Time course of endothelial-neutrophil interaction in splanchnic artery ischemia-reperfusion

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Hayward, Reid, and Allan M. Lefer. Time course of endothelial-neutrophil interaction in splanchnic artery ischemia-reperfusion. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H2080–H2086, 1998.—Splanchnic artery occlusion and reperfusion (SAO/R) results in a severe form of circulatory shock that has a high mortality rate. To examine the time course of the early events involved in SAO/R, occlusion of the superior mesenteric artery (SMA) and the celiac artery (120 min) were followed by reperfusion periods of 0, 2.5, 5, 20, 30, 60, or 120 min. Relaxation of isolated SMA vascular rings to the endothelium-dependent vasodilator ACh was unimpaired following 120 min of ischemia (86 ± 5%); however, significant (P < 0.01) reductions in endothelium-dependent vasorelaxation were observed following 2.5 min (53 ± 6%) of reperfusion with severe dysfunction (P < 0.001) observed at 20 min (29 ± 4%). Neutrophil adherence to the endothelium increased as a function of reperfusion time with a 2.3-fold increase observed at 20 min (P < 0.01) and a 3.4-fold increase observed at 120 min (P < 0.001). Intestinal myeloperoxidase activity was significantly increased 30 min after reperfusion (P < 0.05), whereas surface expression of P-selectin progressively increased at 5 (P < 0.05) and 30 min (P < 0.001) postreperfusion. These findings demonstrate that endothelial dysfunction is a very early event in the pathophysiology of SAO/R, subsequently resulting in increased surface expression of P-selectin and the adherence of neutrophils to the endothelium that leads to neutrophil accumulation in the splanchnic viscerosa.

occlusion and reperfusion (SAO/R) provokes tissue injury at remote sites. SAO/R results in severe lung injury characterized by an increase in pulmonary permeability and neutrophil accumulation (9). The vascular dysfunction following SAO/R is extensive, occurring in both the superior mesenteric (15, 21) and pulmonary arteries (9).

It is well established that occlusion and reperfusion of the splanchnic circulation results in a severe form of circulatory shock (6, 11, 14). Occlusion and reperfusion of the splanchnic arteries leads to systemic as well as local derangements that are associated with high mortality rates. During the early stages of reperfusion, the mucosal architecture of the small intestine is distorted with the intestinal villi appearing denuded (7). Focal injury of the intestine appears scattered throughout the intestine at the end of ischemia and steadily worsens as reperfusion progresses (16).

Whereas several mechanisms have been identified in the development and progression of reperfusion injury, one of the key cellular elements involved in this process is neutrophil-mediated tissue dysfunction and injury. Butcher (4) described neutrophil extravasation from the vascular lumen to the site of inflammation as a three-step process. The process begins with neutrophils recognizing and transiently interacting with the vascular endothelium, resulting in what has been characterized as leukocyte rolling (13). This is followed by a strengthening of adhesive forces, which results in the capture and firm adherence of neutrophils to the endothelium. Finally, many of the adherent neutrophils extravasate through the endothelium and come in close proximity to the target tissue where they mediate their injurious effects (17). In particular, ischemia and reperfusion of the splanchnic circulation results in the accumulation of large numbers of neutrophils in the splanchnic visceral organs (18, 26).

Interventions targeted to the early stages of reperfusion injury appear to provide the greatest benefits in splanchnic ischemia-reperfusion. Adding physiological amounts of nitric oxide during SAO/R improves posts ischemic hemodynamics and inhibits surface expression of P-selectin on endothelial venules (1, 10). In addition, infusion of a monoclonal antibody directed against P-selectin increased survival and inhibited leukocyte rolling and adherence in the rat mesenteric microvasculature (6). Therefore, administration of an exogenous source of nitric oxide or a P-selectin blocker attenuates leukocyte-endothelial cell interactions and prolongs survival in SAO/R. Although the inflammatory response associated with ischemia and reperfusion of the mesenteric circulation is well known, the time course and sequence of events involved in the recruitment of

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neutrophils into the gut have not been previously characterized. Therefore, the purpose of this investigation was to determine the time course and sequence of early events involved in the inflammatory response following SAO/R, with particular emphasis on neutrophil-to-endothelial cell interactions and P-selectin expression.

