Downstream effects of splanchnic ischemia-reperfusion injury on renal function and eicosanoid release

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Rothenbach, Patricia, Richard H. Turnage, Jose Igle- sias, Angela Riva, Lori Bartula, and Stuart I. Myers. Downstream effects of splanchnic ischemia-reperfusion injury on renal function and eicosanoid release. J. Appl. Physiol. 82(2): 530–536, 1997.—This study examines the hypothesis that intestinal ischemia-reperfusion (I/R) injury contributes to renal dysfunction by altered renal eicosanoid release. Anesthetized Sprague-Dawley rats underwent 60 min of sham or superior mesenteric artery (SMA) occlusion with 60 min of reperfusion. The I/R groups received either allopuri-

nol, pentoxifylline, 1-benzylimidazole, or carrier before SMA occlusion. In vivo renal artery blood flow was measured by Transonic flow probes, the kidneys were then perfused in vitro for 30 min, and the effluent was analyzed for eicosanoid release and renal function. Intestinal I/R caused a twofold increase in the ratio of renal release of thromboxane B$_2$ to prostaglandin E$_2$ and to 6-ketoprostaglandin F$_{1\alpha}$ compared with the sham level, with a corresponding 25% decrease in renal sodium and inulin clearance and renal blood flow. Pentoxifylline or allopurinol pretreatment restored renal eicosanoid release and renal sodium and inulin clearance to the sham level but did not alter renal blood flow. Pretreatment with 1-benzylimidazole restored renal function, eicosa- noid release, and renal blood flow to sham levels. These data suggest that severe intestinal I/R contributes to the downregulation of renal function. The decrease in renal function is due in part to toxic oxygen metabolites, which occur in the milieu of altered renal eicosanoid release, reflecting a decrease in vasodilator and an increase in vasoconstrictor eicosanoids.

renal eicosanoid release; renal blood flow

THE KIDNEY has been previously shown to be a signifi- cant site of vasoactive prostanoid release, and endog- enous renal prostanoids have been implicated as mediators of renal vascular resistance in normal and pathological states (7, 29, 30, 54). Several animal models have shown that the kidney responds to many varied types of injury (hemorrhagic shock, hydrenephro- sis, hypoxia, acute tubular necrosis, and sepsis) by altering net endogenous eicosanoid release to reflect a net increase in the synthesis of vasoconstrictor eicosanoids compared with endogenous synthesis of vasodilata- tor eicosanoids (7, 29, 30, 40–42, 54, 55). The findings of these studies support the notion that vasodilator eicosanoids contribute to maintaining normal intrarenal blood flow and that either loss of endogenous vasodila- tor eicosanoids or increased synthesis of vasoconstric- tor eicosanoids (or a combination of both scenarios) contributes to decreased intrarenal blood flow in pathological states (6, 21–23, 30, 33, 34, 40–42, 49, 55).

Pentoxifylline (Ptx) has been shown to increase sur- vival and preserve renal function after ischemia-reperfusion (I/R) injury. Although the specific protective mechanism of Ptx on renal function and eicosanoid release after intestinal I/R injury has not been investi- gated, several studies have suggested that the benefi- cial effect of Ptx includes increased tissue oxygenation, increased oxygen consumption, and decreased leuco- cyte adhesiveness and subsequent prevention of the release of toxic substances from leukocytes, including oxygen-derived free radicals (5, 16, 39, 51).

Allopurinol has been shown in studies investigating I/R injury to prevent superoxide radical release after conversion of hypoxanthine to xanthine in ischemic tissue during reperfusion (4, 8, 15, 17–20, 28, 43). Several studies have shown that xanthine oxidase was present in renal and intestinal tissue and that allopuri- nol could prevent injury to both organs after I/R injury (17–20, 28).

Several published reports have documented distant organ dysfunction after acute trauma or injury (10, 21, 23a, 24, 38, 45). The majority of these studies have shown that distant trauma or injury leads to progres- sive pulmonary injury. The pulmonary injury after remote injury was associated with increased pulmo- nary vasoconstrictor eicosanoid release (10, 23, 45). These studies hypothesized that burn or intestinal I/R injury induces the release of various mediators such as eicosanoids, activated neutrophils, and cytokines, which contribute to distant lung injury. These studies have not determined whether burn injury or intestinal I/R injury can alter renal eicosanoid release and renal function. This study examines the hypothesis that severe mesenteric I/R injury alters renal eicosanoid release and renal function.

