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# HYPERTONIC SALINE SOLUTION REDUCES MESENTERIC MICROCIRCULATORY DYSFUNCTIONS AND BACTERIAL TRANSLOCATION IN A RAT MODEL OF STRANGULATED SMALL BOWEL OBSTRUCTION

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ABSTRACT—We examined the effects of hypertonic saline (HS) on inflammatory, metabolic variables, and bacterial translocation (BT) in rats submitted to intestinal obstruction and ischemia (IO). Male Wistar rats were submitted to IO and treated, 2 h thereafter, with lactated Ringer's (LR) (4 mL/kg per 5 min, i.v.) or HS (7.5% NaCl, 4 mL/kg per 5 min, i.v.). Twenty-four hours after IO, rats were also submitted to enterectomy/enteroanastomosis to resection of necrotized small bowel. Leukocyte-endothelial interactions were investigated by intravital microscopy and the expression of P-selectin and intercellular adhesion molecule 1 by immunohistochemistry. Bacterial cultures of mesenteric lymph nodes, liver, spleen, and blood were used to evaluate BT. Levels of chemokines (cytokine-induced neutrophil chemoattractants 1 and 2), insulin, and corticosterone were determined by enzyme-linked immunosorbent assay. Intestinal histology, serum urea and creatinine levels, and hepatic enzymes activities were performed to evaluate local and remote damage. Relative to IO and LR-treated rats, which exhibited increases in the number of rolling (1.5-fold), adhered (3.5-fold) and migrated (9.0-fold) leukocytes, and increased expression of P-selectin (3-fold) and intercellular adhesion molecule 1 (3-fold) on mesenteric microcirculation, treatment with HS followed by enterectomy reduced leukocyte-endothelial interactions and expression of both adhesion molecules to values attained in sham rats. Serum chemokines were normalized after treatment with both solutions followed by enterectomy. Hypertonic saline–treated rats demonstrated a significant reduction in BT to 50% in liver and spleen samples and bacteremia (14%), compared with 82% of BT in liver and spleen samples of IO and LR-treated rats and bacteremia (57%). Local intestinal damage was attenuated, and renal and hepatic function preserved by treatment with HS followed by enterectomy. Survival rate increased to 86% up to 15 days. Data presented suggest that HS solution followed by enterectomy reduces mesenteric microcirculatory dysfunctions and BT, attenuating local and remote damage in a model of strangulated small bowel obstruction.

KEYWORDS—Gut, sepsis, microcirculation, leukocyte-endothelial interactions, chemokines

## INTRODUCTION

Severe sepsis and septic shock are high-morbidity and -mortality conditions associated with a variety of situations, such as trauma, burns, and extensive surgical procedures. In these conditions, the splanchnic hypoperfusion results in the gut becoming a cytokine-generating organ, which is followed by intestinal mucosal injury and loss of gut-barrier function (1).

Ischemia-reperfusion injury of the intestinal tract is an important factor associated with high morbidity and mortality of patients in septic and hypovolemic shock. The reperfusion injury often exceeds the initial cellular damage and is characterized by intense inflammatory reaction involving macrophages, endothelial cells, neutrophils, lymphocytes, platelets and parenchymal cells, the complement system, the coagulation cascade, reactive oxygen and nitrogen species, and proinflammatory and anti-inflammatory cytokines (2).

The treatment of strangulated bowel obstruction is based on the surgical correction of the obstruction restoring intestinal transit and blood flow, associated with aggressive crystalloid fluid resuscitation to correct hypovolemia and dehydration, and appropriate antibiotics. Small volumes of hypertonic saline (HS) solution have the ability to promote immediate blood volume expansion, restore cardiac output and regional blood flows, improve microcirculation, and modulate immune responses, attenuating the inflammatory response induced by shock and trauma (3). The physical and physiological effects of small volumes of HS resuscitation have short duration (4), whereas late effects are related to benefits in the immune response (5, 6) and reduction of the inflammatory response (7, 8).

The present study aimed to investigate the effects of HS solution on mesenteric microcirculatory dysfunctions, bacterial translocation (BT), hemodynamic/metabolic disturbances, and inflammatory response associated with sepsis and multiple organ dysfunction development in a rat model of strangulated small bowel obstruction.

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## MATERIALS AND METHODS

### Animals

Male Wistar rats weighing 250 to 300 g at the beginning of the experiments were used. The animals were maintained at 23°C ± 2°C under a 12-h light-dark cycle and were allowed access to food and water *ad libitum*. All experiments were in accordance with the ethical principles in animal research adopted by the Brazilian College of Animal Experimentation. Approval of the Animal Subject Committee of the Heart Institute (InCor), University of São Paulo Medical School, was obtained before initiating the experiments.

### Anesthesia and monitoring

Rats were anesthetized with i.p. sodium pentobarbital (50 mg/kg). The carotid artery and jugular vein were cannulated with a polyethylene (PE-10) catheter to monitor arterial pressure (AcqKnowledge System MP100; Biopac, Goleta, Calif) and to collect blood samples.

### Intestinal obstruction and ischemia

A rat model of strangulated small bowel obstruction (9) was used. In brief, under anesthesia and 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- to 10-cm length of ileal loop. Midline incision was closed in two layers with a 4–0 suture (Ethicon, Somerville, NJ). After surgical procedures, the animals were kept warm for 1 h at 37°C, returned to their cages, and allowed to recover with free access to food and water.

### Clinical treatment

The experimental protocol is illustrated in Figure 1. Rats were randomly divided into four groups: (*a*) sham—laparotomy without IO; (*b*) IO—intestinal obstruction and ischemia; (*c*) LR—IO rats treated, 2 h thereafter, with lactated Ringer's (LR) solution (4 mL/kg per 5 min, i.v.); and (*d*) HS—IO rats treated, 2 h thereafter, with HS solution (7.5% NaCl 2,400 mOsm/L, 4 mL/kg per 5 min, i.v.).

### Necrotic small bowel resection and peritoneal lavage

Twenty-four hours after IO, removal of the necrotized small bowel loop and irrigation of the peritoneal cavity were performed. Animals were anesthetized with i.p. sodium pentobarbital (50 mg/kg), and the midline abdomen incision was reopened. The necrotic ileum was resected (enterectomy), enteroanastomosis was performed to restoring the intestinal transit, and the bowel was carefully returned into the abdomen. The peritoneal cavity was lavaged with warmed isotonic saline solution (0.9% NaCl). The midline incision was closed in two layers with 4–0 suture (Ethicon). The animals were kept warmed for 1 h at 37°C and returned to their cages with free access to food and water.