METHODS

Experimental protocol. Male Sprague-Dawley rats weighing 273–300 g were anesthetized with pentobarbital sodium (60 mg/kg) injected intraperitoneally. The trachea was cannulated to maintain a patent airway throughout the experiment. A polyethylene catheter was inserted into the carotid artery to monitor mean arterial blood pressure (MABP), which was recorded on a Grass model 7 oscillographic recorder using Statham P23 AC pressure transducers (Gould, Cleveland, OH). The abdominal cavity was opened via a midline laparotomy, and the celiac and superior mesenteric arteries (SMA) were isolated near their aortic origin.

After stabilization occurred, the celiac and SMA were completely occluded for 120 min using nontraumatic arterial clamps. At the end of the ischemic period, the clamps were removed, and the splanchnic circulation was reperfused. Rats were observed for 2 h after reperfusion or until their MABP declined to 50 mmHg, at which time the experiment was terminated. Rats experiencing massive acute circulatory collapse (i.e., MABP < 50 mmHg) within the first 30 min postreperfusion were excluded from the study. At the time of circulatory collapse or at the end of the 2-h reperfusion period, the SMA was removed and studied using isolated vascular ring and leukocyte adherence studies, and biopsies of ileal tissue were removed and analyzed for myeloperoxidase (MPO) activity and surface expression of P-selectin on the venular endothelium.

We also determined separately whether the systemic hypotension associated with reperfusion of the ischemic splanchnic circulation exerted any direct effects on the variables observed in this investigation. Toward this end, a group of rats was subjected to hemorrhage from a cannulated carotid artery without reperfusion of shed blood volume, during which a MABP of 65 mmHg was set. This corresponds to the collapse phase. Additional volume of blood was removed as necessary to maintain the MABP at 65 mmHg for 30 min. After 30 min, measurements of endothelial function, neutrophil adherence, and ileal MPO activity were conducted as outlined above for SAO/R rats.

Isolated SMA ring studies. At the end of each experiment, the SMA was rapidly removed from animals and placed into warmed Krebs-Henseleit (KH) buffer consisting of (in mmol/l): 118 NaCl, 4.75 KCl, 2.54 CaCl2·2H2O, 1.19 KH2PO4, 1.19 MgSO4·7H2O, 12.5 NaHCO3, and 10.0 glucose. Isolated vessels were carefully freed of connective tissue and cut into rings 2–3 mm in length. The rings were then mounted on stainless steel hooks, suspended in a 10-ml tissue bath, and connected to FT-03 force displacement transducers (Grass Instrument, Quincy, MA) to record changes in force on a Grass model 7 oscillographic recorder. The baths were filled with KH buffer and aerated at 37°C with 95% O2-5% CO2. A resting force of 0.5 g was applied to SMA rings, and then the rings were equilibrated for 90 min. During this period, the buffer in the tissue bath was replaced every 15–20 min, and the resting force of the vascular rings was adjusted until 0.5 g of preload was maintained. This resting force was selected because it neither injures the endothelium nor interferes with the release of nitric oxide in response to the endothelium-dependent vasodilators. After equilibration was completed, the rings were exposed to 100 nM U-46619 (11,12-epoxy methano-PGH2, Biomol Research Laboratories, Plymouth Meeting, PA), a thromboxane A2 mimetic, to generate ~0.5 g of developed force. Once a stable contraction was obtained, acetylcholine, an endothelium-dependent vasodilator, was added to the bath in cumulative concentrations of 0.1, 1, 10, and 100 nM. After the cumulative response stabilized, the rings were washed and again allowed to equilibrate to baseline. The procedure was repeated with an endothelium-independent vasodilator, acetylsalicylic acid, dissolved in KH buffer and aerated at 37°C with 95% O2-5% CO2. The bath was then centrifuged for 10 min at 3,600 × g. The red blood cell–poor supernatant was collected, underlayered with Ficoll-hypaque solution and centrifuged at 360 g for 30 min. Residual red blood cells were removed from the resuspended pellet by hypotonic lysis for 30 s, and the pellet was resuspended in phosphate-buffered saline. Cells were washed five times before being labeled.

After the washing cycles were completed, isolated neutrophils were then labeled with a Zynaxis PKH-2 cell linker (Zynaxis Cell Science, prepared for Sigma Immunodiagnostic, Malvern, PA) based on the procedure of Yuan and Fleming (37). Two milliliters of diluted and 10 µl of dye were added to a loose cell pellet containing ~40 × 106 cells. After a 7-min incubation period, 200 ml of 0.2% bovine serum albumin were added to stop the reaction, and 2 ml of phosphate-buffered saline were added to underlay the suspension. The mixture was then centrifuged for 10 min at 1,800 revolutions/min. Cells were washed three times after which the cells were resuspended in phosphate-buffered saline, counted, and utilized in adherence procedures. The procedure of cell isolation and cell labeling yields relatively unstimulated cells having normal function (35, 37).