MATERIALS AND METHODS

Surgical Model

Male Sprague-Dawley rats (~300 g) were housed and used in compliance with the regulations of the animal care facility of the University of Texas Southwestern. All animals were allowed food and water ad libitum before the experiment. The animals were anesthetized with methoxyflurane by inhala- tion (37, 39). The abdomen was washed with Betadine, and a midline laparatomy was performed. Each animal received 50 U/kg of heparin. The animals were divided into five experimen- tal groups: sham, 60 min of ischemia followed by 60 min of reperfusion [superior mesenteric artery (SMA) I/R], 60 min of ischemia followed by 60 min of reperfusion with Ptx pretreat- ment (SMA I/R + Ptx), 60 min of ischemia followed by 60 min of reperfusion after allopurinol pretreatment (SMA I/R +
allopurinol), and 60 min of ischemia followed by 60 min of reperfusion with pretreatment with 1-benzylimidazole [thromboxane synthase inhibitor (see Refs. 40–42, 54, 55); SMA I/R + imidazole]. Systolic arterial blood pressure and arterial blood flow were monitored from 15 min before I/R until 15 min after reperfusion (38). Blood pressure was monitored by using Digi-Med recorders (Louisville, KY).

Preparation of the Rat for I/R Injury and In Vivo Aortic and Renal Artery Blood Flow

The animals of the sham group received an injection of 0.05 ml saline carrier 10 min before SMA occlusion and 1 min before clip removal (31, 39). The PtX I/R groups received either an injection of 0.05 ml saline carrier (SMA I/R) or 0.05 ml of saline containing 50 mg/kg PtX iv (SMA I/R + PtX) 10 min before microvascular clipping of SMA and occlusion of collateral vessels and 1 min before clip removal, as previously described (31, 39). The imidazole group of animals received 0.5 ml of saline or saline containing 50 mg/kg of 1-benzylimidazole 2 min before SMA clipping (SMA I/R + imidazole) (40–42, 54, 55). The allopurinol groups received either saline carrier (pH adjusted to 10.5 with 1 N NaOH) or allopurinol 50 mg/kg by gavage for 3 days. After the dose of allopurinol on the third day, rats underwent intestinal I/R as described in Surgical Model (SMA I/R + allopurinol). All rats were studied for identical time periods regardless of group assignment. The SMA clip was removed after 60 min. At this time, a second dose of 0.05 ml saline was given to the sham and SMA I/R and the 0.05 ml of PtX (50 mg/kg) was administered to the SMA I/R + PtX group, and the bowel was reperfused for 60 min (39).

All animals had measurement of renal and abdominal aortic blood flow by mean transmittable Doppler flowmeters (1RB109 and 2SB73, Transonic systems, Ithaca, NY) as described by Bailey et al. (2), Drost (11), and Myers and Hernandez (37). Blood flow measurements were made before intestinal I/R and then at 15 min after intestinal I/R (total of 135 min) and were recorded as milliliters per minute. The data are presented as renal blood flow as a percentage of aortic blood flow (means ± SE).

Quantification of Renal Function in the Isolated Perfused Rat Kidney

The renal function of the isolated perfused rat kidneys was analyzed by examining renal sodium clearance, renal inulin clearance, and fractional sodium excretion, as previously described, at 30 min of in vitro perfusion (26, 32, 40, 46, 50).

Sodium clearance. Sodium from the urine and perfusate (Kreb’s buffer) was measured on Beckman Astra-8–3000 (Beckman Instruments, Houston, TX), and sodium clearance was calculated as (urine sodium; mmol/l)·(volume of urine; ml/min)/(Kreb’s sodium; mmol/l) and expressed as means ± SE (ml/min).

Inulin clearance. Inulin (Sigma Chemical, St. Louis, MO) stock solution was prepared in water and subsequently diluted with Krebs-Henseleit buffer to a concentration of 0.2 mg/ml. The inulin-Kreb’s solution was infused at a rate that maintained renal perfusion pressure at 100 mmHg (3 ml/min), and urine was collected for 10 min at 30 min of perfusion. The urine volumes were measured and diluted 1:10 in 10% trichloroacetic acid and centrifuged at 2,000 revolutions/min for 10 min. The supernatant was used to assay for inulin. Anthrone reagent (Sigma Chemical) was prepared in 70% sulfuric acid at a concentration of 2 mg/ml. Then, 200 µl of unknowns, standards, and Krebs blank were added to 2 ml of the Anthrone reagent and heated for 10 min at 57°C. Assay tubes were then cooled to room temperature and read at 620 nm on a Beckman DU 50 spectrophotometer. Unknown sample concentrations were extrapolated from a standard inulin curve, and glomerular filtration rate was...
calculated as [urine inulin (mg/ml)·volume urine (ml/min)]/Kreb's inulin (mg/ml) and expressed as means ± SE (ml/min).
Fractional sodium excretion. Fractional sodium excretion is expressed as a percentage in this study and was calculated as sodium clearance/inulin clearance x 100 and expressed as means ± SE.

