Effects of Inhibition of PAF, ICAM-1 and PECAM-1 on Gut Barrier Failure Caused by Intestinal Ischemia and Reperfusion

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Background: The role of cell adhesion molecules and transmigration of PMNs through the endothelial barrier is probably essential in intestinal ischemia and reperfusion (I/R)-induced gut barrier dysfunction. Although cytokines are released in I/R, it is unclear whether cytokines directly increase permeability or if this phenomenon requires both expression of cell adhesion molecules and PMN adhesion-activation. Endothelial barrier dysfunction plays an important role in the pathogenesis of multiple organ dysfunction syndrome, inducing gut barrier failure, but the mechanisms are not fully understood. The purpose of this study was to evaluate the potential therapeutic value of inhibition of platelet activating factor (PAF), intercellular adhesion molecule-1 (ICAM-1), and platelet endothelial cell adhesion molecule-1 (PECAM-1) in gut barrier dysfunction induced by intestinal I/R. Methods: A PAF antagonist (lexipafant, BB-882) and monoclonal antibodies against rat ICAM-1 (anti-ICAM-1-MAb) and PECAM-1 (anti-PECAM-1-MAb) were used in a model of gut barrier dysfunction caused by intestinal ischemia for 40 min and concomitant reperfusion for 12 h in the rat, and endothelial permeability, myeloperoxidase activity, interleukin-1β, and protease inhibitor levels were evaluated. Results: The endothelial permeability and tissue leukocyte recruitment in the distal small intestine significantly increased in rats with I/R treated with saline. Proteolytic activity in plasma was evident by low levels of the three measured plasma protease inhibitors. These changes were, to different degrees, reduced by treatment with lexipafant, anti-ICAM-1-MAb, or anti-PECAM-1-MAb. Alterations in systemic levels of interleukin-1β paralleled the changes found in gut barrier permeability and leukocyte trapping. Conclusions: Our results suggest that treatment with the PAF inhibitor lexipafant and monoclonal antibodies against ICAM-1 or, seemingly most efficient, PECAM-1 reduces the severity of I/R-associated intestinal dysfunction, associated with a decrease in systemic concentrations of IL-1β local leukocyte recruitment, and partly restoring plasma protease inhibitor levels.

Key words: Adhesion molecules; barrier; cytokine; endothelium; epithelium; leukocyte; permeability; protease inhibitors

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Intestinal ischemia, e.g. presented as acute mesenteric ischemia, is still associated with a high mortality (1). Restoration of blood flow may save the intestine, but could also result in the development of multiple organ dysfunction and death (2). Neutrophil (PMN)–endothelial cell (EC) interactions are supposed to play a central role in the pathogenesis of gut barrier failure following ischemia and reperfusion (I/R) and multiple organ dysfunction syndrome (MODS) (3, 4). In previous in vivo studies we have shown that primed circulating neutrophils, platelet activating factor (PAF) and some cytokines, such as interleukin-1β (IL-1β) and interleukin-6 (IL-6) might be involved in the development of gut barrier dysfunction (5). It has also been suggested that cytokine-stimulated ECs activate quiescent PMNs to disrupt the endothelial integrity, thereby causing an increase in microvascular permeability and, in addition, potentially promoting progressive organ inflammation by exposing thrombogenic surfaces, leading to further accumulation of PMNs and other proinflammatory agents (6).

The role of cell adhesion molecules and transmigration of PMNs through the endothelial tight junctions is probably essential in intestinal I/R-induced gut barrier dysfunction. Although cytokines are released in I/R, it is unclear whether cytokines directly increase permeability or if this phenomenon requires both expressions of cell adhesion molecules and PMN adhesion-activation (7).