The SMA were removed from rats undergoing SAO/R, placed in warmed oxygenated KH buffer, and cleaned of all external adipose and loose connective tissue. Artery segments were then sectioned into 2- to 3-mm rings, opened, and placed into conical wells containing 2 ml of KH buffer. SMA segments were then incubated luminal side up with PKH-2-labeled neutrophils (106 cells) for 20 min at 37°C in a shaker bath under elliptical rotation (120 revolutions/min). After the 20-min incubation period, sections were washed in KH buffer and placed endothelial side up on glass microscope slides. Neutrophils adhering to the endothelium were counted using epifluorescence microscopy (Nikon Diaphot, Nikon, Garden City, NY). Five different fields of each endothelial surface were counted, and the results were expressed as adherent neutrophils per squared millimeter of endothelial surface. Sham-operated control segments were obtained from sham-operated control SAO/R rats following a 240-min observation period. In additional experiments, SMA segments were obtained from control rats not undergoing the 240-min observation but were stimulated with 2 U/ml thrombin for 10 min. After thrombin stimulation, segments were incubated with...
labeled polymorphonuclear leukocytes (PMN) for 20 min and counted as described above.

Determination of intestinal MPO activity. MPO activity, an enzyme occurring virtually exclusively in PMN, was determined in the ileum using the method of Bradley et al. (3) as modified by Mullan et al. (25). A hemorrhage-free area of the ileum ~8–10 cm in length, at least 30 cm distal to the stomach, was dissected and carefully rinsed in 0.9% NaCl. The sample was then homogenized in 0.5% hexadecyltrimethylammonium bromide (HTAB, Sigma, St. Louis, MO, which was dissolved in 50 mM potassium phosphate buffer at pH 6.0) using a Polytron (PCU-2) homogenizer (Kinematica, Lucerne, Switzerland). Homogenates were centrifuged at 12,500 g at 4°C for 30 min. The supernatants were then collected and reacted with 0.167 mg/ml of o-dianisidine dihydrochloride (Sigma) and 0.0005% H2O2 in 50 mM phosphate buffer at pH 6.0. The resultant change in absorbance was determined spectrophotometrically at 460 nm. One unit of MPO is defined as that quantity of enzyme hydrolyzing 1 mmol of peroxide/min at 25°C.

Immunohistochemical localization of P-selectin. Immunohistochemical localization of P-selectin was determined using monoclonal antibody PB1.3, which only detects surface expression of P-selectin. After 0, 5, 20, 60, and 120 min of reperfusion, both the SMA and superior mesenteric vein were rapidly cannulated for perfusion fixation of the small bowel. The ileum was first washed free of blood by perfusion with KH buffer warmed to 37°C, bubbled with 95% O2-5% CO2, and fixed in 4% paraformaldehyde for 90 min at 4°C. Immunohistochemical localization of P-selectin was accomplished using the avidin-biotin immunoperoxidase technique (Vectorstain ABC Reagent, Vector Laboratories, Burlingame, CA) as previously described by Weyrich et al. (34). Positive staining was defined as a venule displaying brown reaction product on >50% of the endothelial circumference. Fifty venules per tissue section were examined, and 20 sections were analyzed per group. The percentage of positive staining venules was tallied and recorded.

Statistical analyses. All values in the text and Figs. 1–5 are presented as means ± SE of n independent experiments. Data were compared by ANOVA using post hoc analysis with Fisher’s correct t-test. Probabilities of ≤0.05 were considered to be significant in all cases.

RESULTS

Effects of SAO/R on MABP. The time course of MABP changes in rats subjected to SAO/R and sham-operated control rats is summarized in Fig. 1. Both groups of rats demonstrated comparable initial values for MABP ranging from 110–135 mmHg. No significant change in MABP was observed in sham-operated controls throughout the entire 240-min observation period. However, in those rats undergoing SAO/R, occlusion of the celiac and SMA resulted in a significant increase in MABP from a preocclusion value of 116 ± 3 to 160 ± 3 mmHg. However, there was a slow but steady decline in MABP during the 120-min occlusion period to values approaching control levels just before reperfusion. After reperfusion, MABP precipitously decreased to ~55–75 mmHg and remained there for the duration of the observation period. Thus occlusion of the mesenteric arteries for 120 min followed by 120 min of reperfusion resulted in severe hypotension characteristic of a severe shock state.