Statistical Analysis
Eicosanoid release data are calculated as nanograms per minute and are reported as the ratio of TxB2 to PGE2 and of TxB2 to 6-keto-PGF1α. Renal function data are presented as milliliters per minute for sodium clearance and inulin clearance and as percent for sodium excretion. In vivo renal artery blood flow is presented as a percentage of total aortic blood flow. Eicosanoid release data and renal function data are presented as means ± SE for six rats at 30 min of perfusion. Statistical significance is accepted at P < 0.05 with comparisons made by using analysis of variance and Duncan’s post hoc test or Student’s t-test. Renal blood flow data are presented as means ± SE for six rats at 15 min after I/R. The immunoblot data are presented as means ± SE for four rats and analyzed by Student’s t-test.

RESULTS
Intestinal I/R produced a ninefold increase in the ratio of the renal release of TxB2 to PGE2 compared with the sham group at 30 min of perfusion (Fig. 1). The increased ratio of release of TxB2 to PGE2 was due to a significant increase in the renal release of TxB2 and to a marked decrease in the renal release of PGE2 (Table 1). In the I/R groups that received Ptx or allopurinol pretreatment, the ratio of renal release of TxB2 to PGE2 remained at the sham level (Fig. 1, Table 1).
Intestinal I/R also caused a twofold increase in the ratio of renal release of TxB2 to 6-keto-PGF1α, compared with the sham group at 30 min of perfusion. This twofold increase in the ratio of renal release of TxB2 to 6-keto-PGF1α was almost entirely secondary to the increased release of TxB2 (Table 1). The increased ratio of the renal release of TxB2 to 6-keto-PGF1α after intestinal I/R was prevented by pretreatment with Ptx or allopurinol (Fig. 2).
Sodium clearance and inulin clearance of the rat kidney perfused in vitro with oxygenated Krebs at 30 min of perfusion after intestinal I/R were significantly decreased compared with the sham group. Ptx and allopurinol pretreatment prevented the decrease in sodium and inulin clearances after intestinal I/R injury (Table 2). Intestinal I/R did not significantly alter percent sodium excretion in the isolated perfused rat kidney at 30 min of perfusion. However, a downward trend was noted (Table 1).

Mean arterial pressures were compared at 120 min in the sham group or at 60 min after reperfusion in the I/R groups. Arterial pressure was 87 ± 6.5 mmHg in the

![Fig. 1. Effect of intestinal ischemia-reperfusion (I/R) injury on ratio of endogenous renal release of thromboxane B2 (TxB2) to prostaglandin E2 (PGE2) in vitro perfused rat kidney. Rats received pentoxifylline (Ptx, stippled bar), allopurinol (crosshatched bar), or carrier (open bar); were subjected to superior mesenteric artery (SMA) occlusion and reperfusion (solid bar) as described in MATERIALS AND METHODS; and were compared with sham controls (open bar). Venous effluent was collected at 30 min of perfusion and assayed for TxB2 (thromboxane A2 metabolite) and PGE2. Values are calculated as picograms of TxB2 or PGE2 released per milliliter and are expressed as a ratio of renal release of TxB2 to PGE2 as a percent. Values are means ± SE for 6 rats. *Significantly different from Ptx, allopurinol, and sham groups, P < 0.05 (by analysis of variance and Duncan’s post hoc test.]

![Fig. 2. Effect of intestinal I/R injury on ratio of endogenous renal release of TxB2 to 6-ketoprostaglandin F1α (6-keto-PGF1α) in vitro perfused rat kidney. Rats received Ptx (stippled bar), allopurinol (crosshatched bar), or carrier (open bar); were subjected to SMA occlusion and reperfusion (solid bar) as described in MATERIALS AND METHODS; and were compared with sham controls (open bar). Venous effluent was collected at 30 min of perfusion and assayed for TxB2 (thromboxane A2 metabolite) and 6-keto-PGF1α (prostaglandin I2 metabolite). Values are calculated as picograms of TxB2 or 6-keto-PGF1α released per milliliter and are expressed as a ratio of renal release of TxB2 to 6-keto-PGF1α as a percent. Values are means ± SE for 6 rats. *Significantly different from Ptx, allopurinol, and sham groups, P < 0.05 (by analysis of variance and Duncan’s post hoc test.)