Endothelial barrier dysfunction plays an important role in the pathogenesis of the systemic inflammatory response syndrome (SIRS) and MODS, by exposing adhesion molecules on the surface of the endothelial membranes, producing
and releasing inflammatory mediators, interacting with circulating leukocytes, and compromising the barrier integrity (8). Plasma extravasation, occurring through the whole molecular weight (MW) range, from proteins such as albumin, with a rather moderate MW of 66,500 Da, to high MW proteins, like alpha-1-macroglobulin with a MW of 725,000, is a critical characteristic of endothelial barrier dysfunction. The leakage is associated with tissue edema, leukocyte recruitment, and organ dysfunction. Inflammatory mediators produced and released, such as interleukins, oxygen free radicals, PAF, polymorphonuclear enzymes, proteases, and adhesion molecules, have been suggested as potential early markers of severity and critical links in the pathogenesis and pathophysiology of disease (8). In previous studies (3), we have found proteolytic activity in plasma, as evidenced by low levels of the main plasma protease inhibitors of several blood cascade systems. In the present study, alterations in gut barrier dysfunction and leukocyte recruitment, systemic levels of interleukins, and plasma protease inhibitors, were evaluated in a model of small intestinal I/R in the rat. Furthermore, the potential therapeutic inhibition of PAF, intercellular adhesion molecule-1 (ICAM-1), and platelet endothelial cell adhesion molecule-1 (PECAM-1) was investigated in I/R-associated gut endothelial barrier dysfunction in rats, by using a PAF antagonist (lexipafant, BB-882) and monoclonal antibodies against rat ICAM-1 and PECAM-1.

Materials and Methods

Animals

Adult male Sprague–Dawley rats, weighing 250 g, were fed standard rat chow (R3, Astra-Ewos, Södertälje, Sweden) and water ad libitum. The rats were allowed to acclimatize to our laboratory conditions for 6 days and were subjected to a regime of 12 h day/night cycle living in mesh stainless-steel cages (3 rats/cage) at constant temperature (22°C). The Animal Ethics Committee approved the protocol at Lund University. All animals were handled in accordance with the guidelines set forth by the Swedish Physiological Society.

Induction of small intestinal I/R

The operations were performed under aseptic conditions using pentobarbital anesthesia (45 mg/kg i.p.). The animals had no access to solid food, but free access to water the last 12 h prior to the experiment. A 5.0 cm long midline laparotomy was performed and the superior mesenteric artery (SMA) was identified after deflecting the loops of intestine to the left with moist gauze swabs. After that the SMA was separated from its accompanying lymphatic trunk, it was temporarily occluded by an atraumatic clamp for 40 min at the origin from the aorta. Immediate blanching of the small intestine and cecum verified that the blood supply to these intestinal segments had been shut off. The abdomen was then covered with a sterile moist gauze pad. After 40 min intestinal ischemia, the clamp was removed from the SMA and, after verifying the return of blood supply to the gut, the laparotomy was closed and the animals were allowed to wake up, followed by 12 h reperfusion. The sham operation consisted of separation of the SMA without clamping for 40 min, followed by 12 h sham reperfusion (3).

The animals were randomly divided into eight groups with 6 rats in each group. All groups had a reperfusion period of 12 h. Group 1: sham operation and 1 ml saline i.p.; group 2: sham operation and lexipafant (BB-882, British Biotech Pharmaceuticals Ltd, Oxford, UK) i.p.; group 3: sham operation and anti-ICAM-1-MAb i.p.; group 4: sham operation and anti-PECAM-1-MAb i.p.; group 5: 40 min ischemia and 1 ml saline i.p.; group 6: 40 min ischemia and lexipafant i.p.; group 7: 40 min ischemia and monoclonal antibodies (M Ab) against rat ICAM-1 i.p. (anti-ICAM-1-MAb), purified from ascites on a Protein A matrix (R&D Systems Inc., Minneapolis, MN, USA); and group 8: 40 min ischemia and MAb against rat PECAM-1 i.p. (anti-PECAM-1-MAb), purified from tissue culture supernate on a Protein A matrix (R&D Systems Inc.); the PAF antagonist lexipafant (BB-882) was derived from British Biotech Pharmaceuticals Ltd, Oxford, UK. Measurements were performed 12 h after the end of the ischomic challenge or sham operation. Sterile saline or saline containing anti-ICAM-1-MAb (0.5 mg/kg), anti-PECAM-1-MAb (0.5 mg/kg), or lexipafant (5 mg/kg) in a volume of 0.2 ml was injected i.p. 15 min after sham operation or the end of ischemia, treatment repeated for lexipafant after 6 h (3).

