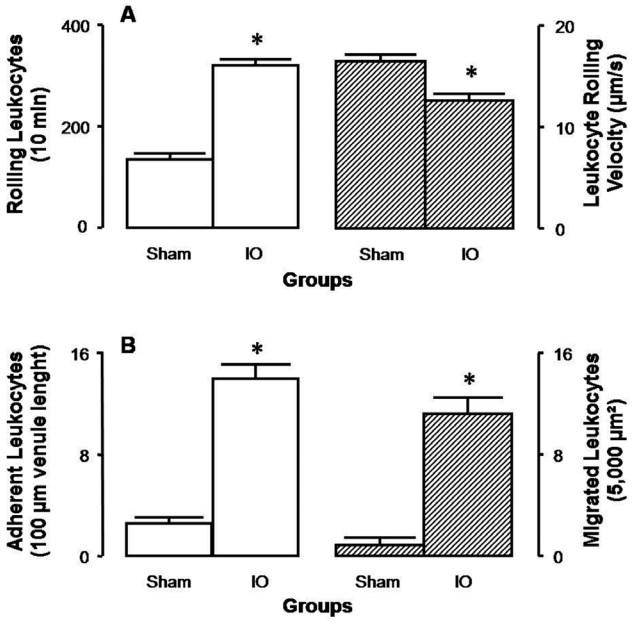

Figure 1 - Intravital microscopy of rat mesentery, 24 h after surgical procedures. A. Number of rolling leukocytes/10 min (open bars) and leukocyte rolling velocity (µm/s, hatched bars); B. number of adherent leukocytes/100 µm venule length (open bars) and number of migrated leukocytes/5,000µm² (hatched bars). Values are means ± SEM for 7 rats in each group. *$P < 0.001$ *vs.* corresponding values in Sham group

Expression of P-selectin and ICAM-1

Twenty-four hours after the surgical procedures, expression of P-selectin was markedly increased in the mesenteric microvessels of IO rats compared to Sham rats, as evidenced by immune staining. Similar results were obtained for ICAM-1 expression on mesenteric microvessels. Representative sections of these preparations and the quantitative evaluation of immune staining are shown in Figure 2.

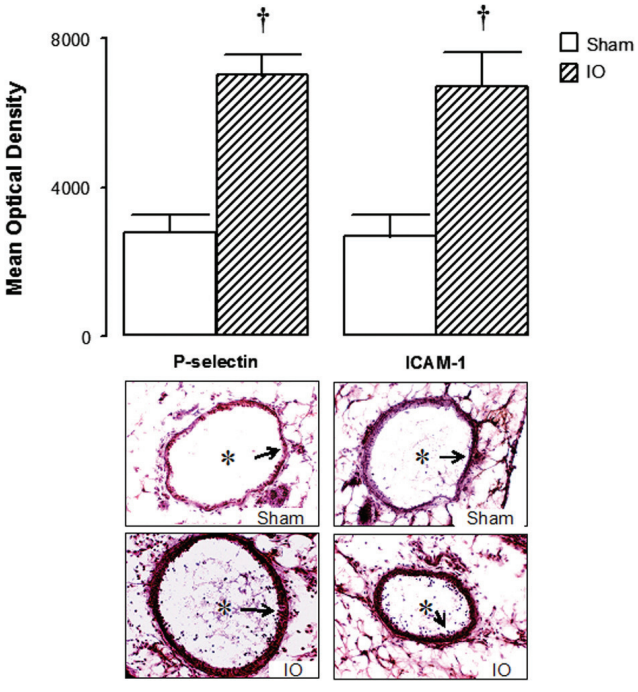

Figure 2 - Microphotographs of the mesentery and quantitative evaluation of immune staining for ICAM-1 and P-selectin on mesenteric microvessels obtained from Sham and IO rats. Mesenteric sections (8 µm) were stained (arrows) for the detection of ICAM-1 and P-selectin. * indicates the vessel lumen (original magnification 1500x). Values are means ± SEM for 8 samples/rat, 3 rats/group. Analyses were performed with the software Image-Pro Plus, version 4.1, Media Cybernetics. † $P < 0.001$ *vs.* Sham group

DISCUSSION

The current model reproduced several features observed in patients presenting mechanical small bowel obstruction. Important inflammatory events were observed in the mesenteric microcirculation, including an increased number of rolling, adherent, and migrated leukocytes accompanied by increased expression of P-selectin and ICAM-1 on mesenteric microvessels and neutrophilia**.** Hyperglycemia, hyperlactatemia, respiratory alkalosis, hyperkaliemia, and increased levels of urea, creatinine and AST and ALP activity reflect metabolic and acid-basic disorders and remote organ damage. In parallel, there was growth of enteric bacteria (*E. coli*) in samples of MLN, liver, spleen, and blood.

