Improvement in Early Symptoms of Shock by Delayed Intestinal Protease Inhibition

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**Hypothesis:** Recent findings indicate that intraintestinal pancreatic protease inhibition before superior mesenteric artery occlusion (SMAO) attenuates inflammation and symptoms of shock. Herein we examine the effectiveness of delayed intestinal protease inhibition during reperfusion after SMAO.

**Subjects:** Three groups of male Wistar rats were studied: a nonshock sham group and 2 groups exposed to SMAO for 100 minutes and treated by delayed intestinal lavage starting 40 minutes after reperfusion with buffer (delayed-lavage group) or with the digestive protease inhibitor gabexate mesilate (FOY) (delayed FOY-lavage group).

**Results:** Arterial pressure during reperfusion was significantly lower in the delayed-lavage animals compared with the sham group. Superior mesentery artery occlusion and reperfusion caused the formation of leukocyte activation factors in intestinal homogenates and in plasma, as well as intestinal injury. The delayed-lavage group had a significant increase in activated leukocytes in venules of cremaster muscle. In contrast, in the delayed FOY-lavage group, lavage 40 minutes after reperfusion led to a significant improvement of blood pressure and decreased formation of intestine-derived leukocyte activation factors and intestinal injury compared with the delayed-lavage group. In addition, the delayed FOY-lavage group exhibited fewer rolling leukocytes in venules and reduced apoptosis in the cremaster muscle microcirculation. Intestinal ischemia-induced endotoxemia was attenuated in the delayed FOY-lavage animals.

**Conclusion:** Delayed intestinal protease inhibition may improve experimental SMAO–induced shock by reducing intestinal injury, decreasing the level of cell activation in plasma and in the microcirculation, and restoring the blood pressure.

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activators, and leukocyte–endothelial cell interaction in the microcirculation of a peripheral muscle as a marker for systemic inflammation. In addition, we examine the role of a possible shock-induced endotoxin translocation and TNF-α activation during intestinal protease inhibition and their relation to leukocyte activation.

**METHODS**

**MATERIALS**

Fifteen male Wistar rats (weight, 180-220 g; Charles River Laboratories, Wilmington, Mass) were maintained on a standard rat chow and water ad libitum. All experiments were reviewed and approved by the University of California, San Diego, Animal Use and Care Committee. After general anesthesia (60 mg of pentobarbital sodium per kilogram of body weight, given intramuscularly), the animals were placed on a heating pad (37°C-38°C). The left femoral artery and vein were cannulated (PE-50 polyethylene tube; Clay Adams, Parsippany, NJ) to record arterial blood pressure and to administer supplemental anesthetic agent and resuscitation fluid (Ringer lactate, 4 mL/kg).

**Experimental Protocol**

The animals were laparotomized, and the superior mesenteric artery was clamped with a vessel clamp for 100 minutes (except in the nonshock sham [SHAM] group). The mortality rate for this model is 90% in 24 hours, with heart and lung failure and intestinal damage as major causes of death. During ischemia, the cremaster muscle microcirculation was exposed as described in the “Cremaster Muscle Preparation” subsection of this section. Animals were randomly divided into 3 groups:

1. Sham animals (n=5) underwent surgery without intestinal lave or ischemia. Rats in the other 2 groups underwent intestinal lave 40 minutes after intestinal reperfusion as described in the next subsection with 1 of 2 solutions:
   2. Krebs-Henseleit buffer solution (150 mL; delayed-lavage [DLAV] group, n=5) or
   3. A serine protease inhibitor comprising 0.37 mM gabexate mesilate (FOY; Ono Pharmaceutical, Tokyo, Japan) in 150 mL of Krebs-Henseleit buffer (delayed-FOY [DFOY] group, n=5), which reversibly blocks pancreatic proteases by binding proteases and thus inhibiting the binding site to activate the corresponding receptor at the effector cell.