### Blood sampling

Arterial blood samples (100 μL) were obtained from the catheter inserted into the carotid artery at three different time points (0, 24, and 48 h) to perform gas analysis. For microbiological and immunoenzymatic assays, serum biochemistry blood samples were obtained from the abdominal aorta at the time the animals were killed, that is, at 24 or 48 h in two independent sets of experiments.

### Microbiological assay

Mesenteric lymph nodes (MLNs), liver, spleen, and blood from the abdominal aorta were obtained 24 h after IO. Tissues were macerated and diluted with 1.0 mL (6.0 mL for liver) 0.9% NaCl. Aliquots of 100 μL were sowed on MacConkey agar (Difco) and incubated for 24 h at 37°C. Blood samples (1 mL) were inoculated into Hemocult I (Laborclin, Paraná, Brazil) under sterile conditions for 24 to 48 h at 37°C. Samples were then sowed on MacConkey agar and incubated for 24 h at 37°C.

### Intravital microscopy of the mesenteric microcirculation

Intravital microscopy of the mesenteric microcirculation was performed only once in each animal 24 h after necrotic small bowel resection, as illustrated in Figure 1. The animals were anesthetized with i.p. sodium pentobarbital (50 mg/kg), and after an abdominal midline incision, the distal ileum and its accompanying mesentery were exposed for *in vivo* microscopic examination of the microcirculation, as previously described (10, 11). The animals were maintained on a specially designed stage warmed by circulating water kept at 37°C. The stage has a transparent platform on which the tissue to be transilluminated was placed. The mesentery was continuously perfused along the study period with a warmed (37°C) 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), 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, 25–30 μm) were selected for each animal. A charge-coupled device color camera (TK-C1380U; JVC Co, Tokyo, Japan) was incorporated to a triocular microscope (Axioplan 2; Carl Zeiss Co, München-Hallbergmoos, Germany) to facilitate the observation of the enlarged image (425×) on a microcomputer monitor. Analyses of leukocyte-endothelium interactions were performed online by using an 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 velocity significantly slower than that of erythrocytes in a given microvessel. 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 on a single animal to avoid sampling variability. Individual leukocyte rolling velocity was calculated from

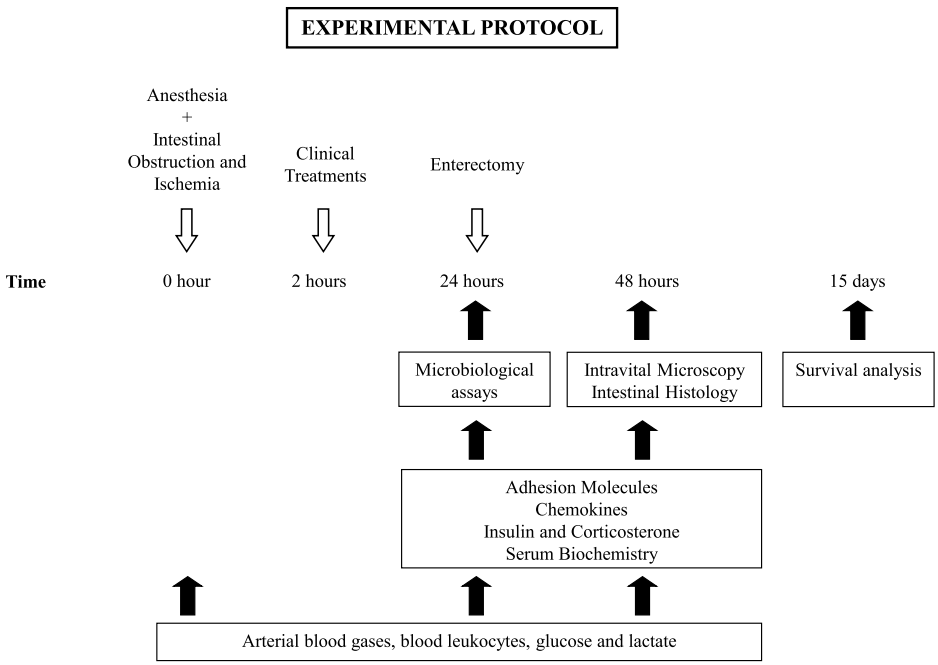



FIG. 1. Schematic representation of the experimental protocol.

the time required for steady rolling leukocyte to travel a defined distance in the microvessel. Rolling velocity in each vessel was determined as the average velocity of 10 leukocytes. Results are presented as micrometers per second. A leukocyte was considered to be adherent to the venular endothelium if it remained stationary for more than 30 s (7). Adherent cells were counted during a 10-min period in a 100-μm segment of the vessel. The number of leukocytes accumulating at the connective tissue, adjacent to the chosen postcapillary venule, was determined in a standard area of 5,000 $\mu m^2$. Two to three different fields were evaluated for each microvessel. Three to five microvessels were selected on a single animal.

### Immunohistochemistry for P-selectin and intercellular adhesion molecule 1

Twenty-four hours after IO and 24 h after necrotic small bowel resection, the animals were anesthetized with i.p. sodium pentobarbital (50 mg/kg) and exsanguinated by abdominal aorta puncture. The mesentery was removed, immersed in hexan, and frozen into liquid nitrogen. Serial 8-μm cryostat sections were placed onto glass slides previously coated with organosilane (Sigma Chemical Co, St Louis, Mo). For the immunodetection of intercellular adhesion molecule 1 (ICAM-1) and P-selectin on mesenteric microvessels, samples were fixed in acetone and exposed to 3% hydrogen peroxide. SuperBlock buffer (Pierce Biotechnology, Rockford, Ill) was used to block nonspecific sites. Tissue sections were incubated overnight at 4°C with a biotin-conjugated mouse monoclonal antibody anti–rat ICAM-1 (CD54; Seikagaku Co, Tokyo, Japan) or a biotin-conjugated mouse monoclonal antibody anti–human P-selectin (CD62P; R&D Systems Inc, Minneapolis, Minn). Both antibodies were diluted 1:100 in phosphate-buffered saline (PBS) containing 0.3% Tween 20. After washing the slides with PBS, sections were incubated with streptavidin-fluorescein (Amersham Pharmacia Biotech, London, UK) diluted 1:200 in PBS for 1 h at room temperature. After further washes, samples were treated with Vectashield mounting medium containing propidium iodide (Vector Labs, Burlingame, Calif) to preserve the fluorescence. Negative control samples were incubated with PBS instead of the primary antibody. Analyses were performed by using the Software Image-Pro Plus, version 4.1 (Media Cybernetics, Silver Spring, Md). Results are presented as mean fluorescence intensity.

