Modulation of P-selectin expression in the postischemic intestinal microvasculature

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Weibel-Palade bodies. In vitro and in vivo experiments have revealed that this preformed pool of P-selectin can be rapidly (within minutes) mobilized to the cell surface after endothelial activation with inflammatory mediators such as histamine, thrombin, oxygen radicals, and leukotrienes (5, 10, 16, 28, 33). P-selectin expression on endothelial cells is also regulated by transcription-dependent mechanisms that function in parallel with, but independent of, the rapidly induced translocation of P-selectin from storage granules in endothelial cells (13). Hence, it has been proposed that the preformed pool of P-selectin participates in the recruitment of leukocytes that occurs within minutes after initiation of an inflammatory response, whereas the transcription-dependent expression of P-selectin contributes to the leukocyte rolling observed several hours after the onset of inflammation (8).

The contention that P-selectin contributes to leukocyte-endothelial cell adhesion (rolling) in inflamed tissues is largely based on studies employing either monoclonal antibodies (MAbs) directed against P-selectin or gene-targeted mice that are deficient in P-selectin (20, 27, 31, 41). For example, it has been demonstrated that both spontaneous and ischemia-reperfusion (I/R)-induced leukocyte rolling are significantly lower in postcapillary venules of P-selectin-deficient mice than in venules of wild-type mice (31, 41). Similarly, P-selectin specific MAbs are effective in reducing the leukocyte adhesion that is observed within minutes after reperfusion of an ischemic venule in cat mesentery (22). However, there are also reports that fail to show an inhibitory action of P-selectin MAbs in reducing leukocyte adhesion in different models of acute inflammation (42). Although the discrepant actions of P-selectin MAbs may reflect intrinsic differences in stimuli used to induce inflammation, it is also possible that the effectiveness of P-selectin MAbs reflects the level of expression of this adhesion molecule in the specific tissue under study. Recent in vivo studies have demonstrated significant regional differences in the magnitude and kinetics of P-selectin after treatment with different inflammatory stimuli (8).

It has been proposed that the recruitment of rolling leukocytes that is elicted in postcapillary venules by I/R is associated with a corresponding elevation in the expression of P-selectin on endothelial cells (6, 20). This contention is supported by reports of an increased number of postischemic microvessels that are immunostain for P-selectin (6, 40). Although immunohistochemical methods provide valuable information regarding the spatial distribution of newly expressed P-selectin in

THE SELECTINS HAVE BEEN implicated in the recruitment of leukocytes into tissues exposed to ischemia and reperfusion (12, 20, 24, 37). This family of adhesion glycoproteins appears to mediate a low-affinity adhesive interaction between leukocytes and endothelial cells that is manifested as a rolling behavior in postcapillary venules. The selectins consist of three (L-, E-, and P-selectin) structurally similar carbohydrate-binding lectins that are comprised of an NH2-terminal lectin domain, an epidermal growth factor domain, and a series of consensus repeats similar to those in complement proteins (15). P-selectin, which is expressed on the surface of stimulated endothelial cells and activated platelets (33), has received the most attention as a mediator of leukocyte recruitment in postischemic tissues (7, 20). This adhesion molecule normally exists in vascular endothelial cells, where it is stored in Weibel-Palade bodies. In vitro and in vivo experiments
postischemic tissues, the resolution of this technique is not sufficient for precise characterization of the magnitude and kinetics of expression of P-selectin in whole organs, nor does it allow for a mechanistic assessment of the action of different pharmacological agents on adhesion molecule expression. A novel method, based on the use of radiolabeled MAbs, was recently developed for quantification of P-selectin expression in different vascular beds of the mouse (8). In the present study, we have employed the dual radiolabeled MAb technique to determine the time course and magnitude of P-selectin expression in murine small intestine exposed to ischemia and reperfusion. We also used this technique to address the contribution of different inflammatory mediators in initiating the rapid, initial increase in P-selectin expression that occurs after I/R.

MATERIALS AND METHODS

MAbs. The MAbs used for the in vivo assessment of P-selectin were RB40.34, a rat immunoglobulin G1 (IgG1) against mouse P-selectin (Pharmingen, San Diego, CA) (2). The antibody RB40.34, directed against P-selectin, has been shown by immunohistochemical staining to be localized on endothelial cells and platelets in blood vessels of wild-type mice and to be absent in P-selectin-deficient mice (3). P-23, a nonbinding murine IgG1 directed against human P-selectin, was also used in the experimental protocols (29).