**Intestinal Lavage**

In the DLAV and DFOY groups, the proximal duodenum was ligated after the pylorus and incised. A polyethylene tube (PE-250, Clay Adams) was inserted through the duodenal lumen into the descending duodenum before the papilla of Vater and fixed with 4-0 silk. Thereafter, the terminal ileum was disconnected from the cecum and incised. Forty minutes after intestinal reperfusion, the upper duodenum was rinsed with 100 mL of Krebs-Henseleit buffer solution (38.0°C, pH 7.6-7.8) with (DFOY group) or without (DLAV group) 0.37 mM FOY to flush out the intestinal content. Thereafter, another polyethylene tube was inserted into the terminal ileum and fixed with 4-0 silk for further intestinal lavage and fluid collection. In the DLAV and DFOY groups, the small intestine was rinsed with an additional 50 mL of washout solution (buffered with and without 0.37 mM FOY for the DFOY and DLAV rats, respectively) 75 minutes after reperfusion.

Preliminary investigation with intravital microscopy of the microcirculation in the intestinal wall revealed that intestinal lave did not alter the microcirculation (capillary and venular perfusion, as well as functional capillary density) with or without FOY. The blood pressure dropped in some rats during the first 5 minutes of lave and returned to normal levels before lave. We did not observe an increase in blood pressure due to intestinal lave alone. In the first minutes during intestinal lave, the gut motility seemed to be increased (by macroscopic observation); after 5 to 10 minutes, the motility decreased again; thereafter, it returned to normal levels. We observed no paralysis of the gut during this experiment.

**Plasma and Tissue Samples**

Before and after the lave, intestinal fluid was collected and centrifuged at 500g for 5 minutes. Aliquots of the supernatant were stored at −70°C until later measurements of protease and endotoxin activity, as further described in this section. Two milliliters of heparinized blood (10 U/mL) was drawn before ischemia and replaced by 4 mL of Ringer lactate, as well as 80 minutes after reperfusion at the end of the experiment. The samples were centrifuged (500g for 5 minutes), and plasma was frozen (at −70°C) for later measurements of leukocyte activation (by pseudopod formation) and endotoxin activity (described in the next subsection). After euthanasia, a 5-cm segment of the small intestine was excised, rinsed with 10mM phosphate-buffered saline (PBS), and fixed in formalin (10%) for 24 hours. The intestinal segment was embedded in resin, sectioned (1-µm thickness), stained with toluidine blue O, and examined under brightfield microscopy by a pathologist at magnifications up to ×300 for tissue damage. Another segment of the terminal ileum (1 g) was excised and rinsed, and all intestinal contents removed. The rinsed ileum segment was placed into 3 mL of a 10mM PBS solution, homogenized, and centrifuged at 1000g for 10 minutes. The supernatants were frozen (at −70°C) until determination of TNF-α formation and cell activation by leukocyte pseudopod formation, as described in the next subsection.

**Leukocyte Activating Factors**

To assess the level of leukocyte activating factors in the tissue samples, the formation of pseudopods by human leukocytes after incubation with 100 mL of intestinal homogenates or plasma was investigated. One hundred microliters of PBS incubation served as a negative control, and exposure to 100 µL of 10−6 M formyl-methionyl-leucyl-phenyalanine (a bacterial tripeptide that activates leukocytes) served as a positive control. Pseudopod formation is a useful clinical index of early leukocyte activation. Leukocytes with pseudopods are stiff and may easily be trapped in the capillary network. Moreover, pseudopods are important for leukocyte migration into the extravascular tissue. Measurements of pseudopod formation on human leukocytes were carried out as described previously.

**Cremaster Muscle Preparation**

Leukocytes and endothelial cells are among the first targets of inflammatory mediators in the bloodstream and are abundant in the microcirculation. To examine peripheral leukocyte or endothelial cell activation, we investigated the microcirculation of the cremaster muscle. This tissue preparation provides a full view of microvascular cells. The cremaster muscle was exposed according to a modified procedure previously described. Throughout the surgical procedure and during the experiment, the preparation was maintained at 37°C and superfused with Krebs-Henseleit bicarbonate-buffered solution saturated with a 95%
nitrogen to 5% carbon dioxide gas mixture to keep the tissue moist and under physiological conditions.