### Enzyme immunoassay for chemokines

The concentrations of cytokine-induced neutrophil chemoattractant 1 (CINC-1) and CINC-2 in the serum of the animals were determined by enzyme-linked immunosorbent assay (ELISA) at 24 h after IO and 24 h after necrotic small bowel resection, using commercially available kits according to the manufacturer's instructions (R&D Systems Inc). The sensitivity of the assay was of 15 pg/mL.

### Intestinal histology

Segments of the distal ileum were removed 24 h after enterectomy. Samples were immersed in 10% paraformaldehyde and prepared for histological analysis. Tissue samples were dehydrated, embedded in paraffin, cut into a series of 4-μm-thick slices, and stained with hematoxylin and eosin. A scoring system to grade the degree of intestinal injury was devised on the basis of the following features: degree of injury to the basal lamina, edema, congestion, and inflammatory infiltrate. Each parameter received a score of 0 (none), 1 (mild), 2 (moderate), or 3 (severe) points. Total scores were calculated as the sum of individual parameters scores for a given sample. Mean scores and SDs were calculated for each group of animals.

### Hematocrit, arterial blood gases, blood lactate, blood glucose, and white blood cell counts

Hematocrit, blood gases, and blood lactate analyses were performed in blood samples obtained from the carotid artery at baseline (0 h), 24 h after IO, and 24 h after necrotic small bowel resection. 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 (Advantage glucose monitor; Lilly, São Paulo, Brazil) were determined in blood samples obtained from the cut tip of the tail at baseline (0 h), 24 h after IO, and 24 h after necrotic small bowel resection. Total cell counts were determined by using a hemacytometer. 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.

TABLE 1. **Analyses of arterial blood gases, hematocrit, blood lactate, and glucose levels**

| Groups | $Pa_{O_2}$, mmHg | $Pa_{CO_2}$, mmHg | pH | Hematocrit, % | Lactate, mmol/L | Blood glucose, mg/dL |
|---|---|---|---|---|---|---|
| Sham | | | | | | |
| Baseline | 77 ± 5 | 49 ± 1 | 7.35 ± 0.03 | 43 ± 2 | 2.4 ± 0.5 | 91 ± 11 |
| 24 h | 69 ± 8 | 45 ± 2 | 7.40 ± 0.02 | 40 ± 2 | 2.4 ± 0.4 | 96 ± 7 |
| IO | | | | | | |
| Baseline | 92 ± 5 | 44 ± 1 | 7.36 ± 0.02 | 42 ± 3 | 2.3 ± 0.5 | 96 ± 6 |
| 24 h | 91 ± 32 | 34 ± 15* | 7.49 ± 0.10* | 44 ± 4 | 4.8 ± 0.7† | 196 ± 39† |
| LR | | | | | | |
| Baseline | 77 ± 21 | 50 ± 4 | 7.33 ± 0.04 | 43 ± 2 | 1.8 ± 0.2 | 93 ± 4 |
| 24 h | 73 ± 15 | 40 ± 5* | 7.47 ± 0.04* | 44 ± 5 | 4.1 ± 0.5† | 126 ± 45* |
| HS | | | | | | |
| Baseline | 76 ± 21 | 50 ± 6 | 7.32 ± 0.03 | 40 ± 5 | 2.0 ± 0.7 | 96 ± 6 |
| 24 h | 76 ± 17 | 40 ± 6* | 7.44 ± 0.05* | 39 ± 8 | 3.2 ± 0.8† | 124 ± 19* |
| Sham | | | | | | |
| 48 h | 70 ± 7 | 44 ± 7 | 7.34 ± 0.08 | 43 ± 6 | 2.2 ± 0.5 | 94 ± 6 |
| IO + enterectomy | | | | | | |
| 48 h | 81 ± 14 | 37 ± 3 | 7.46 ± 0.03 | 41 ± 2 | 2.8 ± 0.7 | 124 ± 13‡ |
| LR + enterectomy | | | | | | |
| 48 h | 75 ± 3 | 41 ± 4 | 7.42 ± 0.05 | 40 ± 3 | 2.1 ± 0.5 | 109 ± 10 |
| HS + enterectomy | | | | | | |
| 48 h | 72 ± 5 | 39 ± 3 | 7.43 ± 0.02 | 39 ± 3 | 1.8 ± 0.1 | 97 ± 11 |

Intestinal obstruction and ischemia (IO); laparotomy without IO (sham); IO rats treated with LR solution (LR); IO rats treated with HS (HS) (7.5% NaCl) solution. Data are presented as mean ± SD for five to seven animals in each group.
*$P < 0.05$ vs. baseline.
†$P < 0.001$ vs. baseline.
‡$P < 0.05$ vs. sham and HS.

### *Serum biochemistry*

Twenty-four hours after IO and 24 h after necrotic small bowel resection, blood was collected from the abdominal aorta for the analyses of urea, creatinine, and the activities of the enzymes alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) determined by commercially available kits (Modular Analytics; Roche Diagnostics GmbH, Mannheim, Germany).

### *Enzyme immunoassay for insulin*

Blood was collected from the abdominal aorta at 24 h after IO and 24 h after necrotic small bowel resection, and cells were removed by centrifugation. Serum samples were kept at −70°C until use. The concentration of insulin was determined by ELISA (SPIbio, Massy, France), based on the competition between unlabeled rat insulin and acetylcholinesterase-linked rat insulin (tracer) for limited specific guinea pig anti–rat insulin antiserum sites. Results are presented as nanograms per milliliter.

### *Immunoassay for corticosterone*

Serum samples, obtained as described above, were kept at −70°C until use. The concentration of corticosterone was determined by ELISA (Cayman Chemical Company Inc, Ann Arbor, Mich), based on the competition between corticosterone present in the samples and a fixed amount of ALP-labeled corticosterone for sites on a sheep polyclonal antibody. Results are presented as picograms per milliliter.

### *Statistical analysis*

Data are presented as means ± SD. Statistical comparisons among groups were performed using ANOVA for repeated-measures and one-way analysis of variance. Differences among groups were tested by Tukey-Kramer or Kruskal-Wallis multiple-comparisons tests. Differences between proportions were evaluated by $\varkappa^2$ analyses. Survival was evaluated by the Kaplan-Meier test. $P < 0.05$ was considered significant.