Measurements of intestinal mucosal endothelial permeability

Red blood cells (RBC) were labeled with 51Cr (New England Nuclear, Boston, Mass., USA) during 20 min incubation at room temperature and then washed three times with physiological saline. The radioactivity was about 2.5 × 10⁶ cpm/ml. Intestinal mucosal endothelial barrier permeability was assessed in rats by the passage of 125I-labeled human serum albumin (HSA, Institutt for engergi-teknikk, Kjeller, Norway) from blood to the intestinal interstitial space. 125I-HSA in 1 ml, with 2.5 × 10⁶ cpm radioactivity, was injected into the femoral vein. One ml blood was drawn from the femoral vein after 1 h equilibration, followed by injection of 51Cr-RBC (2.5 × 10⁶ cpm/ml). The animals were killed by an overdose of ether 2 min after the RBC injection. The intestines were harvested and cleared of external blood by blotting dry. The radioactivity of 125I and 51Cr in blood and tissue samples was measured in a gamma-counter (1272 Clinigamma, LKB, Wallac OY, Finland). After that the samples were weighed for determining wet tissue weight, then all samples were put into the oven in 50°C for 7 days in order to obtain the dry weight.

Intestinal endothelial permeability was assessed by leakage of radiolabeled albumin from blood into the interstitial space and expressed as isotopic flux, defined as the proportion of 125I-radioactivity per gram tissue sample compared with per
gram blood as described previously (9). To assay possible redistribution of tissue blood, tissue blood content (TBC) was calculated by the proportion of counts $^{51}$Cr reference per gram tissue sample and counts $^{51}$Cr reference per gram blood sample. In order to correct for potential differences in the vascular surface area available for exchange of albumin, the albumin leakage index was calculated by dividing the extravascular protein accumulation in each tissue by assuming that all $^{51}$Cr-labeled RBC remain intravascularly, using the formula: albumin leakage index (ALI) = (extravascular tissue $^{125}$I counts/$^{125}$I counts per gram blood)/tissue blood content. Tissue blood content was calculated as $^{51}$Cr-RBC counts per gram tissue/$^{51}$Cr-RBC counts per ml blood. Extravascular tissue $^{125}$I counts was obtained by the subtraction of counts $^{125}$I reference per gram blood sample multiplied by tissue blood content from counts $^{125}$I reference per gram tissue (5).

Assays of IL-1β and IL-6

Plasma for assays were obtained by centrifuging blood for 15 min at 3500 rpm, 4 °C, and then kept at −70 °C until assays were performed. Plasma levels of IL-1β and IL-6 were determined by use of an enzyme linked-immuno-sorbent assay (ELISA) specific for rat IL-1β and IL-6 (BioSource International, Camarillo, CA, USA). Antibodies specific for rat IL-1β and IL-6 were coated onto the wells of the microtiter strips provided. The samples, including standards of known rat IL-1β and IL-6 content (BioSource), were pipetted into 96-well microtiter plates and all samples were run in duplicate. A standard curve was made by serial dilutions of known cytokine concentrations, provided in the ELISA kits, and the concentrations in the samples were calculated from this. All plates were read in a microplate reader (Multiskan PLUS Spektrofotometer, Labsystem AB, Lund, Sweden) at 450 nm.

Measurement of leukocyte recruitment

After that the small intestine was perfused with heparinized PBS to exclude tissue blood, a loop of distal ileum (10–12 cm proximal from the cecum), approximately 5 cm in length, was harvested, frozen immediately in liquid nitrogen and stored at −70 °C until measurements. For myeloperoxidase (MPO) measurement we used a modified method described by Komatsu et al. (10). All chemicals were purchased from Sigma Chemical Co., St. Louis, MO, USA. The samples were weighed (100–150 mg), put in 1 ml ice-cold potassium phosphate buffer (20 mM, pH 7.4), homogenized (Homogenizer Omni 1000, Lambda Polynom, Sollentuna, Sweden) for 15 sec on ice and then centrifuged at 10,000 rpm for 15 min at 4 °C (Sorvall RC-5B, Refrigerated superspeed centrifuge, Lambda Polynom). The supernatant was discharged to avoid the hemoglobin influence. The participates were rehomogenized for 15 sec in 0.5 ml 50 mM PBS, pH 6.0, with 0.5% HTAB (hexadecltrimethylammonium bromide) and 10 mM EDTA, followed by sonication on ice for 20 sec (Sonifier 250, KEBO Lab AB, Lund, Sweden), freezing and
thawing and homogenizing twice in order to permeate the cellular membrane. The samples were then centrifuged at 15,000 rpm for 15 min at 4°C. Of the final supernatant, 0.1 ml was put into 1.0 ml reaction solution consisting of 80 mM PBS, pH 5.4, with 1.6 mM TMB (tetra methyl benzidine), 0.5% HTAB and 0.3 mM H₂O₂. After incubation for 3 min at 37°C, the reaction was terminated by adding 4 ml 0.2 mM sodium acetate, pH 3.0, with the samples kept on ice. The tissue myeloperoxidase activity in the samples, defined as the change in absorbance at 655 nm in 1 min per gram tissue (A₆₅₅/min/g) (Hitachi U 2000, KEBO Lab) (10).