**Intravital Microscopy**

After exteriorization of the cremaster muscle, the rat was placed on an in vivo microscope, and 3 postcapillary venules in the muscle were selected for observation. The tissue was examined with an ×40 water immersion lens (numerical aperture 0.8; Carl Zeiss, Munich, Germany). Brightfield and fluorescent images were recorded with a color-coupled charge device camera (VI-470; Optronics, Goleta, Calif) at a frame rate of one sixtieth of a second for brightfield and one fourth of a second for fluorescent light and stored on a videotape recorder (AG3600; Panasonic Matsushita Electric, Tokyo).

After 80 minutes of ischemia (which was selected to coincide with a 40-minute equilibration period) and 40 and 80 minutes after reperfusion, the areas of interest were viewed and recorded on videotape.

**Leukocyte and Endothelial Cell Activation in the Microcirculation**

The red blood cell velocity was measured online using the photometric cross-correlation technique (Vista Electronics Company, Ramona, Calif). The vessel diameter and lengths were measured from the transillumination images during videotape playback. Thereafter, the number of rolling and adherent leukocytes was determined in each vessel for each time point. The number of rolling cells was expressed as cells per minute. A leukocyte was considered as an adherent cell when it did not move for at least 30 seconds during the observation period. The number of adherent cells was expressed as cells per square millimeter. White blood cell velocity was measured on a frame-by-frame basis (1 frame=1/30th of a second). The leukocyte adhesion index (LAI) was calculated as the ratio between white blood cell velocity and red blood cell velocity in the same vessel segment during the same period. An LAI of 1 indicates no membrane adhesion, and an LAI of 0 indicates complete membrane adhesion of leukocytes to the endothelium without rolling. A decline in LAI results from an increase in leukocyte or endothelial cell adhesion, expression of membrane adhesion molecules, and pseudopod formation.

**Apoptosis in the Cremaster Muscle**

The life-or-death indicator propidium iodide (PI) (1µM; Sigma-Aldrich Corp, St Louis, Mo) was added to the superfusate to display nuclei of dead cells. At each time point, a selected tissue area was videotaped for 1 minute using transillumination. Propidium iodide fluorescence was detected during epillumination using a fluorescent filter set attachment (Ploemper, Leitz, Germany) with a 100-W mercury lamp. After 80 minutes of ischemia (which was selected to coincide with a 40-minute equilibration period) and 40 and 80 minutes after reperfusion, selected tissue areas were viewed and recorded on videotape. At the end of the experiment, ethyl alcohol (100%) was added to the tissue perfusate to determine the total number of cells in each observation field and to compute the fraction of cells with PI-positive nuclei.

**Endotoxin Activity**

Endotoxin activity was assayed by the limulus amebocyte lysate test, with a kinetic modification of the test kit procedure. Samples were diluted 1:10 in pyrogen-free water and heated to 75°C for 5 minutes to remove nonspecific inhibitors. A quantitative chromatogenic kinetic method was developed based on limulus amebocyte lysate and a chromogen substrate (Coatest Endotoxin; Chromogenix, Stockholm, Sweden) using a microplate reader (ATTCC340; SLT, Grodö, Austria), allowing kinetic and isothermic applications for kinetic measurements at 37°C for 90 minutes. A commercial software program (Magellan; Tecan, Salzburg, Austria) with a curve fit test was used to calculate sample concentrations on the microplate. Endotoxin concentrations were computed from maximal changes of optical density per measurement cycle, with reference to a standard curve of Escherichia coli B26:06 endotoxin (Difco, Detroit, Mich) within a range of 1 to 10³ endotoxin units (EU)/mL. The detection limit of the method was 0.007 EU/mL of test sample.