Radioiodination of MAbs. The binding (RB40.34) and nonbinding MAbs (P-23) were labeled with 125I and 131I (Du Pont-New England Nuclear, Boston, MA), respectively, using the iodogen method. In brief, iodogen (Sigma T-0656) was dissolved in chloroform at a concentration of 0.5 mg/ml, and 250 µl of this solution were placed in glass tubes and evaporated under nitrogen. A 250-µg sample of MAb was added to each iodogen-coated tube, and either 125I or 131I with a total activity of 250 µCi was added. The mixture was incubated in ice, with periodic stirring for 20 min. The total volume was brought to 2.5 ml by adding phosphate-buffered saline (PBS; pH 7.4). Thereafter, the coupled MAb was separated from free 125I or 131I by gel filtration on a Sephadex PD-10 column. The column was equilibrated and then eluted with PBS containing 1% bovine serum albumin. Two fractions of 2.5 ml were collected, the second of which contained the radiolabeled antibody. Absence of free 125I or 131I was ensured by extensive dialysis of the protein-containing fraction. Less than 1% of the activity of the protein fraction was recovered from the dialysis fluid (35).

Animal procedures. Male C57Bl/6j mice (n = 94) were used in the radiolabeled antibody experiments. The mice were anesthetized subcutaneously with a mixture of ketamine and xylazine at doses of 100 and 5 mg/kg, respectively. An abdominal incision was performed to expose the superior mesenteric artery (SMA). Splanchnic ischemia of the small intestine was produced by occlusion of the SMA with an artery clamp for a period of 20 min; thereafter, the tissue was permitted to reperfuse by removing the clamp. Before the abdominal incision was sutured, 2 ml of 0.9% saline were infused into the peritoneal cavity. In mice subjected to ≥5 h of reperfusion, recovery from anesthesia was allowed, and the animals were reanesthetized with a mixture of ketamine and xylazine at a dose of 150 and 7.5 mg/kg, respectively. Thereafter, the left jugular vein and right carotid artery were cannulated with polyethylene tubing (PE-10). In mice exposed to 20 min of SMA occlusion and 30 min of reperfusion, jugular vein and carotid artery catheters were inserted before SMA occlusion, and the abdominal incision was not sutured after reperfusion due to the short period of reperfusion. Sham-treated (control) mice were exposed to the surgical conditions described for mice exposed to 30 min of reperfusion except the SMA was not occluded.

To measure P-selectin expression, a mixture of 10 µg of 125I-labeled P-selectin MAb (RB 40.34) and a dose (0.5–5.0 µg) of 131I-labeled nonbinding MAb (P-23) were injected through the jugular vein catheter. It was previously shown that 10 µg of 125I-labeled P-selectin MAb (RB 40.34) is sufficient to saturate the P-selectin expressed on the vascular endothelium after exposure to inflammatory stimuli (8). A blood sample was obtained through the carotid artery catheter at 5 min after injection of the MAb mixture. The animals were then heparinized (30 units heparin sodium) and rapidly exsanguinated, by perfusion of bicarbonate-buffered saline (BBS) through the jugular vein catheter with simultaneous blood withdrawal through the carotid artery catheter. This was followed by perfusion of 10 ml BBS through the carotid artery catheter after the inferior vena cava was severed at the thoracic level. Entire organs were harvested and weighed.

Calculation of P-selectin expression. The method for calculating the expression of P-selectin expression has been previously described (8, 35). In brief, the 125I (binding MAb) and 131I (nonbinding MAb) activities in different tissues and in 50-μl samples of cell-free plasma were counted in a 14800 Wizard 3 gamma counter (Wallac, Turku, Finland), with automatic correction for background activity and spillover. The total injected activity in each experiment was calculated by counting a 2-μl sample of the radiolabeled MAb mixture. The radioactivities remaining in the tube used to mix the MAbs and the syringe used to inject the mixture were subtracted from the total injected activity. The accumulated activity of each MAb in an organ was expressed as the percent of the injected activity per gram of tissue. P-selectin expression was calculated by subtracting the accumulated activity per gram tissue of the nonbinding MAb (131I-P-23) from the activity of the binding anti-P-selectin MAb (125I/RB 40.34), respectively. Previous studies have shown that MAbs retain their functional activity after radioiodination as evidenced by a similar effectiveness of labeled and unlabeled MAbs to block leukocyte adherence in rat mesenteric venules (35). In addition, it has been reported that constitutive, histamine-induced, and endotoxin-induced expression of P-selectin in the intestine is largely abolished in P-selectin-deficient mice, but not in their wild-type counterparts (8).