Measurement of albumin and plasma protease inhibitors

Three ml of blood was taken from each animal at the end of the experiment. EDTA plasma was obtained by centrifugation and then kept at −70°C until analysis. Albumin and the plasma protease inhibitor alpha-1-macroglobulin were measured with electroimmunoassay (11), using specific antisera against the rat proteins, prepared in our laboratory. The functional activities of the plasma protease inhibitors antithrombin III (S-2765; N-alpha-Cbo-D-Arg-pNA-2HCl) and alpha-2-antiplasmin (S-2251; G-D-Val-Leu-Lys-pNa-2HCl) were measured using chromogenic peptide substrates (12). The assay method given by the manufacturer (Chromogenix, Mölndal, Sweden) for humans could be used unchanged for rat antithrombin III, while it had to be partly modified for rat alpha-2-antiplasmin. Using the microtiter plate assay method for alpha-2-antiplasmin, the diluted samples were incubated with the substrate (S-2251) for 30 sec (30–60 sec in manual) and with plasmin for 7 min (5 min in manual), to give the best reproducible results. The standard curve for hirudinized, as well as for citrated and EDTA, rat plasma turned out to be much more flat that the human counterparts, almost horizontal between 50% and 125%, when using the proposed sample dilution of 1/40. Using a sample dilution of 1/80 instead resulted in a straight and steep standard curve also for rat plasma for 0%–75%, whereafter the curve was slightly bent for 75%–125%. Since these conditions gave the most precise and reproducible results out of several tested modifications, they were used throughout the study. All values are given as a percentage of normal in a reference plasma pool of 10 normal rats, of the same weight and age as the experimental animals.

Statistics

Unpaired Student t test or non-parametric test (Mann-Whitney rank sum test) was used after ANOVA. A probability of <0.05 was considered as significant. Values are expressed as mean ± sₓ.

Results

Values in between the different sham operated groups did not differ significantly and they were thus pooled as controls. The intestinal mucosal water content (Fig. 1) significantly
increased in animals with I/R challenge ($P < 0.01$), to different degrees restored in animals with I/R and treatment with lexicapant ($P < 0.05$), anti-ICAM-1-MAb ($P < 0.05$) or anti-PECAM-1-MAb ($P < 0.05$) as compared to controls. Small intestinal blood content (Fig. 2) in rats with intestinal I/R and saline was significantly lower as compared to controls ($P < 0.01$), but returned to control levels in the group with I/R and PECAM-1-MAb ($P < 0.01$). Administration of lexicapant or anti-ICAM-1-MAb tended to increase small intestinal blood content, though it still remained substantially lower than seen in controls.

HSA leakage index (Fig. 3) significantly increased in the small intestinal mucosa in intestinal I/R animals treated with saline ($P < 0.01$ versus controls). HSA leakage index in the small intestinal mucosa in intestinal I/R animals treated with lexicapant ($P < 0.01$), anti-ICAM-1-MAb ($P < 0.01$), or anti-PECAM-1-MAb ($P < 0.01$) were lower than in animals with I/R and saline, and still higher (lexipafant, $P < 0.05$; anti-ICAM-1-MAb, $P < 0.05$) than in controls.