**TNF-α Levels**

Tumor necrosis factor α was measured by enzyme-linked immunosorbent assay as described previously.13

**Protease Activity Measurements**

Protease activity in intestinal fluid was measured with a kit (Enz-Check; Molecular Probes, Eugene, Ore). Briefly, samples were mixed with 100 µL of digestion buffer and 100 µL of BODIPY-casein working solution (both provided with the kit) in a 96-well microplate and incubated for 90 minutes at 38°C. BODIPY labeled fluorescent dye labeling active peptides at excitation and emission maxima of approximately 505 and 513 nm and 589 and 617 nm, respectively. The proteases that are detected by this kit include elastase, trypsin, chymotrypsin, and pepsin. After incubation, the fluorescence intensity was measured by a photometer. Enzyme activity is expressed in fluorescent units (FU), and the lower detection limit lies between 1.0×10³ and 4.4×10³ FU.

**STATISTICAL ANALYSIS**

Data are presented as mean±SD. Repeated measurements of blood pressure and of leukocyte activation in the microcirculation in a single group were made using the repeated 1-way analysis of variance. To assess differences in blood pressure and microcirculatory measurements, leukocyte activation and endotoxins, and TNF-α levels at the same time point between all groups, the 1-way analysis of variance was used. Analysis of variance was followed by the Newman-Keuls correction test. Correlation between endotoxins, TNF-α, and leukocyte activating factors was done by Spearman rank order test. Trypsin values in intestinal fluids and before-and-after values of leukocyte activation by plasma were compared using the unpaired and paired t test. P<.05 was considered to be significant, as measured by SigmaStat (Jandel Scientific, San Rafael, Calif). P values are given in the figure legends. The power of the tests was 80% or higher.

**MEAN ARTERIAL BLOOD PRESSURE**

The mean arterial blood pressure (Figure 1) significantly decreased during reperfusion in the DLAV (72±13 mm Hg) and DFOY (83±7 mm Hg) animals compared with the SHAM group (94±8 mm Hg). The mean arterial blood pressure remained reduced throughout the experiment in the DLAV group (80 minutes after reperfusion, 66±11 mm Hg). However, protease inhibition starting 40 minutes after reperfusion in the DFOY animals significantly increased mean arterial blood pres-
During reperfusion, the microcirculation in the cremaster muscle exhibited enhanced levels of cell activation (Figure 3). Eighty minutes after reperfusion, the DLAV group had significantly increased numbers of rolling leukocytes (300±51 cells/mm per minute) and adherent leukocytes (18±7 cells/mm²) and a decreased LAI (0.05±0.02) compared with their values during ischemia (168±78 cells/mm per minute; 0 cells/mm²; and LAI, 0.14±0.02) or the values of the SHAM group (110±35 cells/mm per minute; 4±3 cells/mm²; and LAI, 0.13±0.01). Cell death in the cremaster muscle of the DLAV group significantly increased during reperfusion (80 minutes, 10%±6% PI-positive cells) compared with ischemia and SHAM values (both, 0% PI-positive cells).

Figure 2. The fraction of human leukocytes activated (as detected by pseudopods longer than 1 µm) by gut homogenates at 180 minutes (A) or heparinized plasma collected before ischemia and 80 minutes after reperfusion (B). Groups are the same as in Figure 1. Phosphate-buffered saline (PBS) serves as a negative control and formyl-methionyl-leucyl-phenylalanine (fMLP) as a positive control. A, Asterisk indicates P<.006 for the DLAV group compared with the SHAM and DFOY groups; dagger sign, P<.001 for fMLP compared with the SHAM, PBS, and DFOY groups. B, Asterisk indicates P<.049 for the DLAV group compared with its baseline value before ischemia and P<.003 compared with the DFOY and SHAM groups at 180 minutes. Error bars are mean ± SD.

LEUKOCYTE ACTIVATING FACTORS IN GUT HOMOGENATE AND PLASMA

After 80 minutes of reperfusion, the supernatant of intestinal homogenates from the DLAV animals (18%±8% activated leukocytes) and their plasma (14%±6% activated leukocytes) significantly activated naive human leukocytes (Figure 2) compared with the SHAM group (intestine, 4%±1% activated leukocytes; and plasma, 3%±1% activated leukocytes). Compared with the DLAV group, the formation of leukocyte activating factors in the intestine 80 minutes after reperfusion was significantly reduced in the DFOY animals (10%±6% activated leukocytes). There was, however, a significant difference between the DFOY and the SHAM animals (Figure 2A). Eighty minutes after reperfusion, the level of leukocyte activating factors in the plasma was almost normalized by delayed intestinal protease inhibition (4%±3% activated leukocytes) compared with the DLAV animals (Figure 2B).