Experimental protocols. The time course and magnitude of I/R-induced P-selectin expression in the small intestine was determined in mice after 20 min of ischemia and variable reperfusion periods, using the radiolabeled MAb procedure. In addition to the small intestine, a variety of tissues, such as lung, heart, liver, large intestine, and stomach, were also harvested from each animal. To assess the mechanisms responsible for the rapid, initial rise in I/R-induced P-selectin expression, measurements were taken from mice receiving pharmacological agents (or gene mutations) that have previously been shown to interfere with I/R-induced leukocyte-endothelial cell adhesion and/or vascular injury. The pharmacological agents tested include diethylenetriamine (DETA)/nitric oxide (NO) (n = 5) (a NO donating agent, 163 mg/ml of extracellular fluid) and DETA (n = 4) (163 mg/ml of extracellular fluid), which were administered intraperitoneally 30 min before reperfusion. The doses of DETA and DETA/NO were calculated to achieve a 1 mM concentration of the drug in the extracellular fluid, where the volume of extracellular fluid in an individual mouse was calculated as 30% of body weight. In addition, either 20 mg/kg diphenhydramine (n = 4;...
an H₁-receptor antagonist) was administered intravenously 10 min before reperfusion, 100 mg/kg oxypurinol (n = 5; a xanthine oxidase inhibitor) was administered intraperitoneally 30 min before ischemia, 30 mg/kg MK-886 (n = 6; a 5-lipoxygenase inhibitor) was administered orally 4 h before ischemia, or 30 mg/kg MK-571 (n = 5; a leukotriene C₄/D₄ antagonist) was administered intravenously 5 min before reperfusion. In all of these experiments, P-selectin expression was measured 30 min after reperfusion.

The effects of I/R on intestinal P-selectin expression was also examined in CuZn-superoxide dismutase (SOD) transgenic mice [n = 3; C57Bl/6TgN(SOD1)3Cje] and in mast cell-deficient mice (n = 3–5; Jackson Laboratories, Bar Harbor, ME). The transgenic mice with the human gene for CuZn-SOD (carried on a C57Bl/6 background) express approximately three times the basal level of CuZn-SOD (9). The mast cell-deficient mice (WBB6F1/J, W/W), are known to express approximately three times the basal level of CuZn-SOD (9). The mast cell-deficient mice (WBB6F1/J, W/W) are exposed to gut I/R (11). In these mice, intestinal P-selectin expression was also determined at 30 min after reperfusion.

To assess the contribution of platelet-bound labeled P-selectin MAb to the total binding of our P-selectin MAb in the intestinal vasculature, an inhibitor of α₁β₃ (the protein component of the adhesion molecule of GPIIb/IIIa) (TP9201; a gift from James O. Tolley, Telios Pharmaceuticals, San Diego, CA) was administered to unstimulated and I/R-stimulated mice. The peptide, TP9201, was previously shown to be a potent inhibitor of platelet aggregation and thrombosis by selectively blocking the binding of α₁β₃ to fibrinogen (32, 34, 38). In control mice (n = 3), a 7-mg/kg dose of TP9201 was administered (intravenously) 15 min before injection of the radiolabeled MAb mixture. In mice exposed to gut I/R (n = 3), 7 mg/kg TP9201 was administered 15 min before the induction of ischemia. In both groups, P-selectin expression was determined at 30 min after reperfusion.