Serum levels of IL-1$\beta$ (Fig. 4) in animals with small intestinal I/R and saline significantly increased as compared to controls ($P < 0.01$), while the levels were significantly lower in animals with intestinal I/R treated with lexicapant ($P < 0.05$), anti-ICAM-1-MAb ($P < 0.01$), or anti-PECAM-1-MAb ($P < 0.01$), as compared with animals subjected to I/R and treatment with saline. Serum levels of IL-6 (Fig. 5) in animals with small intestinal I/R and saline treatment was higher ($P < 0.01$ as compared to controls) and treatment with lexicapant ($P < 0.05$) or anti-PECAM-1-MAb ($P < 0.05$) reduced the I/R-induced increase in IL-6.

Tissue leukocyte recruitment as measured by the intestinal myeloperoxidase content significantly increased in the small intestinal mucosa after induction of gut I/R and saline treatment (Fig. 6; $P < 0.01$ versus controls). Treatment with lexicapant ($P < 0.05$), anti-ICAM-1-MAb ($P < 0.01$) or anti-PECAM-1-MAb ($P < 0.01$) in I/R animals significantly reduced tissue MPO content in the small intestinal mucosa as compared to animals with I/R and saline, but the levels were still significantly higher than in controls ($P < 0.05$).

Albumin and all three measured plasma protease inhibitors decreased during I/R challenge (Fig. 7). Albumin decreased from 91% to 55% in parallel with the results found for the small intestinal mucosal albumin leakage index (Fig. 2). Two of the measured protease inhibitors, i.e. the high MW alpha-1-macroglobulin and the low MW alpha-2-antiplasmin (MW as for albumin), decreased to very low levels, about 50% of normal plasma levels. Antithrombin III (MW as for albumin) also decreased, although not that pronounced, to 58% of normal levels. All treatment regimens protected rather well against the fall in albumin and antithrombin III levels (Fig. 7). Lexicapant treatment was more effective as compared to anti-PECAM and anti-ICAM monoclonal antibody treatment regarding the other two measured inhibitors, i.e. alpha-1-macroglobulin and alpha-2-antiplasmin.
Fig. 4. Serum levels of interleukin-1β (IL-1β) in controls and the groups subjected to small intestinal ischemia for 40 min and reperfusion for 12 h, treated with saline, the PAF inhibitor lelipafant, and monoclonal antibodies against intercellular adhesion molecule-1 (anti-ICAM1-MAb), or platelet endothelial cell adhesion molecule-1 (anti-PECAM1-MAb). * and ** stand for \( P < 0.05 \) and 0.01, respectively, as compared with controls. + and ++ stand for \( P < 0.05 \) and 0.01, respectively, as compared with I/R animals treated with saline.

Fig. 5. Serum levels of interleukin-6 (IL-6) in controls and the groups subjected to small intestinal ischemia for 40 min and reperfusion for 12 h, treated with saline, the PAF inhibitor lelipafant, and monoclonal antibodies against intercellular adhesion molecule-1 (anti-ICAM1-MAb), or platelet endothelial cell adhesion molecule-1 (anti-PECAM1-MAb). * and ** stand for \( P < 0.05 \) and 0.01, respectively, as compared with controls. + stands for \( P < 0.05 \) as compared with I/R animals treated with saline.
Fig. 6. Tissue myeloperoxidase content in the ileum in controls and the groups subjected to small intestinal ischemia for 40 min and reperfusion for 12 h, treated with saline, the PAF inhibitor lexiapafant, and monoclonal antibodies against intercellular adhesion molecule-1 (anti-ICAM1-MAb), or platelet endothelial cell adhesion molecule-1 (anti-PECAM1-MAb). * and ** stand for $P < 0.05$ and $0.01$, respectively, as compared with controls. + and ++ stand for $P < 0.05$ and $0.01$, respectively, as compared with I/R animals treated with saline.