## RESULTS

### *Systemic variables*

Values of mean arterial pressure, recorded during the first 120 min before treatment, were stable and similar among groups (data not shown). At 140 min, that is, 20 min after treatment, there were no differences in mean arterial pressure. Values (mean ± SD) for five animals in each group were as follows: 113 ± 9 mmHg in sham group, 117 ± 3 mmHg in the IO group, 124 ± 2 mmHg in LR group, and 120 ± 12 mmHg in HS group. All animals presented similar arterial oxygenation 24 h after IO, as depicted by the values for $Pao_2$ in Table 1. Intestinal obstruction and ischemia, LR, and HS groups presented decreased $Paco_2$ and alkalosis, and an increase in blood lactate and blood glucose levels, 24 h after IO. After necrotic small bowel resection (48 h), $Paco_2$, pH, blood lactate, and glycemia were normalized, with the exception of IO rats, which were still hyperglycemic. As shown in Table 2, the activity of ALT, AST, and ALP and levels of creatinine and urea significantly increased 24 h after IO compared with the sham group. At this time point, LR and HS treatments were efficient to prevent increases in serum creatinine levels and hepatic enzyme activity. Levels of urea, however, were still elevated in the LR group. Even after enterectomy serum activities of AST and ALP and levels of creatinine and urea remained increased in the IO group. The LR group exhibited normal levels of urea but increased levels of creatinine and elevated activities of ALT, AST, and ALP, whereas treatment of IO rats with HS solution reduced the activities of ALT and ALP and levels of creatinine and urea. Aspartate aminotransferase activity was still elevated (Table 2).

### *Effect of HS solution on* Escherichia coli *translocation*

Results, summarized in Table 3, showed that, 24 h after IO, samples of MLN were positive for the presence of *E. coli* in 86% to 100% of the animals submitted to intestinal obstruction and ischemia (IO, LR, and HS groups), against 14% in the sham group. Whereas there was no growth of *E. coli* in liver, spleen, and blood samples from the sham group, liver and spleen samples, as well as hemocultures, were positive for *E. coli* in 86% of IO rats and 79% of LR rats. In contrast, in the HS group, 50% of liver and spleen samples and 14% of blood samples were positive for *E. coli*. Furthermore, the number of *E. coli* colony-forming units (CFU)/g tissue was significantly reduced in liver and spleen after treatment of the animals with HS solution.

TABLE 2. **Serum biochemistry**

| Groups | Urea, mg/dL | Creatinine, mg/dL | ALT, IU/L | AST, IU/L | ALP, IU/L |
|---|---|---|---|---|---|
| 24 h | | | | | |
| Sham | 44 ± 4 | 0.25 ± 0.04 | 43 ± 13 | 124 ± 17 | 130 ± 15 |
| IO | 111 ± 59* | 0.63 ± 0.21† | 84 ± 39* | 302 ± 158* | 274 ± 174* |
| LR | 86 ± 18* | 0.40 ± 0.08 | 47 ± 7 | 174 ± 50 | 154 ± 27 |
| HS | 71 ± 25 | 0.38 ± 0.05 | 52 ± 16 | 211 ± 69 | 164 ± 35 |
| 48 h | | | | | |
| Sham | 39 ± 3 | 0.27 ± 0.04 | 41 ± 6 | 120 ± 18 | 117 ± 5 |
| IO + enterectomy | 71 ± 9‡§‖ | 0.70 ± 0.08‡§ | 41 ± 4 | 228 ± 42‡ | 189 ± 37‡ |
| LR + enterectomy | 55 ± 10 | 0.76 ± 0.05‡§ | 60 ± 10‡¶ | 257 ± 21‡ | 261 ± 45‡¶ |
| HS + enterectomy | 42 ± 1 | 0.37 ± 0.08 | 47 ± 2 | 203 ± 28‡ | 139 ± 6 |

Intestinal obstruction and ischemia (IO); laparotomy without IO (sham); IO rats treated with LR solution; IO rats treated with HS (7.5% NaCl) solution. Data are presented as mean ± SD for five animals in each group.
*$P < 0.05$ vs. sham.
†$P < 0.01$ vs. sham.
‡$P < 0.001$ vs. sham.
§$P < 0.001$ vs. HS.
‖$P < 0.01$ vs. LR.
¶$P < 0.001$ vs. IO.

TABLE 3. **Microbiological assays 24 h after IO**

| | MLN | | Liver | | Spleen | | Blood |
|---|---|---|---|---|---|---|---|
| Groups | +/n | CFU/g | +/n | CFU/g | +/n | CFU/g | +/n |
| Sham | 1/7 (14%) | 57 | 0/7 (0%) | NG | 0/7 (0%) | NG | 0/7 (0%) |
| IO | 6/7 (86%) | 2,939 ± 4,289* | 6/7 (86%) | 953 ± 1,285* | 6/7 (86%) | 4,616 ± 4,832* | 4/7 (57%) |
| LR | 7/7 (100%) | 1,862 ± 3,117* | 5/7 (71%) | 3,080 ± 4,096* | 6/7 (86%) | 4,376 ± 5,671* | 6/7 (86%) |
| HS | 6/7 (86%) | 2,371 ± 3,555* | 3/7 (43%) | 104 ± 117*† | 4/7 (57%) | 174 ± 184*† | 1/7 (14%) |

Mesenteric lymph nodes; number of animals with positive cultures for *E. coli*/total number of animals (+/n); no growth (NG); intestinal obstruction and ischemia (IO); laparotomy without IO (sham); IO rats treated with LR solution; IO rats treated with HS (7.5% NaCl) solution. Samples were obtained 24 h after IO. Data are presented as mean ± SD for seven animals in each group.
*$P < 0.05$ vs. sham.
†$P < 0.05$ vs. IO.
‡$P < 0.05$ vs. LR.

### *Effects of HS solution and enterectomy on leukocyte-endothelial interactions and expression of P-selectin and ICAM-1*

For the observation of mesenteric microcirculation, single and unbranched postcapillary venules were selected; their diameters ranged from 25 to 30 μm in all groups. Leukocyte rolling velocity and numbers of rolling, adhered, and migrated leukocytes are presented in Table 4. Compared with sham rats, leukocyte rolling velocity was significantly reduced in IO rats submitted to enterectomy. Intestinal obstruction and ischemia and LR rats submitted to enterectomy presented increased numbers of rolling (~1.5-fold), adhered (~3.5-fold), and migrated leukocytes (~9.0-fold) compared with sham rats. In contrast, treatment with HS solution followed by enterectomy restored leukocyte rolling velocity and significantly reduced the number of rolling leukocytes. The number of adhered and migrated leukocytes matched the values attained in sham group. Representative photomicrographs from each group are shown in Figure 2. Total blood leukocyte counts did not differ among groups. However, a significant increase in neutrophil-lymphocyte ratio was observed in the LR group compared with sham group. Results, illustrated in Figure 3A, showed that the expression of P-selectin was 3-fold increased in mesenteric microvessels of IO rats compared with sham rats. Similar results were observed after administration of LR solution to IO rats. In contrast, treatment of the animals with HS solution reduced P-selectin expression to values observed in sham group. Expression of ICAM-1 significantly increased (3-fold) in the IO group compared with sham group and further increased after treatment of IO rats with LR, as illustrated in Figure 3C. After enterectomy the expression of P-selectin and ICAM-1 remained increased in IO and LR rats, compared with sham rats. Values attained in HS rats matched those observed in sham rats (Fig. 3, B and D).