Ribonuclease protection assay analysis of I/R-induced P-selectin mRNA. Total RNA was isolated from murine small bowel from a section of either control or I/R-treated small bowel. Approximately 20 mg of total RNA were obtained from the samples. The small intestine was homogenized in lysis buffer containing guanidinium thiocyanate as described by Chomczynski and Sacchi (4) and phenol-chloroform extracted buffer containing guanidinium thiocyanate as described by the manufacturer. In brief, the mixture of total RNA and radiolabeled probe was coethanol precipitated by adjusting the final ammonium acetate concentration to 0.5 M using the 5 M stock provided and then adding 2.5 vol of 100% ethanol. The mixture was placed in –20°C for 15 min. The probe and sample RNA were pelleted for 15 min by microcentrifugation at 4°C. The ethanol supernatant was aspirated, and samples were allowed to air dry for 5 min at room temperature. Ten microliters of HybSpeed hybridization buffer, preheated to 95°C, was added to each pellet and incubated in a water bath at 95°C. Samples were vortexed for 15 s and placed in the water bath to resolubilize the RNA/probe mixture. After the last vortex, samples were incubated at the 95°C bath for 3 min and then transferred to a 68°C water bath for 10 min, for hybridization of the probe to the sample RNA. One hundred microliters of RNAseA/T1 mix were added to each tube, and the samples were placed at 37°C for 30 min. A working solution of RNase was prepared by diluting stock RNAseA/T1 mix 1:100 with HybSpeed RNase digestion buffer. After 150 µl of HybSpeed inactivation/precipitation mix were added to each tube, the samples were vortexed briefly. Tubes were transferred to –20°C for 30 min to precipitate the protected fragments.

Samples were then removed from the freezer and microwaved for 15 min at maximum speed at 4°C, and the supernatant was carefully aspirated. Pellets were dissolved in 10 µl of solution E (provided with kit) by vigorous vortexing and then heated for 5 min at 95°C and vortexed again. Samples were loaded on a 5% denaturing polyacrylamide gel and electrophoresed at 250 V for 1.5 h in 1× tris(hydroxymethyl)aminomethane borate EDTA buffer. The gel was placed on Whatman paper and dried for autoradiography. The protected P-selectin and β-actin fragments were examined by scanning densitometry (HP ScanJet 4C and ImageQuant software). P-selectin mRNA levels were normalized to β-actin and expressed as percent of control (n = 3).

RESULTS

Kinetics of I/R-induced P-selectin expression in the small intestine. Figure 1A illustrates the magnitude and kinetics of P-selectin expression, and Fig. 1B illustrates P-selectin mRNA in the small intestine after 20 min of ischemia and variable periods of reperfusion. Significant levels of P-selectin were observed in the small intestine under baseline conditions. Within 10
min of reperfusion, P-selectin expression increased twofold (P < 0.001). A greater level of P-selectin expression was noted at 30 min of reperfusion, with a further increase in expression occurring at 5 h of reperfusion. Thereafter, P-selectin expression in the gut vasculature declined and reached the constitutive value at 24 h of reperfusion. Significant increases in intestinal P-selectin mRNA were not observed until 3 h of reperfusion with a further rise noted at 5 h of reperfusion. In all other tissues examined, an invariant change in P-selectin expression was observed after reperfusion of the small intestine (P > 0.05).

Role of oxygen radicals and mast cell-derived histamine. Table 1 summarizes the responses of I/R-induced P-selectin expression (measured at 30 min of reperfusion) to interventions directed at interfering with oxygen radical production/accumulation and mast cell products. The data obtained from these experiments indicate that the I/R-induced increase in intestinal P-selectin expression is not attenuated by treatment with the xanthine oxidase inhibitor oxypurinol, nor is it reduced in mice that overexpress the superoxide scavenging enzyme CuZn-SOD. Similarly, mast cell-deficient (W/W^*) mice did not exhibit a blunted I/R-induced P-selectin response relative to their wild-type (+/−) controls. The H_1 histamine receptor antagonist, which has been shown to completely block histamine-induced P-selectin expression in murine intestine (8), also did not alter the upregulation of P-selectin elicited by I/R.

Role of 5-lipoxygenase products. Figure 2 summarizes the effects of a 5-lipoxygenase inhibitor, MK-886, and an antagonist of leukotriene C_4/D_4, MK-571, on the rapid, initial rise in P-selectin elicited by I/R. MK-886 pretreatment resulted in a 60% reduction (P < 0.05) in intestinal P-selectin expression at 30 min after reperfusion. However, MK-571 had no beneficial effect on P-selectin expression after reperfusion.

Effects of a NO donor. Figure 3 summarizes the responses of gut I/R-induced P-selectin expression to administration of the NO-releasing compound DETA/NO. Although the carrier molecule DETA had no effect on I/R-induced P-selectin upregulation, mice pretreated with DETA/NO did not exhibit a significant increment in P-selectin expression, i.e., the value obtained in the DETA/NO group was not significantly greater than constitutive expression in untreated mice (P > 0.05) but was significantly lower than the values obtained in I/R alone or DETAO + I/R groups.