Table 1. Effect of severe splanchnic ischemia-reperfusion on renal eicosanoid release with the sham group at 30 min of perfusion. This twofold increase in the ratio of renal release of TxB2 to 6-keto-PGF1α was almost entirely secondary to the increased release of TxB2 (Table 1). The increased ratio of the renal release of TxB2 to 6-keto-PGF1α after intestinal I/R was prevented by pretreatment with Ptx or allopurinol (Fig. 2).

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>SMA I/R</th>
<th>SMA I/R + Ptx</th>
<th>SMA I/R + allopurinol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thromboxane B2</td>
<td>44.3 ± 6</td>
<td>93.3 ± 20*</td>
<td>40 ± 2.5</td>
<td>40 ± 5</td>
</tr>
<tr>
<td>PGI2</td>
<td>196 ± 29</td>
<td>181 ± 35</td>
<td>168 ± 25</td>
<td>152 ± 43</td>
</tr>
<tr>
<td>PGE2</td>
<td>132 ± 11</td>
<td>49 ± 20*</td>
<td>120 ± 23</td>
<td>146 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE given in pg/ml for 6 rats. I/R, ischemia-reperfusion; Sham, sham occlusion; SMA, superior mesenteric artery occlusion; Ptx, pentoxifylline; PGI2, prostaglandin I2; PGE2, prostaglandin E2. PGI2 was assayed as 6-ketoprostaglandin F1α. *P < 0.02 compared with Sham, SMA I/R + Ptx, and SMA I/R + allopurinol by analysis of variance and Duncan’s post hoc test.
arterial pressure (56 mmHg). Treatment of the sham group treated with Ptx and 107 ± 8 mmHg in the sham group treated with imidazole. Systolic pressure was not significantly altered by I/R (89 ± 6 mmHg). Treatment of the I/R groups with Ptx significantly decreased arterial pressure to 48 ± 8 mmHg compared with the sham group treated with Ptx or the I/R groups (P < 0.05). Treatment of the I/R groups with either allopurinol or imidazole did not significantly alter arterial pressure (56 ± 12 and 72 ± 7 mmHg, respectively) compared with sham groups without drug treatment or sham groups treated with either allopurinol or imidazole (90 ± 7 mmHg). Intestinal I/R injury decreased in vivo renal artery blood flow by 25% when compared with sham-operated controls (Fig. 3). Ptx or allopurinol pretreatment did not alter renal artery blood flow after I/R injury (Fig. 3).

Treatment of the sham animals with 1-benzylimidazole decreased endogenous renal release of TxB2 but did not alter release of PGE2 or 6-keto-PGF1a. Treatment of the sham animals with 1-benzylimidazole did not alter renal function or in vivo renal blood flow (Table 3). A separate group of animals was subjected to intestinal I/R with saline carrier for comparison with the intestinal I/R group treated with 1-benzylimidazole. Intestinal I/R treated with saline carrier increased renal release of TxB2 and decreased renal release of PGE2 concomitant with decreases in inulin and sodium clearance and in vivo renal blood flow. These changes in renal eicosanoid release, renal function, and renal blood flow were reversed by 1-benzylimidazole pretreatment (Table 3).

Intestinal I/R decreased the cyclooxygenase-1 content in the renal medulla by 30% compared with the sham group. Pretreatment with Ptx (or allopurinol, data not shown) did not prevent the decrease in cyclooxygenase-1 content in the intestinal I/R group (Table 4). Intestinal I/R did not alter thromboxane synthase content in the cortex or medulla. Renal cortical and medullary prostacyclin synthase content was below the level of detection, as was cortical cyclooxygenase-1 content (Table 4).

DISCUSSION

Over the past 20 years, several studies have suggested that endogenous renal eicosanoids influence both renal vascular tone and urinary diuresis (6, 7, 23, 25, 29, 30, 33, 40–42, 49, 54, 55). These studies have shown PGE2 to be the primary eicosanoid synthesized and released by the kidney (7, 29). Several chronic models of renal pathology have demonstrated that the kidney responds to injury by an increased level of endogenous renal eicosanoid synthesis with an increased synthesis of TxA2 (vasoconstrictor eicosanoid) and a corresponding decrease in synthesis of PGE2 and PGJ2 (vasodilator eicosanoids) (34, 41, 42, 54, 55). These studies support the notion that relative changes in vasoconstrictor and vasodilator eicosanoid release contribute to the increase in renal vascular resistance.