LEUKOCYTE AND ENDOTHELIAL CELL ACTIVATION IN THE PERIPHERAL MICROCIRCULATION

During reperfusion, the microcirculation in the cremaster muscle exhibited enhanced levels of cell activation (Figure 3). Eighty minutes after reperfusion, the DLAV group had significantly increased numbers of rolling leukocytes (300±51 cells/mm per minute) and adherent leukocytes (18±7 cells/mm²) and a decreased LAI (0.05±0.02) compared with their values during ischemia (168±78 cells/mm per minute; 0 cells/mm²; and LAI, 0.14±0.02) or the values of the SHAM group (110±35 cells/mm per minute; 4±3 cells/mm²; and LAI, 0.13±0.01). Cell death in the cremaster muscle of the DLAV group significantly increased during reperfusion (80 minutes, 10%±6% PI-positive cells) compared with ischemia and SHAM values (both, 0% PI-positive cells).

Figure 1. Mean arterial blood pressure during ischemia and reperfusion. After reperfusion, the blood pressure dropped significantly in animals subjected to superior mesenteric artery occlusion at 120 and 140 minutes; asterisks indicate P<.002 for the delayed-lavage (DLAV) group and P=.001 for the delayed-FOY (protease inhibitor gabexate mesilate) (DFOY) group. Intestinal lavage with the protease inhibitor FOY at 140 minutes (in the DFOY group) attenuated the blood pressure reduction at 180 minutes compared with the DLAV group; dagger sign indicates P<.001. SHAM indicates the nonshock sham group. Error bars are mean ± SD.
of cell death may be attenuated by delayed pancreatic enzyme inhibition.

**HISTOLOGICAL EXAMINATION OF THE SMALL INTESTINE**

Histological sections of the intestine revealed ischemia and reperfusion-induced injury in the form of a complete loss of villi, ulcerations, and a high number of infiltrating leukocytes into the mucosa and submucosa (Figure 4C and D) in the DLAV group. The intestinal injury was improved in the DFOY group as seen in the form of intact villi and no ulcerations. However, there were several leukocytes infiltrating the mucosa and the submucosa (Figure 4E and F).

**ENDOTOXIN ACTIVITY AND TNF-α LEVELS**

Delayed intestinal lavage did not reduce endotoxin activity within the intestinal fluid, with or without the protease inhibitor FOY (Figure 5A). Intestinal ischemia and reperfusion were followed by endotoxemia 80 minutes after reperfusion in the DLAV animals. Intestinal protease inhibition 40 minutes after reperfusion significantly reduced plasma endotoxin activity in the DFOY group (Figure 5B). However, plasma endotoxin activity did not significantly correlate with the amount of leukocyte activation produced by the plasma (Figure 5C), especially in samples from shock-induced animals that produce high levels of cell activation.

Tumor necrosis factor (TNF-α) levels in the intestinal homogenate significantly increased after intestinal ischemia and 80 minutes of reperfusion in all groups (Figure 6A). Eighty minutes after reperfusion, the TNF-α levels in the plasma did not correlate with the level of cell activation produced by the same plasma samples (Figure 6B).

**INTESTINAL PROTEASE ACTIVITY**

After 80 minutes of reperfusion, the intestinal protease activity was significantly reduced when delayed lavage
was carried out at 40 minutes of reperfusion with FOY (188±33 FU compared with prelavage values of 2512±353 FU or the values in the DLAV rats 80 minutes after reperfusion of 895±586 FU) (P<.05).