Fig. 7. Plasma levels of albumin and the three plasma protease inhibitors alpha-1-macroglobulin ($\alpha$-1-M), alpha-2-antiplasmin ($\alpha$-2-APL) and antithrombin III (AT III) in controls and the groups subjected to small intestinal ischemia for 40 min and reperfusion for 12 h, treated with saline, the PAF inhibitor lexiapafant, and monoclonal antibodies against intercellular adhesion molecule-1 (anti-ICAM1-MAb), or platelet endothelial cell adhesion molecule-1 (anti-PECAM1-MAb). * and ** stand for $P < 0.05$ and $0.01$, respectively, as compared with controls. + and ++ stand for $P < 0.05$ and $0.01$, respectively, as compared with I/R animals treated with saline.
Discussion

The multiple organ dysfunction syndrome (MODS) is considered to be a consequence of the generalized and autodestructive inflammatory response of the host to different types of challenge, mediated by the massive activation of inflammatory cells that release endogenous humoral mediators (13). Research into the modulation of inflammatory mediators with the aim of preventing the potential further development of MODS is currently in progress. The inflammatory and immunological response that follows the challenges are the result of cellular interactions provoked by cascade activations of different intermediary systems, among which the cytokine system stands out. This system is highly effective both in local and systemic control of the inflammatory process (14).

Endothelial cells play a key role in the formation of an effective vascular barrier against high MW proteins, lipids, and various cells of the immune system. Modulation of this barrier function occurs during a number of physiological and pathological conditions including inflammation and I/R injury. In these conditions, neutrophils and other inflammatory cells gain access to local tissue and promote the formation of an inflammatory response (7). It is established that a class of cell surface molecules, the cell adhesion molecules (CAMs), is essential for neutrophil adhesion and activation (15). At least three types of CAMs are involved in the process: the selectins (endothelial-leukocyte adhesion molecule-1 (ELAM-1)), the integrins (intracellular adhesion molecule-1), and platelet-endothelial cell adhesion molecule-1 (PECAM-1) (16). It has also been shown that several cytokines secreted during I/R can both attract PMNs and increase the expression of endothelial receptors (17).

Leukocyte adherence to the microvascular endothelium appears to be an early and rate-limiting step in the development of I/R injury. Available data suggest the leukocyte adhesion glycoprotein complex termed CD11/CD18 as the primary mediator of the adherence of neutrophils to the postischemic intestinal microvasculature (18). The use of monoclonal antibodies against different adhesion molecules may help our understanding of the molecular events that mediate the leukocyte–endothelial cell interactions during I/R. In the present study, the use of a PAF antagonist, anti-ICAM-1-MAb, and anti-PECAM-1-MAb reduced the recruitment of PMNs, decreased the plasma cytokine levels, and decreased the otherwise occurring endothelial permeability impairment in rats subjected to intestinal I/R.

Endothelial barrier dysfunction, characterized by an increase in endothelial permeability, release of inflammatory mediators, and exposure of adhesion molecules on the cellular surface, plays an important role in development of MODS (19). The maintenance of gut endothelial barrier integrity is critical in preventing the formation of gut barrier dysfunction (20). In the present study, I/R-associated intestinal endothelial barrier dysfunction was evident by the exudation of plasma albumin referred with tissue blood content in distal small intestine 12 h after ischemia. Treatment with leuipafant, anti-ICAM-1-MAb, or anti-PECAM-1-MAb to varying degrees reduced the severity of the I/R-induced gut mucosal barrier impairment. Plasma levels of antiprotease levels were also deranged with plasma extravasation through the whole molecular weight range. All the three measured plasma protease inhibitors decreased following I/R challenge. The levels for antithrombin III paralleled albumin levels, and could thus partly be due to intestinal barrier leakage, since antithrombin III has about the same MW as albumin. Theoretically, proteolytic destruction instead of consumption could explain the low levels for both antithrombin III and alpha-2-antiplasmin, since these inhibitors were measured using functional assays. Liberated leukocyte elastase may, for example, directly inactivate protease inhibitors, such as alpha-2-antiplasmin and the C1-esterase inhibitor (21). Also, oxygen free radicals may directly inactivate several important protease inhibitors, e.g. alpha-2-antiplasmin, antithrombin III, and C1-esterase inhibitor (22). Alpha-1-macroglobulin, however, was measured using an immunological method, where also destructed inhibitor complexes are measured. Thus, the low levels found for alpha-1-macroglobulin are due to complex formation and clearance of the complexes from the circulation. The low levels of alpha-2-antiplasmin could be caused by several mechanisms, like macrophage overactivation with the release of fibrinolytic active products. Furthermore, remote trauma results in increased pulmonary fibrinolytic activity, since vascular endothelial cells, particularly in the lung, release fibrinolytic active products (23). The fibrinolytic system seems thus to be heavily overactivated following I/R, and the extremely low levels of alpha-2-antiplasmin may allow excessive and unlimited plasmin activity, and thus excessive fibrinolysis. Plasmin is a potent proteolytic enzyme which may catalyze the degradation of fibrin and also, directly or indirectly, most extracellular proteins (24), resulting in damage of endothelial barrier integrity and the potential development of multiple organ dysfunction. Alpha-1-macroglobulin, with its broad inhibitory capacity, is a potent inhibitor of pancreatic and leukocyte elastase, leukocyte cathepsins B and G, trypsin and chymotrypsin (25). Thus, also the low levels of alpha-1-macroglobulin indicate possible macrophage overactivation, with resultant proteolytic activity. Moreover, since alpha-1-macroglobulin and its protease complexes are reported to play an important role in immunoregulation (26), consumption of the inhibitor and large amounts of the protease–alpha-macroglobulin complexes may influence the outcome of the challenge, also due to such effects.