To assess the potential influence of platelet retention in the vasculature on P-selectin expression in small
P-selectin-deficient mice, that the accumulation of the Recently, the dual radiolabeled MAb technique was attained using immunohistochemical methods (8, 35). for quantifying the in vivo expression of endothelial cell 125I-labeled P-selectin MAb in different vascular beds DISCUSSION Radiolabeled MAbs have proven to be a valuable tool for quantifying the in vivo expression of endothelial cell adhesion molecules with a precision not previously attained using immunohistochemical methods (8, 35). Recently, the dual radiolabeled MAb technique was used to quantify both constitutive and induced P-selectin expression in a mouse model of inflammation (8). It was demonstrated, using both wild-type and P-selectin-deficient mice, that the accumulation of the 125I-labeled P-selectin MAb in different vascular beds stimulated with either histamine or endotoxin was due to the specific binding of the MAb to its ligand. In the present study, we employed the dual radiolabeled MAb technique to assess the influence of, and mechanisms underlying, another stimulus of P-selectin expression in the intestine, that is, I/R.

The existing literature on P-selectin expression in microvessels exposed to I/R largely consists of reports that describe qualitative and/or semiquantitative assessments of immunostained microvessels or the responses of rolling leukocytes in postcapillary venules to blocking doses of P-selectin MAbs (6, 40). The present study provides quantitative data that demonstrate a significant increase in P-selectin expression over constitutive levels in the intestinal vasculature exposed to I/R. Our observation that the gut vasculature normally exhibits a significant level of constitutive P-selectin expression agrees favorably with the results of a previous report that demonstrated P-selectin expression in unstimulated small intestine of wild-type, but not P-selectin-deficient, mice (8). This observation is also consistent with reports describing an attenuation of leukocyte rolling in unstimulated postcapillary venules of mice receiving a blocking MAb to P-selectin (27).

Our study also provides quantitative data suggesting that P-selectin expression more than doubles in the intestinal vasculature within 10 min after reperfusion. This rapid increase in P-selectin expression after I/R is consistent with published evidence of a preformed storage pool of the adhesion molecule within granular structures (Weibel-Palade bodies) of the endothelial cell. It has been demonstrated using monolayers of cultured endothelial cells that the stored pool of P-selectin is mobilized to the cell surface within a few minutes after exposure to agents such as histamine, thrombin, leukotrienes, and hydrogen peroxide (5, 15, 28, 36). The rapidity by which I/R elicits a rise in P-selectin expression in the gut vasculature suggests that this early response likely represents the mobilization of a preformed pool of P-selectin. This possibility is supported by reports demonstrating the accumulation, in postischemic intestine, of different mediators (histamine and leukotrienes) that are known to elicit the translocation of P-selectin from Weibel-Palade bodies (1, 26). Furthermore, our finding that P-selectin is rapidly upregulated in postischemic intestine is consistent with reports demonstrating that the increased (~3-fold) flux of rolling leukocytes observed in postcapillary venules after 10 min of reperfusion is nearly completely prevented by a P-selectin-specific MAb (20). The latter observation, coupled to the findings of the present study, suggest that the two- to threefold increase in P-selectin expression observed 10–30 min after reperfusion is sufficient to sustain significant increases in the number of rolling leukocytes on endothelial cells.

The kinetics of P-selectin expression noted in the murine intestine suggest that translocation of a preformed pool of the adhesion molecule may not be the only mechanism that contributes to the overall response elicited by I/R. Our previous experience with histamine-induced P-selectin expression in murine intestine suggests that the preformed pool achieves maximal expression on the endothelial cell surface in 10 min and returns to the baseline (constitutive) value within 3 h after endothelial cell stimulation (8). Such a time course contrasts with that observed in the gut after I/R, where there is a further rise in P-selectin expression at 5 h of reperfusion and a continued increase in surface expression at 8 h after reperfusion (Fig. 1). A likely explanation for the greater and more prolonged increase in P-selectin expression observed several hours after reperfusion is enhanced transcription-dependent production of the adhesion molecule. There is evidence derived from cultured endothelial cells that supports the possibility of transcriptionally regulated P-selectin expression by cytokines (13). Because serum levels of several cytokines, including tumor necrosis factor, are elevated after intestinal I/R (39), the presence of transcription-dependent promoters of P-selectin expression in the postischemic gut appears likely. Support for this possibility is provided by the increased P-selectin mRNA detected in the intestine at 3 and 5 h after reperfusion.