![Fig. 1. Time course of mean arterial blood pressure (MABP) for rats subjected to sham operation (Sham) or to splanchic artery occlusion and reperfusion (SAO/R). All values are means ± SE of surviving animals at each time point. Numbers represent number of surviving rats in each group. *P < 0.05; **P < 0.01; ***P < 0.001 vs. Sham.](image)

Effects of SAO/R on SMA endothelial dysfunction. Endothelial dysfunction is a key event in the development and progression of several pathophysiological conditions. Therefore, we studied the time course of endothelial dysfunction following SAO/R by comparing the vasorelaxant responses of isolated SMA rings to endothelium-dependent and endothelium-independent vasodilators (Fig. 2). The endothelium-dependent vasodilator ACh as well as the endothelium-independent vasodilator NaNO2 produced full relaxation of SMA rings isolated from sham-operated control rats. Similarly, SMA rings undergoing 120 min of ischemia without reperfusion exhibited normal endothelial function comparable to values obtained in sham-operated control rats. However, 2.5 min of reperfusion was sufficient to significantly (P < 0.01) diminish the vasorelaxant response to ACh in the absence of a diminished NaNO2 response, and longer periods of reperfusion resulted in even further decrements in endothelial function.
function. Twenty minutes after reperfusion, a severe endothelial dysfunction was attained, and this dysfunction was sustained over the remainder of the 120-min postreperfusion period. This endothelial dysfunction was not due to hypotension per se, because rats that hemorrhaged to a MABP of 65 mmHg for 30 min exhibited a vasorelaxation response of 90 ± 2% to ACh, a value not significantly different from sham-operated controls. At all postreperfusion times, SMA rings exhibited full relaxation to the endothelium-independent vasodilator NaNO₂, indicating that the ischemia and subsequent reperfusion did not result in any dysfunction of the underlying vascular smooth muscle. Thus there appears to be a significant degree of endothelial dysfunction that is observed as early as 2.5 min after reperfusion and plateaus ∼20 min after reperfusion.

Effects of SAO/R on neutrophil adherence. Another early event in neutrophil-mediated reperfusion injury is increased adherence of neutrophils to the reperfused SMA endothelium. We therefore measured the adherence of unstimulated neutrophils to the ischemic-reperfused SMA. Sham-operated control rats demonstrated minimal endothelial adhesiveness as evidenced by a low number of adherent neutrophils (i.e., 10–15 PMNs/mm² surface area of endothelium) (Fig. 3). Similarly, low numbers of neutrophils were observed to be adherent following 120 min of ischemia and 0, 2.5, or 5 min of reperfusion. However, from 20 to 120 min after reperfusion, the reperfused SMA endothelium became progressively more adhesive. The magnitude of the increase in adherence observed at 20 min postreperfusion was 61% above control values (P < 0.01). Peak adherence following 120 min of reperfusion was 139% above the initial control values (P < 0.001). SMA segments isolated from rats that hemorrhaged to 65 mmHg exhibited a minimal degree of adherence (15.6 ± 2.1 PMNs/mm²), which was not significantly different from sham-operated controls. This indicates that the increased PMN adherence following reperfusion of the ischemic splanchnic vasculature was not due to the acute hypotension observed on reperfusion of the ischemic splanchnic vasculature. In addition, incubation of control SMA segments with thrombin resulted in a comparable degree of stimulation as occurred with 120 min of reperfusion.

Effects of SAO/R on intestinal MPO activity. Adherence of neutrophils to the dysfunctional endothelium and their subsequent accumulation in affected tissues have been shown to be one of the primary mechanisms involved in reperfusion injury. Because MPO is found virtually exclusively in neutrophils, we assessed MPO activity as an index of PMN accumulation in intestinal tissue (Fig. 4). No significant differences in ileal MPO activity were observed between sham-operated control rats and those rats undergoing 120 min of ischemia without reperfusion. Likewise, no significant increase in MPO activity was observed with reperfusion periods up to and including 20 min, suggesting that very few neutrophils accumulate in the intestine during the very early postreperfusion period. However, 30 min of reperfusion resulted in a 3.7-fold increase (P < 0.05) in intestinal MPO activity, which increased seven- and eightfold (P < 0.001) after 60 and 120 min of reperfusion, respectively. These data indicate that significant PMN accumulation occurs 30–120 min after reperfusion. In contrast, rats hemorrhaged to 65 mmHg for 30 min exhibited low intestinal MPO activities (i.e., 0.60 ± 0.20 IU/g tissue), a value not significantly different from that observed in sham-operated controls.