In the present study, MPO content in the small intestine significantly increased, in parallel with the increase in small intestinal albumin leakage in rats subjected to SMA I/R. Leukocyte–endothelial cell interactions lead to excessive trafficking of leukocytes to the tissue, as well as endothelial
barrier compromise, responsible for the initiation of the inflammatory reaction, serious tissue injury, and concomitant organ dysfunction. Leukocyte recruitment is thought to include four steps: 1) transient tethering and rolling of the leukocytes along the endothelial surface of the vessel wall; 2) triggering of signals that activate the upregulation of leukocyte integrins; 3) leukocyte tight adhesion to the vascular endothelium; and 4) transendothelial migration (27). PAF, a biologically active phospholipid stored in precursor form within cell membranes, is an intercellular signal responsible for cell communications, and an inflammatory mediator. During the inflammatory response, PAF activates the leukocyte system, which can produce and release a variety of secondary inflammatory mediators and provokes the exposure of adhesion molecules on the surface of endothelial cells and leukocytes, which then can cause leukocyte adhesion and rolling on the endothelium and migration through interendothelial cells to the interstitium (28, 29). Data from the present study demonstrate that treatment with lexipafant ameliorates the severity of gut leukocyte recruitment after induction of small intestinal I/R, in parallel with improvement of the impaired intestinal barrier function. It might be that lexipafant neutralizes PAF receptors on the surface of endothelial cells, reduces circulating PAF as intercellular signals, downregulates the activation of leukocytes/macrophages and the interaction between leukocytes and endothelial cells, and maintains the integrity of the endothelial barrier.

The immunoglobulin-like cell adhesion molecules (IgCAMs) are a family of membrane-bound adhesion molecules, consisting of ICAM-1, -2, and -3, VCAM-1, PECAM-1, and MAdCAM-1. IgCAMs are active in the inflammatory process by arresting and firmly binding rolling leukocytes, flattening the arrested leukocytes, and mediating their transendothelial migration (30). Among other cell types, ICAM-1 (CD54) is expressed by activated and non-activated endothelial cell and by lymphocytes, monocytes, and epithelial cells (31). Its expression is stimulated by, for example, IL-1, IL-17, TNF-α, IFN-γ, and LPS (32), and the amount of ICAM-1 remains upregulated on continued stimulation. Ultrastructurally, ICAM-1 is found in the endoplasmic reticulum and in the extracellular matrix, suggesting active secretion, shedding, or release on cell lysis (as either phagocytosis or apoptosis) (33). In the present study, the treatment with anti-ICAM1-MAb ameliorated the severity of tissue edema and gut leukocyte recruitment after induction of small intestinal I/R, in parallel with an improvement of the impaired intestinal barrier permeability. It is possible that monoclonal antibodies may neutralize adhesion molecule receptors on the surface of endothelial cells, thereby reducing CAMs as intercellular signals, downregulating the activation of leukocytes/macrophages, including reduced PAF activation, accumulation of leukocytes in the tissue, and interaction between leukocytes and endothelial cells, and maintaining the integrity of the endothelial barrier. This might explain why monoclonal antibodies have a similar effect as the PAF antagonist.