### *Effect of HS solution and enterectomy on chemokines release*

Increases in serum concentrations of CINC-1 (8.6-fold) and CINC-2 (16.6-fold) levels were observed 24 h after IO compared with sham group (Fig. 4, A and C). Relative to IO rats, treatment of the animals with LR significantly reduced (45%) the levels of CINC-2, but not CINC-1. When treated with HS solution, IO rats exhibited a significant reduction in CINC-1 (55%) and CINC-2 (65%) levels, as demonstrated in Figure 4, A and C. After enterectomy, the levels of both chemokines, CINC-1 and CINC-2, were markedly reduced in all groups as illustrated in Figure 4, B and D. Notwithstanding that, levels of CINC-2 were still higher in the IO group compared with sham group. In the HS-treated group, CINC-2 concentrations were reduced to the levels observed in sham rats (Fig. 4D).

### *Intestinal histology*

Relative to sham group, intestinal damage was observed in the IO group and LR- and HS-treated groups (Fig. 5A), which displayed significantly higher total scores, including basal lamina injury, edema, congestion, and presence of inflammatory

TABLE 4. **Leukocyte-endothelial interactions at mesenteric microcirculation**

| | | IO | LR | HS |
|---|---|---|---|---|
| Groups | Sham | Enterectomy | | |
| Leukocyte rolling velocity, μm/s | 19 ± 4 | 13 ± 1* | 16 ± 2 | 21 ± 2 |
| Rolling leukocytes/10 min | 128 ± 10 | 182 ± 7† | 193 ± 20† | 88 ± 15* |
| Adhered leukocytes/100-μm venule length | 3 ± 0 | 11 ± 2† | 10 ± 2† | 5 ± 1 |
| Migrated leukocytes/5,000 $\mu m^2$ | 1.0 ± 0.5 | 10.0 ± 1.6† | 8.0 ± 0.7† | 3.0 ± 0.5 |
| Blood leukocyte count, /μL | 13,664 ± 1,591 | 15,800 ± 2,263 | 15,493 ± 1,696 | 14,079 ± 1,198 |
| Neutrophil-lymphocyte ratio | 0.30 ± 0.04 | 0.70 ± 0.34 | 1.40 ± 1.09* | 0.80 ± 0.62 |

Intestinal obstruction and ischemia (IO); laparotomy without IO (sham); IO rats treated with LR solution; IO rats treated with HS (7.5% NaCl) solution. Intravital microscopy was performed 24 h after enterectomy. Data are presented as mean ± SD for five to seven animals.
*$P < 0.01$ vs. sham.
†$P < 0.001$ vs. sham.

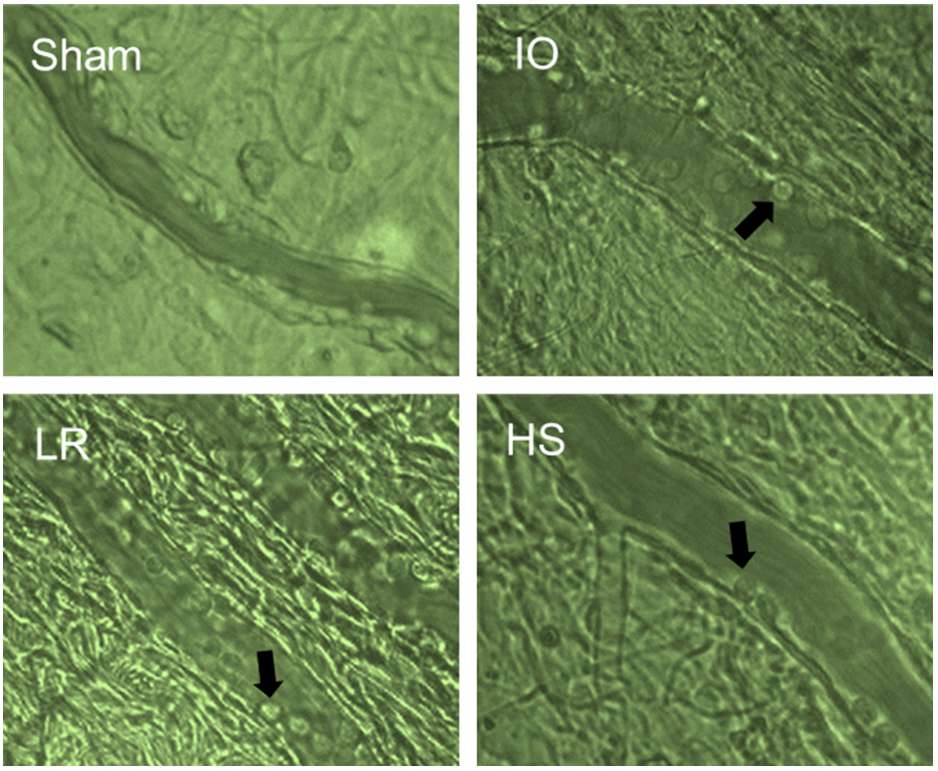



FIG. 2. **Representative photomicrographs of mesenteric microcirculation**. Increased leukocyte-endothelial interactions (arrows) are observed in rats submitted to IO, and IO rats treated with LR solution (LR), compared with IO rats treated with HS solution and sham-operated rats (sham). Final magnification ×425.

cells (Fig. 5B). Despite that, the total score for HS-treated rats was significantly lower when compared with LR-treated rats.

### *Serum insulin and corticosterone levels*

Results, illustrated in Figure 6A, showed that IO rats exhibited a significant reduction in serum insulin concentrations, which was prevented by treatment of the animals with LR or HS solutions. After enterectomy, there were no significant differences among groups (Fig. 6B). Relative to sham group, IO, LR, and HS groups presented a significant increase in serum corticosterone levels (Fig. 6C). Increased levels of corticosterone were still observed in IO and LR rats after enterectomy. Values were normalized after treatment with HS solution (Fig. 6D).

### *Survival rate*

Results, illustrated in Figure 7, showed that compared with sham group, which presented 100% survival, all rats of the IO group died by 72 h. Enterectomy and peritoneal lavage increased survival rate to 43% in the IO and LR groups. In HS-treated group, survival increased up to 86%. After enterectomy, IO (n = 3) and LR (n = 3) rats that have survived for 15 days exhibited lower body weight gain (11 ± 13 g/15 days and 14 ± 4 g/15 days, respectively) when compared with sham rats (43 ± 8 g/15 days, n = 7; $P < 0.05$). In the HS group, the gain in body weight (32 ± 18 g/15 days, n = 6) was similar to values attained in sham group (n = 7, $P > 0.05$).