Figure 4. Histological sections of the small intestine from the nonshock sham group (A and B) and from rats undergoing intestinal ischemia or reperfusion and intestinal lavage with buffer (delayed-lavage group, C and D) or with the protease inhibitor gabexate mesilate (delayed-FOY group, E and F) 40 minutes after reperfusion. Sections were taken 180 minutes after the onset of intestinal ischemia and reperfusion. Arrows mark leukocytes.
In intensive care, treatment modalities for shock followed by a systemic inflammatory response syndrome and multiple organ failure are limited. The original trigger mechanism for shock (hemorrhage, sepsis, heart failure, or trauma) seems to be followed by a cascade of processes, including leukocyte and endothelial cell activation. The origin of this cascade has remained uncertain.

Recent evidence suggests that pancreatic proteases may be important for leukocyte and endothelial cell activation and organ dysfunction.6-8,14-18 This leads to the hypothesis that IPI starting before intestinal ischemia and reperfusion maintains blood pressure. This approach has been tested and provides significant protection from early symptoms of shock.7,8,15,16,19,20 In the present study, we tested for the first time (to our knowledge) the hypothesis that delayed blockade of pancreatic enzymes may prevent rapid cessation of inflammatory mediator production in the ischemic intestine. We observed an increase in arterial blood pressure by IPI when started at a delayed time point during reperfusion, suggesting that this treatment may be an option in clinical situations without opportunity for pretreatment.

The mechanism by which IPI attenuates mean arterial blood pressure reduction during reperfusion in this model appears to involve blockade of the production of inflammatory mediators by digestive enzymes in the wall of the intestine. This is suggested by the reduction of leukocyte activating factors in gut homogenates after delayed FOY lavage, although TNF-α was not reduced in the DFOY group. This evidence indicates that IPI is reducing the activity of thus far unknown inflammatory mediators that stimulate leukocytes. We concluded that
inflammatory mediators may be an important target of IPI therapy. Tumor necrosis factor α, however, seems not to be involved. The biochemical characterization of the inflammatory mediators in plasma is the focus of investigations.19,21,22 Hydrophobic and hydrophilic inflammatory mediators, many of which have a molecular weight below about 10 kDa, have been identified. In the presence of digestive enzymes in the wall of the intestine, continued generation of proinflammatory mediators may occur during later periods of shock. Interruption of this process by delayed IPI may lower the levels of inflammatory mediators in systemic plasma. The reduction of plasmatic leukocyte activation has been demonstrated with preshock IPI8 and delayed IPI, as shown in this study. However, reduction of intestinal protease activity attenuates not only intestinal and plasmatic leukocyte activation8,10 but also myocardial depression.7,18,23 a key facet that may also account for the improved blood pressure.

The benefit was less likely obtained by improved perfusion of the intestine, because the level of microvascular perfusion in the wall of the intestine was unaffected by the intraintestinal lavage, with or without protease inhibition (F.F., unpublished intravital microscopy observations, 2000). Intestinal lavage per se, without inhibition of digestive enzymes, did not increase mean arterial pressure.

The microcirculation may benefit from the increased arterial blood pressure and the reduced inflammatory mediators in the blood, as low-flow states in the microcirculation24 and leukocyte and endothelial cell activation by inflammatory mediators are responsible for reduced capillary function. The microcirculation is one of the first targets affected by activated leukocytes and endothelial cells, resulting in tissue injury followed by peripheral hyperperfusion, hypoxia, or ischemia.25-27 The muscle microcirculation with the highest capillary density is affected by leukocyte and endothelial cell activation.12 Endothelial cells are known to produce cytokines and adhesion molecules and promote inflammation. In this respect, we believe that the skeletal muscle microcirculation plays an important role during shock and sepsis. Therefore, we explored leukocyte and endothelial cell activation in the microcirculation of a peripheral muscle as a tool to identify in vivo some of the earliest manifestations of activation.