PECAM-1 (CD31) is also a member of the immunoglobulin superfamily expressed on the surface of circulating platelets, monocytes, neutrophils, and selected T cell subsets, and a major constituent of the interendothelial junction. Its expression is stimulated by cytokines such as TNF-α, IL-1β, and IFN-γ (34). The functions of PECAM-1 during leukocyte–endothelial cell interaction include homophilic engagement of PECAM-1 leading to upregulation as an agonist receptor, integrin engagement leading to upregulation of PECAM-1 activity, and major control of signal triggering and leukocyte migration through interendothelial cells (35). PECAM-1 has also been reported to be upregulated in non-inflamed areas adjacent to the inflamed tissue, indicating that its upregulation is a precondition for the development of inflammatory lesions (34).

The results in the present study demonstrate that treatment with anti-PECAM-1-MAb following intestinal I/R ameliorates the severity of intestinal barrier damage, seemingly even better than after treatment with lexipafant and anti-ICAM1-MAb. A potential explanation could be that anti-PECAM-1-MAb not only stops the adhesion of leukocytes to the endothelial surface, but also the binding of platelets to the injured endothelium and blocks the passage of leukocytes between endothelial cells (16).

Gut leukocyte recruitment, and plasma exudation in animals with gut I/R and treatment with anti-ICAM-1-MAb tended to be higher as compared to treatment with anti-PECAM-1-MAb administration. Other adhesion molecules, such as vascular cell adhesion molecule-1, intercellular adhesion molecule-2, and mucosal addressing cell adhesion molecule-1, may have similar functions as ICAM-1, and/or the number of exposed ICAM-1 after gut I/R may be higher than other adhesion molecules. The effectiveness of treatment with anti-PECAM-1-MAb could be due to a decrease in production and release of intercellular signals during the leukocyte–endothelial cell interaction and blocking of the leukocyte migration between interendothelial junctions.

Adhesion of inflammatory cells to endothelial cells and the relation to endothelial barrier dysfunction and organ failure is considered to be associated with a variety of inflammatory mediators and/or cytokines. Cytokine-regulated expression of ICAM-1, and vascular cell adhesion molecule-1 in cultured human intestinal microvascular endothelial cells showed a dose-dependent fashion and a time-related response (28). The present study demonstrates a strong correlation between the plasma level of IL-1β, local leukocyte recruitment, and the exudation of various plasma proteins into the interstitium. Treatment with lexipafant, anti-ICAM-1-MAb, and anti-PECAM-1-MAb all reduced the gut I/R induced increase in plasma levels of IL-1β. IL-1β appears in serum later than TNFα, and is detectable 1 h after the challenge (36). IL-6 increases progressively, reaching its maximum value after the IL-1β increase (37). The increase in IL-6 is mediated by both
TNFα and IL-1β, which induce the expression of the gene that codes for the synthesis of IL-6. At the same time, IL-1β stimulates the CD4 and CD8 T lymphocytes to synthesize IL-6 (36). It could be that the time period (12 h reperfusion) in the present study does not include the peak of IL-6.

In the present study, treatment with the PAF inhibitor leuipafant, anti-ICAM-1-MAb, and anti-PECAM-1-MAb all resulted in a reduction of leukocyte accumulation, improvement of the impaired gut mucosal permeability, and decrease in plasma cytokine levels. These molecules may block the different steps of the cascade during I/R-induced gut barrier dysfunction. Another possible explanation is that adhesion molecule gene expression in response to a number of cytokines may involve a common pathway. Collins et al. described that the transcription factor nuclear factor kappa B (NF-kB) and a related group of transcriptional activators are required for gene expression (38). Inhibition of the proteasome involved in the activation of NF-kB decreased TNFα-induced cell-surface expression of E-selectin, VCAM-1, and ICAM-1 (39). Since NF-kB is activated by both TNFα and IL-1β, it is possible that competitive inhibition at this key step may limit the maximal expression of the receptors.

In conclusion, gut I/R-associated intestinal barrier dysfunction was characterized by increased exudation of plasma albumin into the interstitium. Treatment with the PAF inhibitor leuipafant and monoclonal antibodies against ICAM-1 or PECAM-1 reduced the severity of gut I/R-associated intestinal barrier dysfunction, associated with a decline in systemic concentrations of IL-1β and local leukocyte recruitment. Antagonists against endothelial adhesion molecules and platelet activating factor might represent potential future therapeutic methods.

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