Effects of SAO/R on P-selectin expression. Immunohistochemical localization of surface-expressed endothelial cell P-selectin was observed on the microvascular endothelium in the rat ileum. Tissue sections obtained from sham SAO/R rats showed minimal baseline stain-
ing, with cytoplasmic immunostaining primarily located at the endothelium in <5% of small intestinal vessels (Fig. 5). Sections obtained from rats undergoing 120 min of ischemia with 0 min of reperfusion demonstrated low levels of surface-expressed P-selectin, which were not significantly different from sham-operated control rats. However, after 5 min of reperfusion there was a threefold increase (P < 0.05) in the number of microvessels staining positively for surface-expressed P-selectin. Intestinal microvessels demonstrated maximal staining following 30 min of reperfusion at which time there was a fourfold (P < 0.001) increase in comparison to time 0. This trend continued throughout the reperfusion period with significant increases in surface expression observed at 60 (P < 0.001) and 120 (P < 0.001) min after reperfusion.

### DISCUSSION

The celiac and SMA supply nearly 90% of the blood flow to the liver, pancreas, and intestine (24), and thus occlusion of the celiac and SMA results in a severe degree of ischemia to these organs. This reduced splanchnic flow leads to an abrupt increase in systemic arterial blood pressure of 25–50 mmHg due primarily to a decrease in the baroreceptor input to the medullary vasomotor center. This increased MABP diminishes gradually during the ischemia and returns to near preoclusion levels by the end of the ischemic period, probably due to transudation of fluid across the microcirculation. On reperfusion of the splanchnic circulation, a precipitous fall in MABP occurs to values of 55–75 mmHg. This is followed by a small increase in blood pressure within the first 30 min of reperfusion, and thereafter a steady decline in MABP occurs throughout the remainder of the reperfusion period to values incompatible with life (i.e., ~45 mmHg). These changes in postreperfusion MABP signify the occurrence of a severe form of circulatory shock.

Endothelial dysfunction has been shown to be a key early event in a variety of forms of reperfusion injury (20) and was observed in the present study as early as 2.5 min after reperfusion of the splanchnic circulation. Endothelial dysfunction worsened by 20 min postreperfusion, and this severe degree of endothelial dysfunction was maintained throughout the 120-min reperfusion period. The early endothelial dysfunction (i.e., 2.5 and 5 min) observed following reperfusion is likely the result of decreased nitric oxide production (36) and the generation of oxygen-derived free radicals (14, 31). Tsao and Lefer (31) demonstrated that rat hearts perfused with oxygenated KH buffer lacking blood cells exhibit a 75% reduction in endothelium-dependent vasorelaxation within 2.5 to 5 min of reperfusion. This response was blocked by administration of human recombinant superoxide dismutase, suggesting that the vascular endothelium is a major source of oxygen-derived free radicals contributing to reperfusion-induced endothelial dysfunction. Direct evidence for superoxide radical formation in these ischemic-reperfused rat hearts was obtained by chemiluminescence 1 min after reperfusion (23). One source of these endothelium-generated superoxide radicals may be xanthine oxidase (14). Elevated xanthine oxidase activities and abrupt reoxygenation result in the generation of oxygen-derived free radicals by endothelial cells (38). Although blood cells and endothelial cells possess several lines of defense against oxidant stress, large amounts of oxygen-derived free radicals can overwhelm these mechanisms, leading to oxidative injury and dysfunction. One consequence of a burst of superoxide radicals is the quenching of endothelial nitric oxide (29). This leads to upregulating endothelial cell adhesion molecules, stimulating transcription factors, and activating plasma chemotactic factors (2). Therefore, several direct actions of superoxide radicals on endothelial nitric oxide production are likely to be responsible for much of the endothelial dysfunction observed within the first 5 min of reperfusion.