After the achievement of peak levels at 5 h, P-selectin expression declines to reach baseline values after 24 h of reperfusion. This time course for restoration of basal levels of P-selectin expression after I/R differs from that observed in the intestine of mice challenged with endotoxin (lipopolysaccharide, LPS) (8). In the latter instance, P-selectin expression also reached a peak (16-fold increase) near 5 h but remained significantly elevated above baseline (8-fold) at 24 h after LPS challenge. The differences in magnitude and time course of P-selectin expression between LPS and I/R stimulation may represent the participation of different mediators and/or merely reflect differences in the intensity of endothelial cell activation between the two models.

Another major objective of this study was to identify the mediators that contribute to the rapid initial increase in P-selectin expression observed in the intestinal vasculature after I/R. The potential mediators examined were chosen based on three criteria: 1)
Evidence for its accumulation (or depletion) in postischemic tissues, 2) an ability of antagonists or inhibitors (or releasing compounds) of the mediator to blunt I/R-induced leukocyte recruitment and/or microvascular injury, and 3) evidence that the mediator alters the expression of P-selectin on endothelial cells. Accordingly, we assessed the possible contribution of mast cell-derived histamine, xanthine oxidase-derived oxidants, leukotrienes, and NO to I/R-induced P-selectin expression. Although there is ample evidence implicating mast cells (19), xanthine oxidase (12), and superoxide (12) in the recruitment of leukocytes in postischemic microvessels, we were unable to demonstrate a role for any of these factors in the P-selectin expression elicited early after reperfusion. The antagonists, inhibitors, and mutant mice employed to test for the involvement of these factors have shown efficacy in other models of I/R and/or acute inflammation, suggesting that the negative responses are not likely due to failure of the agent/intervention to exert its desired action.

In contrast to the negative responses obtained with the aforementioned mediator-specific interventions, significant attenuation of I/R-induced P-selectin expression was noted in animals pretreated with a 5-lipoxygenase inhibitor (MK-886). The inhibitory action of MK-886 on P-selectin expression is consistent with reports describing P-selectin-dependent recruitment of rolling leukocytes in postcapillary venules exposed to leukotrienes, such as leukotriene C₄/D₄ (18). However, our finding that MK-571, a leukotriene C₄/D₄ antagonist, does not afford the same inhibitory effect as MK-886 suggests that another 5-lipoxygenase product (e.g., leukotriene B₄ or a leukotriene C₄/D₄-independent product) is responsible for the rapid initial increase in P-selectin expression elicited by I/R. Because lipoyxgenases are multifunctional enzymes that also participate in processes such as membrane modeling (23), it is conceivable that MK-886 exerts its effect via a process that is independent of leukotriene biosynthesis.

The NO-donating compound DETA/NO was also effective in attenuating I/R-induced P-selectin expression. This protective action of an NO donor in our model is not surprising in view of reports describing 1) an ability of NO synthase inhibitors to increase the number of venules immunostained for P-selectin in rat ileum (7), 2) an attenuation of I/R-induced leukocyte adhesion and P-selectin-mediated leukocyte-platelet aggregation by NO donors (22, 25), and 3) an inhibition of P-selectin-dependent oxidant-induced leukocyte rolling (14). Given the myriad of biological actions of NO, there are several mechanisms that may explain the ability of an NO donor to attenuate I/R-induced P-selectin expression. These include an attenuation of I/R-induced mast cell degranulation (25), scavenging of oxidants (14), and inhibition of lipoyxgenase activity (30). Our finding that I/R-induced P-selectin expression is not attenuated in either CuZn-SOD transgenic mice or mast cell-deficient mice would argue against the first two possibilities in our model. However, recent descriptions of a potent inhibitory effect of NO on lipoyxgenase activity (17, 30), coupled to our demonstration of an inhibitory action of MK-886 on I/R-induced P-selectin expression, would argue in favor of lipoyxgenases as a primary target for NO in our model.

In conclusion, the results of this study indicate that gut I/R is associated with a rapid increase in vascular P-selectin expression that remains elevated for as long as 8 h. The early increase in expression appears to involve translocation of a preformed pool of P-selectin to the endothelial cell surface, whereas the later expression may be linked to transcription-dependent biosynthesis of new protein. The early initial I/R-induced increase in intestinal P-selectin expression appears to be mediated by a 5-lipoxygenase-dependent, NO-inhibitable mechanism.

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