Experimental models, primarily in rodents, have been used to study intestinal BT phenomenon such as obstructive jaundice,[21] pancreatitis,[22] cirrhosis,[23] alterations of intestinal flora,[24] thermal injury,[25] intestinal ischemia-reperfusion,[26] hemorrhagic shock,[27] and intestinal obstruction.[5-7,28] The current model of intestinal obstruction and ischemia is an uncomplicated surgical procedure that does not require special conditions (e.g., bacterial inoculum) and has several features observed in surgical patients.

As described previously,[1] bacterial translocation was characterized by the growth of enteric bacteria (*E. coli*) in samples of MLN, liver, spleen, and blood. In a rat model of intestinal obstruction without ischemia, Çevikel et al.[6] demonstrated the occurrence of BT in samples of MLN, liver, and blood in 67% of animals. In the same study, the rate of BT increased to 100% of MLN and 75% of liver samples when the rats were submitted to strangulated obstruction. Results presented in our study showed that samples of MLN, liver, and spleen were positive for *E. coli* in 86% of IO rats. Positive hemocultures were observed in 57% of IO rats. Similar rates of BT were observed in the present model. In Sham-operated rats, a positive bacterial culture was observed in only one MLN sample, but low *E. coli* CFU/g counts were observed compared to rats submitted to intestinal obstruction and ischemia. Translocation of commensal bacteria can be considered a normal physiological phenomenon, considering that the majority of bacteria that cross the intestinal mucosa are destroyed by the gut-associated lymphoid tissue.[29] Similar findings were observed in BT models beyond those using intestinal obstruction and ischemia, such as in models of intestinal bacterial *inoculums*,[24] thermal injury,[25] and obstructive jaundice.[21] BT has been shown to occur in healthy patients and is elevated in critically ill patients with trauma, shock, sepsis, or thermal injury.[30] The main mechanisms involved in promoting BT are an alteration in indigenous gastrointestinal microflora, which results in bacterial overgrowth; physical disruption of the gut mucosal barrier by reduced blood flow to the intestine; and impairment of the host defenses.[30]

It has been recognized that major trauma, shock, or burn injury can lead to an MOD syndrome that is associated with a high mortality rate. Deitch *et al.*[31] emphasize the role of the gut in the development of injury and shock-induced MOD. Indeed, sustained vasoconstriction after hemorrhagic shock, or blood flow redistribution associated with septic shock, may cause splanchnic hypoperfusion,[32,33] disruption of the gut mucosal barrier, and BT. In addition, the gut and gut-associated lymphoid tissues produce cytokines and other inflammatory mediators, which may contribute to systemic inflammation and MOD.[13,31] Results presented here demonstrate the translocation of bacteria to the liver accompanied by the presence of hepatic injury; this was indicated by the increased serum activity of AST and ALP and the increased levels of total bilirubin. In a rat model of endotoxemia,[34] ALT and AST activities increase significantly 24 h after injection of LPS, reflecting the hepatic injury. In addition, significant increases in the levels of serum urea and creatinine were observed in our study, suggesting the presence of acute renal failure, a frequent remote complication after sepsis and trauma.[35]

In critically ill patients, hyperglycemia is a common finding and is associated with increased mortality.[36,37] The exact mechanisms of this hyperglycemia are unclear, but it may result from the effects of counter-regulatory factors such as elevated levels of cortisol and cytokines.[38] In a trauma animal model, control of the hyperglycemic state with insulin attenuates the pulmonary injury.[39] One study found a correlation between higher blood glucose levels and an increased mortality rate in a hyperglycemic septic model.[40] In the present study, rats submitted to intestinal obstruction and ischemia presented hyperglycemia (~200 mg/dL) 24 h after the surgical procedure. Some biomarkers (such as serum lactate) can be used as predictors of mortality in patients with infection.[41,42] Rats submitted to intestinal obstruction and ischemia presented a 2-fold increase in lactate levels (~5 mmol/L) 24 h after surgery. In parallel, mortality rates increased from 58% at 48 h to 83% at 72 h following intestinal obstruction and ischemia.