The secondary effects of free radical production by the endothelium provoke neutrophil-induced exacerbation of endothelial dysfunction (12, 30). During the early phase or reperfusion, an increase in oxygen-derived free radicals generated by endothelial cells leads to an upregulation in the surface expression of several adhesion molecules, which can be sustained for several hours (27, 33). Activation of the endothelium promotes the release of inflammatory mediators, which in turn activates circulating PMN. Because unactivated PMN do not contribute to endothelial dysfunction, PMN activation is an important step in this sequence of events (32). Activated PMN have been shown to exacerbate the endothelial dysfunction in isolated perfused hearts or isolated arterial rings, and this form of endothelial dysfunction can be inhibited by human recombinant superoxide dismutase (31). In vivo, activated neutrophils become adherent to the vascular endothelium and release a number of proin-
flammatory and cytotoxic substances such as proteolytic enzymes, leukotriene B₄, platelet-activating factor, as well as superoxide radicals particularly in an oxygen-rich medium (12). Therefore, there are two phases of endothelial dysfunction, the effects of which appear to be additive. First, early dysfunction can be attributed to the generation of oxygen-derived free radicals, which quenches endothelium-derived nitric oxide. Second, decreased nitric oxide leads to the upregulation of adhesion molecules resulting in increased leukocyte-endothelial cell interactions (20). Such interactions place leukocytes in close proximity to the endothelium, allowing leukocyte-derived factors to contribute to the dysfunction.

In the present study, we have obtained direct evidence that adherence of neutrophils to the SMA endothelium as well as the accumulation of neutrophils in the reperfused tissues occurs within 20–30 min of reperfusion of the ischemic splanchnic viscera. Neutrophil adherence to the endothelium during ischemia-reperfusion of the mesenteric circulation can occur on both the arterial and venous side even under physiologically relevant shear conditions (22). Although the number of neutrophils that adhere to the SMA is less than the number that adhere to the mesenteric vein, neutrophil adherence to the SMA occurs in the splanchnic circulation. The role of neutrophils in the development and progression of reperfusion injury has been clearly established (6, 8, 18). In fact, limiting leukocyte-endothelial cell interactions following SAO/R significantly prolongs survival, increases MABP, preserves endothelial function, and attenuates neutrophil accumulation into reperfused tissues (6, 22). Ileal MPO activity remained at or near control levels during the first 20 min of postreperfusion. However, by 30 min of postreperfusion, significant increases were observed. These data suggest that as early as 20 min following reperfusion of the splanchnic circulation, a significant number of neutrophils have become adherent to the endothelium and that many of these neutrophils accumulate in the reperfused tissues starting at 30 min postreperfusion.

The link between endothelial dysfunction and neutrophil adherence can be made by the upregulation of P-selectin following splanchnic ischemia-reperfusion. Our results show that P-selectin is significantly upregulated as early as 5 min postreperfusion and remains upregulated for at least 120 min. This is in agreement with other studies in the feline myocardium (33). Maximal surface expression of endothelial P-selectin occurs ~10–20 min after reperfusion and remains elevated, although at subpeak levels, for as long as 270 min (33). The relatively short period of time necessary for upregulation of P-selectin may be attributed to the early burst of oxygen-derived free radicals by the vascular endothelium (31, 38). Endothelial cell surface expression of P-selectin has been shown to be critical in the progression of the inflammatory response (13) in that neutralization of this adhesion molecule results in a marked blunting of leukocyte-to-endothelial cell interactions and the subsequent tissue inflammation that accompanies reperfusion injury (6, 33). The administration of PB1.3, a P-selectin-neutralizing antibody, has been proven to be effective in limiting endothelial dysfunction and neutrophil accumulation in the ischemic-reperfused myocardium (34) and ileum (6). Therefore, the surface expression of P-selectin is an early and critical event in the pathophysiology of ischemia and reperfusion of the splanchnic circulation and helps explain the progression of events from endothelial dysfunction to PMN adherence to the dysfunctional endothelium, to PMN infiltration, and to tissue injury.

In conclusion, we have shown that 120 min of splanchnic artery occlusion followed by reperfusion results in a severe shock state characterized by hypotension, endothelial dysfunction, and marked accumulation of PMN into ischemic-reperfused tissues. Our results are in agreement with other studies of ischemia-reperfusion in other organs (28, 30, 31). The time course of these events shows that endothelial dysfunction occurs as early as 2.5 min following reperfusion and is likely the result of decreased endothelial nitric oxide production. This endothelial dysfunction promotes P-selectin upregulation on the endothelium, resulting in increased leukocyte-to-endothelial cell interaction. Increased leukocyte-to-endothelial cell interaction contributes to PMN activation and adherence to the endothelium exacerbating the existing endothelial dysfunction and the recruitment of additional PMN into the affected region. These extravasated PMN accumulate in reperfused tissues and mediate a significant degree of cellular and tissue injury, leading to the onset of circulatory shock.

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