## DISCUSSION

Data presented herein showed that HS solution is an effective treatment for the attenuation of local and systemic inflammation and translocation of indigenous bacteria in a rat model of strangulated small bowel obstruction. This is supported by the following observations: (i) HS solution reduced leukocyte-endothelial interactions at the mesenteric

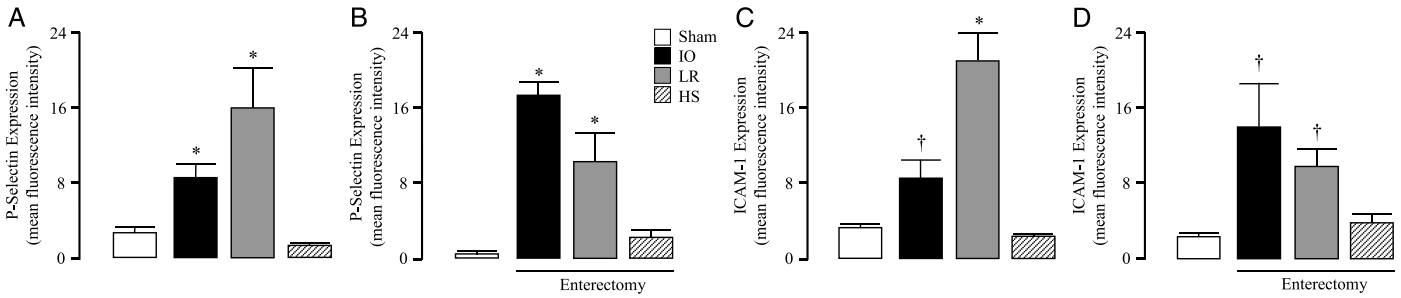



FIG. 3. **Quantitative evaluation of immunofluorescence for P-selectin (A, B) and ICAM-1 (C, D) expression on mesenteric microvessels in rats submitted to IO, laparotomy without IO (sham), IO rats treated with LR solution (LR), and IO rats treated with HS (7.5% NaCl) solution at 24 h (A, C) and 48 h (B, D)**. Values are means ± SD for eight samples/rat, three rats/group. Analyses were performed by using the software Image-Pro Plus, version 4.1, Media Cybernetics. $^{*}P < 0.001$, $^{\dagger}P < 0.005$ vs. sham.

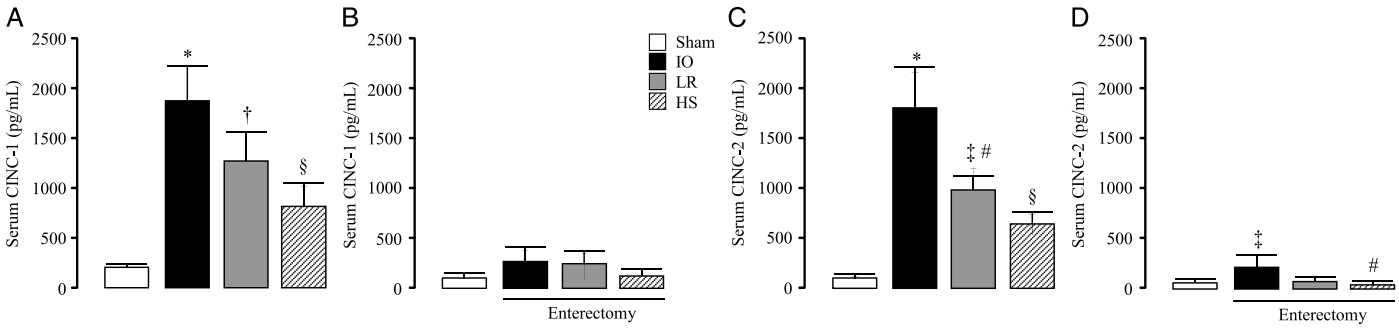


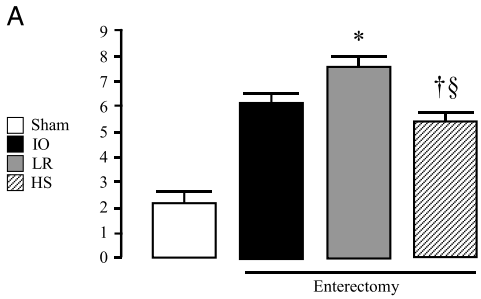

FIG. 4. **Serum concentrations of CINC-1 (A, B) and CINC-2 (C, D) in rats submitted to IO, laparotomy without IO (sham), IO rats treated with LR solution (LR), IO rats treated with HS (7.5% NaCl) solution at 24 h (A, C) and 48 h (B, D)**. Values are means ± SD for five animals in each group. $^{*}P < 0.001$, $^{\dagger}P < 0.01$, $^{\ddagger}P < 0.05$ vs. sham; $^{\S}P < 0.01$, $^{\#}P < 0.05$ vs. IO.

microcirculation, expression of endothelial cell adhesion molecules (P-selectin and ICAM-1), and serum levels of chemokines (CINC-1 and CINC-2), which emphasize its anti-inflammatory actions; (ii) there was a reduction on BT to blood, liver, and spleen; (iii) a local and remote organ-protective effect was depicted by a lower intestinal damage and by normalization of serum levels of urea, creatinine, and hepatic enzymes; and (iv) there was a significant increase in survival of rats treated with HS solution associated with enterectomy.

The current model reproduces several features observed in patients presenting mechanical and strangulated small bowel obstruction and sepsis, as previously demonstrated (9). Aiming to investigate the potential effects of HS solution on microcirculatory dysfunctions and BT, the animals were submitted to treatment in the early inflammatory phase (2 h after insult) followed by enterectomy 24 h thereafter.