Microcirculation is essential for efficient delivery of oxygen to the cells. In critically ill patients, multiple organ dysfunction is associated with microcirculatory alterations and death.[43,44] The crucial role of the interactions between endothelial cells and leukocytes is further emphasized by the beneficial effects of therapeutic interventions acting at this level.[45] During an inflammatory response, leukocytes roll along the lining endothelium of post-capillary venules and eventually become firmly attached to the vascular

wall before migrating into tissues. Specific adhesion glycoproteins expressed on the surface of leukocytes and endothelial cells play a relevant role in the accumulation of leukocytes in the inflammatory lesion.[46,47] Members of the selectin family of cell adhesion molecules are thought to mediate leukocyte rolling along the walls of the microvasculature.[46] Glycoproteins of the CD11/CD18 complex ($\beta_2$integrins) that are expressed on leukocytes interact with ligands such as ICAM-1 on the endothelial cells to mediate leukocyte adhesion and migration.[47]

Microcirculatory dysfunctions similar to those observed in humans have been shown to occur in experimental models of sepsis.[16,48,49] Direct assessment of microcirculatory perfusion has been studied extensively *in vivo* in animals using intravital microscopy. In attempting to examine the intestinal microvasculature following a regional ischemia/reperfusion in the mesenteric vessels in hamsters, Boyd *et al.*[50] observed that a greater accumulation of leukocytes occurs in the mucosa. These authors also found that the crypt layer accounted for the majority of infiltrated cells as compared with the serosal layer and the mesentery. Farquhar *et al.*[51] demonstrated a decrease in the number of perfused capillaries in the small bowel mucosa of rats, using intravital microscopy in a normotensive sepsis model induced by cecal ligation and puncture (CLP). In a rat model of peritonitis, Lehmann *et al.*[52] have shown an increased number of leukocytes sticking within venules of the intestinal submucosal layer and a decrease in the functional capillary density of the intestinal wall. Compared to the current model of strangulated small bowel obstruction, the CLP model induces inflammatory disorders with similar magnitude, as demonstrated by Nakagawa *et al.*[17] However, unlike the CLP model, the strangulated small bowel obstruction model induced translocation of indigenous bacteria without surgically induced contamination of the peritoneal cavity.

In the current study, intravital microscopy was used to observe leukocyte-endothelial interactions in the mesenteric post-capillary venules of rats submitted to intestinal obstruction and ischemia. The real-time data presented here demonstrate a reduction in leukocyte rolling velocity and significant increases in the number of rolling, adherent, and migrated leukocytes 24 h after injury. In parallel, the expression of P-selectin and ICAM-1 in the mesenteric microvessels of these animals was markedly increased compared to Sham-operated rats. The accumulation of leukocytes in the inflamed tissues is preceded by leukocyte rolling and adhesion to the vascular endothelium. Leukocytes roll along the walls of post-capillary venules, mediated by the selectin family of adhesion molecules.[46] The interaction between ICAM-1 on the endothelial cells and β_2 integrins (CD11/CD18) on the leukocytes allows the leukocytes to become firmly adherent to the vascular wall.[47] The crucial role of adhesion molecules in leukocyte recruitment has been further emphasized by studies carried out on P-selectin-deficient mice[53] and ICAM-1-deficient mice,[54] both of which exhibit impaired neutrophil migration in response to noxious stimuli. The upregulation of P-selectin and ICAM-1 on mesenteric microvessels after intestinal obstruction and ischemia, as demonstrated in this study, suggests that generalized endothelial cell activation and the associated inflammatory response might precede the phenomenon of BT and MOD. Samel *et al.*[28] used intravital microscopy to assess the translocation of inoculated viable fluorescent *E. coli* under conditions of intestinal obstruction and ischemia in rats. This study demonstrated that the segmental ischemia of the obstructed small bowel accelerates translocation of bacteria into the submucosa and muscularis propria and further translocation to extraintestinal organs such as the liver and spleen. In this study [28], green fluorescent protein-transfected *E. coli* were visible in the submucosa and muscularis 10 and 60 min after *E. coli* administration, respectively. In the present study, bacterial translocation was evaluated 24 h after intestinal obstruction and ischemia, as previously demonstrated.[5-7]

Intestinal obstruction and ischemia in rats is a relevant model for the *in vivo* study of mesenteric microcirculatory dysfunction and the occurrence of bacterial translocation and parallels the events implicated in MOD and death.

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