In contrast to pretreatment with IPI,8 delayed IPI reduced only the number of rolling leukocytes in the peripheral microcirculation, while there was a nonsignificant trend to attenuate leukocyte adhesion. This evidence suggests that leukocytes already adhering to the endothelium are not significantly affected in their membrane adhesion mechanism. Delayed IPI may be better able to prevent an increase in the number of circulating leukocytes, so that fewer leukocytes have the possibility to enter the cremaster microcirculation, resulting in a reduced number of rolling leukocytes on postcapillary venules. Fewer circulating leukocytes may be possible because of reduced cell release from the bone marrow due to less inflammatory stimuli in the blood (reduced number of leukocyte activating factors by IPI18 and lower circulating interleukin 6 levels3). The cells that entered into the cremaster microcirculation during reperfusion after delayed IPI exhibited the same levels of rolling (a process that largely depends on P-selectin expression and is determined by LAI) and adhesion (eg, due to intercellular adhesion molecule 1 expression). Therefore, the evidence supports the hypothesis that the level of adhesion molecule expression is not affected by delayed IPI. In contrast, IPI applied before ischemia may reduce expression of adhesion molecules, as seen in a previous study.8

Intraintestinal pancreatic protease inhibition before reperfusion significantly reduces the level of apoptosis.8 With delayed IPI, we observed a trend to prevent further elevation of apoptosis. Because PI-positive cells cannot be resuscitated, delayed IPI reduces further spreading of apoptosis. This observation is in line with the hypothesis that IPI reduces the level of inflammatory and potentially toxic mediators in the plasma.

There was a nonsignificant trend toward improvement in leukocytes’ rheology and apoptosis in the muscle microcirculation. In this model, it was not possible to observe these variables several hours after ischemia; therefore, further studies are needed to explore this issue.

The presence of bacterial and endotoxin translocation in critically ill patients has been demonstrated as early as 1970.28 Recent clinical research did not confirm this observation,29 nor has the use of antiendotoxin and anticytokine strategies been protective and safe in clinical settings.1,2 Experimental studies30,31 indicate that intestinal damage and the subsequent release of leukocyte activating factors from the small intestine during intestinal ischemia and reperfusion or hemorrhage do not correlate with bacterial or endotoxin translocation. Therefore, in patients without an identifiable infection, other factors may be responsible for the systemic inflammatory response.

Toxic mediators, other than endotoxins, are abundant in posthemorrhagic plasma4 and mesenteric lymph.3,32 These mediators increase lung permeability and endothelial cell damage during hemorrhage and after resuscitation. In the present study, no strong correlation between endotoxin activity or TNF-α levels and leukocyte activation could be identified, which is in line with earlier observations.33 Although delayed IPI did not change intestinal endotoxin activity, the intervention significantly decreased endotoxin translocation. The implication of this observation in our study remains unclear. The mechanism by which IPI prevents leukocyte activation may be less dependent on endotoxins or TNF-α in plasma, as these variables did not correlate with each other.

The clinical implications of this experimental study remain to be explored. Therapeutic interventions to improve survival are dependent on a patient’s stage of shock (inflammatory or immunodepressed stage or early- or late-stage shock). Inhibition of intestinal proteases and indirect interference with the inflammatory response may be less stage-dependent and may have fewer adverse effects. Gut lavage may have a disadvantage in that it reduces its motility. Although during our experiment gut motility was not compromised according to macroscopic observation, long-term studies should further examine this issue. It also remains to be determined whether survival is improved by IPI. In our study, we examined only the early effect of this intervention. Because we in-
cised the small intestine and the duodenum to cannulate the gut and block intestinal proteases by FOY lavage, no long-term survival study was carried out. A different approach to inhibit intestinal proteases needs to be devised to determine survival rates.

In conclusion, the present results indicate that, even at a delayed phase of shock, inhibition of pancreatic enzyme activity in the lumen of the intestine may restore hypotension after intestinal ischemia and attenuate the levels of leukocyte activating factors in the intestine and in plasma. Shock-induced intestinal ulcerations and loss of villi architecture are significantly improved in animals treated with delayed IPI. Although for about 3 hours leukocytes that adhere to endothelium of postcapillary venules in a peripheral skeletal muscle are not affected by delayed IPI, such inhibition seems to attenuate the number of peripheral leukocytes and enhance cell death in microcirculation during shock. Our results suggest that shock-induced leukocyte activation may be less dependent on endotoxin activity and TNF-α levels.

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REFERENCES