Global oxygenation was similar in IO, LR, and HS groups. There was a significant increase in blood pH associated with a decrease in $Paco_2$, probably because of a respiratory alkalosis with hyperventilation, which corrected and exceeded the lactic acidosis typically observed in sepsis. The measurement of blood lactate is an important parameter in evaluating tissue hypoperfusion and/or hypoxia (12). Results presented herein showed that treatment with LR or HS solutions in association with enterectomy was efficient to normalize blood lactate levels, situation that was not observed in IO rats submitted to small bowel resection only. The intestinal hypoperfusion might be the main source for the elevation of blood lactate. Indeed, microcirculatory disorders have been demonstrated *in vivo* by intravital microscopy in experimental models of sepsis (10, 13). Boyd et al. (14) observed an intense accumulation of leukocytes in the mucosa after regional ischemia and reperfusion, with the majority of cells present in the crypt compared with the serosa and mesentery. Similar increases in the number of leukocytes adhering to the intestinal submucosa venules and a decrease in functional capillary density of the intestinal wall were observed after peritonitis in rats (15). In a model of normotensive sepsis induced by cecal ligation and puncture, Farquhar et al. (16) demonstrated a reduction in the number of perfused capillaries in the mucosa of the rat small intestine. In addition, increased numbers of rolling, adhered, and migrated leukocytes are observed by intravital microscopy of the mesenteric microcirculation, 24 h after induction of sepsis by cecal ligation and puncture in rats (11). Similar results were observed after IO, as demonstrated herein. Treatment with HS associated with enterectomy was efficient to reduce the inflammatory response at mesenteric microcirculation, restoring leukocyte rolling velocity and significantly reducing the number of rolling, adhered, and migrated leukocytes. In contrast, enterectomy alone or previous treatment with LR was not effective to prevent the local inflammatory reaction. In a study on peritonitis-induced septic shock, treatment of rats with HS solution prevents circulatory failure and organ dysfunction, decreasing mortality rate (17).

Leukocyte-endothelial interactions depend on the expression of specific adhesion glycoproteins on leukocytes and endothelial cells, which play a relevant role in the accumulation of leukocytes in the inflammatory lesion (18). In the current study, treatment of IO rats with HS reduced the expression of both adhesion molecules, P-selectin and ICAM-1, to values observed in sham rats when evaluated at two time points, before and after surgical treatment. In contrast, LR treatment potentiated the expression of both molecules above values observed in non-treated IO rats, remaining increased after enterectomy. In a




| Feature | Sham | IO | LR | HS |
|---|---|---|---|---|
| Basal lamina injury | 0.4 | 1.6 | 2.0 | 1.4 |
| Edema | 1.0 | 1.6 | 2.4 | 1.2 |
| Congestion | 0.4 | 1.8 | 2.0 | 1.6 |
| Inflammatory cells | 0.4 | 1.2 | 1.6 | 1.2 |
| **Total** | **2.2 ± 0.3** | **6.2 ± 0.3** | **7.8 ± 0.3** | **5.4 ± 0.2** |

FIG. 5. **Total intestinal histologic injury score (A) and average intestinal injury score for each estimated feature (B) in rats submitted to IO, laparotomy without IO (sham), IO rats treated with LR solution (LR), IO rats treated with HS (7.5% NaCl) solution at 48 h**. Values are means ± SD for five animals in each group. $^{*}P < 0.001$, $^{\dagger}P < 0.01$ vs. IO; $^{\S}P < 0.05$ vs. LR.

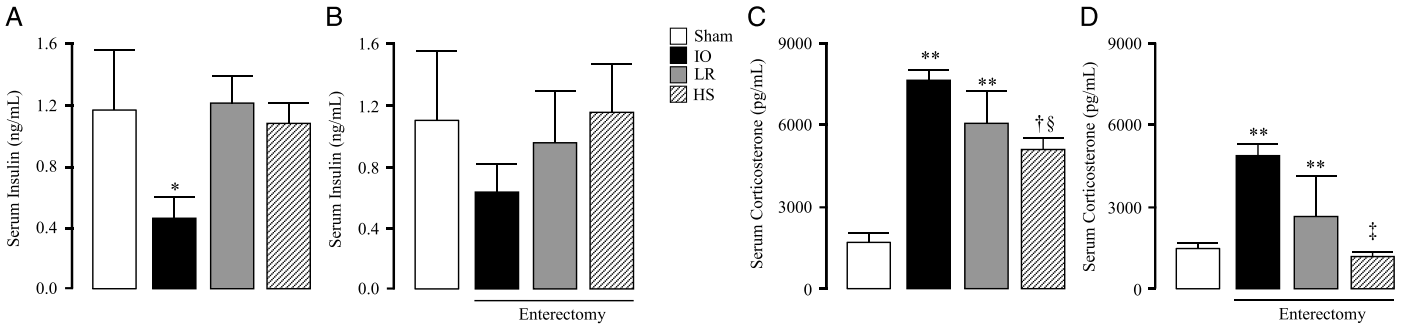



FIG. 6. **Serum concentrations of insulin (A, B) and corticosterone (C, D) in rats submitted to IO, laparotomy without IO (sham), IO rats treated with LR solution (LR), IO rats treated with HS (7.5% NaCl) solution at 24 h (A, C) and 48 h (B, D)**. Values are means ± SD for five animals in each group. $*P < 0.05$ vs. other groups; $**P < 0.001$, $^{\dagger}P < 0.01$ vs. sham; $^{\S}P < 0.05$ vs. IO; $^{\ddagger}P < 0.001$ vs. IO and LR.

previous study on hemorrhagic shock in rats, HS resuscitation downregulates leukocyte-endothelial cell interactions and lung ICAM-1 expression (7). In a double-injury model, hemorrhagic shock followed by lipopolysaccharide-induced lung inflammation in rats, treatment with HS reduces albumin leak and the number of neutrophil counts in bronchoalveolar lavage fluid, induces shedding of L-selectin, and prevents lipopolysaccharide-stimulated expression and activation of CD11b on neutrophils, when compared with LR resuscitation (19). In addition, HS associated with pentoxifylline significantly reduces the concentrations of tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) in bronchoalveolar lavage of rats subjected to hemorrhagic shock (8). The capacity of leukocytes to interact with the endothelium depends on the activation of endothelial cells by cytokines, such as TNF-α and IL-1β. This activation results in the upregulation or de novo synthesis of a variety of chemokines and adhesion molecules, including ICAM-1 (20). It has been shown that endothelial IL-8 plays a crucial role in the recruitment of neutrophils in sepsis (21). Data presented herein showed that HS solution significantly reduced serum levels of CINC-1 and CINC-2, analogs of the IL-8 family in humans, compared with LR treatment. Indeed, CINC-1 and possibly other CXC chemokines have an important role during intestinal ischemia and reperfusion injury (22).

In an *in vitro* study, hyperosmolarity induced by HS solution reduces the expression of ICAM-1 and IL-8 secretion, enhances phosphorylation of p38 MAPK, and reduces phosphorylation of IκB in TNF-α–stimulated human lung endothelial cells (23). In another *in vitro* study, the addition of dextran 70 + 7.5% NaCl to a human endothelial cell culture, primed with plasma from septic patients, reduces the adhesion of leukocytes to endothelial cells and the activation of both leukocytes and endothelial cells (24). At the clinical scenario, many of the effects and benefits observed in animal studies are not confirmed. However, in a recent study with patients with septic shock, it was demonstrated that treatment with 250 mL of 6% hydroxyethyl starch in 7.2% NaCl (Hyperhes) improves cardiac contractility and vascular tone compared with 500 mL 6% hydroxyethyl starch in 0.9% NaCl. However, hypertonic fluid was not superior to isotonic fluid with regard to gastric mucosal metabolism perfusion ratio and sublingual microcirculation (25).

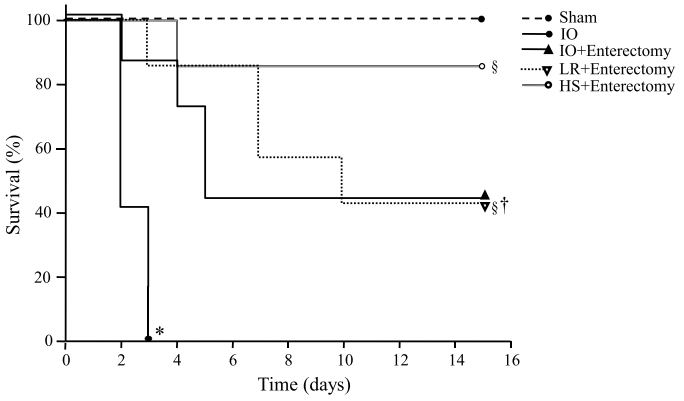



FIG. 7. **Kaplan-Meier survival analysis of animals**. Rats submitted to IO, laparotomy without IO (sham), IO rats treated with LR solution (LR), IO rats treated with HS (7.5% NaCl) solution were observed for 15 days. $*P < 0.001$, $^{\dagger}P < 0.05$ vs. sham; $^{\S}P < 0.01$ vs. IO.

The mechanisms that delineate microcirculatory dysfunction and organ failure are triggered by changes in microvascular blood flow and tissue oxygenation (26). The splanchnic vasoconstriction after hemorrhagic shock or over the redistribution of blood flow in septic shock causes splanchnic hypoperfusion, leading to ischemia of intestinal mucosa and predisposing to BT (27). Bacterial translocation, characterized by the growth of enteric bacteria (*E. coli*) in samples of MLN, liver, spleen, and blood, was observed in experimental models of sepsis (9, 28, 29). Data presented herein demonstrated that a massive number of CFUs were found in samples of different tissues in both groups IO nontreated and LR-treated rats. In contrast, HS solution was very effective in the containment of BT to liver and spleen. This suggests a restrictive activity of HS solution on BT and bacterial growth in infected tissues. Accordingly, HS might reduce the impact of bacteremia, a fundamental step for the infection of multiple organs and systems, to the genesis of systemic inflammatory response syndrome and the fatal outcome of sepsis. In rats submitted to hemorrhagic shock, HS resuscitation improves the barrier function (30) and reduces apoptosis of the intestinal mucosa (31). In a mouse model of thermal injury, the introduction of HS treatment reduces BT to MLNs and, in parallel, enhances host response to bacterial challenge by increasing Toll-like receptors 2 and 4 expression on inflammatory cells (32). In experimental sepsis by i.v. infusion of *E. coli*, resuscitation with HS solution has protective effect on the intestinal mucosa (33). Experimental evidence indicates that whereas physical and physiological effects of the administration of small volumes of HS have short duration, late effects are related to benefits in the immune response (3).

Stress-induced hyperglycemia is a common feature observed in intensive care unit patients and is associated with adverse outcome (34). In addition, insulin resistance often occurs following injury and/or critical illness (35). Control of blood glucose by intensive insulin therapy reduces morbidity and mortality among critically ill patients in the surgical intensive care unit (36). The postoperative hypercatabolic period, characterized by an increase in processes such as glycogenolysis and gluconeogenesis, among others, may be the cause of the hyperglycemia observed in rats that underwent IO. Stress associated with critical illness is characterized by activation of the hypothalamic-pituitary-adrenal axis with the release of cortisol from the adrenal gland (37). In addition to increased cortisol secretion, the stress response is characterized by a marked increase in the release of norepinephrine and epinephrine as well as glucagon and growth hormone (38). Indeed, elevated serum corticosterone levels were observed in IO, LR, and HS rats, 24 h after small bowel obstruction. In parallel, all of these groups presented hyperglycemia. Whereas IO rats exhibited a significant reduction in serum insulin concentrations, treatment of the animals with LR or HS prevented the insulinopenic state 24 h after small bowel obstruction and, in combination with enterectomy, reduced blood glucose to normal values. Serum corticosterone concentration was normalized only in the IO rats treated with HS followed by enterectomy. Furthermore, the gut secretes hormones or incretins, such as glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1, which potentiate meal-induced insulin secretion (39). Studies in rats demonstrated that parasympathetic stimuli regulate glucose-dependent insulinotropic polypeptide and glucagon-like peptide secretion (40). In the critically ill state, characterized by a stress response with higher plasma levels of catecholamines (41), the suggestion is that the release of incretins, by cholinergic stimuli, may be decreased, leading to a reduction in insulin secretion and hyperglycemia.

To investigate the changes that IO promotes locally and in distant organs and how treatments with HS or LR interfere with these changes, serum biochemistry and intestinal histology were performed. Both solutions, HS and LR, were effective to prevent increases in serum creatinine levels and hepatic enzyme activity. The local intestinal damage was attenuated by treatment with HS solution.

Finally, in association with the early benefits on the gut barrier function by reducing BT, and on the course of the inflammatory response by attenuating leukocyte-endothelial interactions, the expression of adhesion molecules, release of chemokines, and reduction of local and remote organ damage, HS treatment followed by enterectomy increased substantially the survival rate up to 15 days. Although it was not possible to relate high survival rate with a specific effect of HS, the transient elevation on plasma tonicity can initiate a series of molecular and cellular effects that reduce the inflammatory reaction and improve the immune response, which could reduce tissue damage and ameliorate the response to infections (42).

Some limitations of our study include the fact that animals were not given an equal amount of $Na^+$ in isovolumetric LR control solution and the inability to perform all analyses (microbiological assay, intravital microscopy, and survival analysis) in the same animal during all the experimental protocol. The lack of monitoring of serum biochemistry and intestinal histology throughout the 15-day interval of survival evaluation is one limitation to analyze the evolution of local and remote organ damage. Finally, further studies are necessary to elucidate the molecular mechanisms of the observed effects of HS solution in this model.

In conclusion, data presented suggest that HS solution followed by enterectomy reduces mesenteric microcirculatory dysfunctions and BT, attenuating local and remote damage in a model of strangulated small bowel obstruction.

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