### Table 2. Effect of severe splanchic ischemia-reperfusion on renal function

<table>
<thead>
<tr>
<th>Sodium clearance, ml/min</th>
<th>Sham</th>
<th>SMA I/R</th>
<th>SMA I/R + Ptx</th>
<th>SMA I/R + allopurinol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.12 ± 0.001</td>
<td>0.08 ± 0.016*</td>
<td>0.11 ± 0.001</td>
<td>0.15 ± 0.007</td>
</tr>
<tr>
<td>Inulin clearance, ml/min</td>
<td>0.17 ± 0.001</td>
<td>0.13 ± 0.002*</td>
<td>0.17 ± 0.004</td>
<td>0.16 ± 0.004</td>
</tr>
<tr>
<td>Sodium excretion, %</td>
<td>83.2 ± 8.5</td>
<td>78.4 ± 4.4</td>
<td>69.0 ± 6.0</td>
<td>69.1 ± 6.0</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6 rats. Renal function was measured with isolated perfused rat kidney. *P < 0.05 compared with Sham, SMA I/R + Ptx, and SMA I/R + allopurinol by analysis of variance and Duncan's post hoc test. Sodium clearance, inulin clearance, and sodium excretion were measured in ml/min and are presented as a percentage of abdominal aortic blood flow at 15 min after reperfusion. Values are reported as means ± SE for 6 rats.

### Table 3. Effect of thromboxane synthase inhibition on renal eicosanoid release, renal function, and renal blood flow after severe splanchic ischemia-reperfusion

<table>
<thead>
<tr>
<th>Prostaglandins</th>
<th>Sham</th>
<th>SMA I/R</th>
<th>SMA I/R + imidazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thromboxane B2, pg/ml</td>
<td>69.6 ± 10</td>
<td>120 ± 18*</td>
<td>17.8 ± 5.0</td>
</tr>
<tr>
<td>PGJ2, pg/ml</td>
<td>273 ± 74</td>
<td>253 ± 43</td>
<td>237 ± 24</td>
</tr>
<tr>
<td>PGE2, pg/ml</td>
<td>219 ± 16</td>
<td>107 ± 30*</td>
<td>180 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6 rats. Renal function was measured with isolated perfused rat kidney. Renal artery blood flow was measured as percentage of aortic blood flow at 15 min after reperfusion. Imidazole is 1-benzylimidazole. Prostaglandins were used to measure eicosanoid release. PGJ2 was assayed as 6-ketoprostaglandin F1α. *P < 0.05 compared with sham and SMA I/R + imidazole by Student's t-test.

### Fig. 3. Effect of intestinal I/R injury on in vivo renal artery blood flow as percentage of abdominal aortic blood flow.

Male Sprague-Dawley rats were anesthetized and underwent midline laparotomy. Renal artery blood flow and abdominal aortic blood flow were measured continuously by Transonic System 1RB 109 and 2SB 73 flow probes in animals subjected to sham (open bar) or SMA I/R (filled bar) injury alone or SMA I/R with Ptx treatment (stippled bar) or allopurinol (crosshatched bar). Renal artery blood flow values are measured in ml/min and are presented as a percentage of abdominal aortic blood flow at 15 min after reperfusion. Values are reported as means ± SE for 6 rats.
This finding was demonstrated in one study that increased TxB2 release from the burn tissue. The synthesis but rather a decreased release of PGE2 and renal synthesis and release of TxA2 (54). In contrast, suggests that 48 h is the time required to upregulate present in these models. This group of studies also hypothesized that acute thermal injury in-
creased burn tissue release of TxB2, which caused
steadily increased burn tissue release of TxB2, which caused secondary injury to the lungs (10). Schmeling et al. (45) examined the effects of intestinal I/R on the lung. In their study, lung injury was assessed by measuring tissue adenosine triphosphate and myeloperoxidase values as well as by histological evaluation. Schmeling et al. demonstrated that intestinal I/R injury caused secondary lung injury by a decrease in tissue ATP, an increase in myeloperoxidase activity, neutrophil sequestration in the lungs, and increased microvascular permeability (45).

The present study utilized a similar approach as described by Schmeling et al. to investigate the effect of severe intestinal I/R on renal function and eicosanoid release. The data showed that severe acute mesenteric I/R injury markedly increased the relative release of endogenous renal vasoconstrictor to vasodilator eicosanoids and caused a parallel decrease in insulin and sodium clearance. Interestingly, acute intestinal I/R injury stimulated an increase in the release of endogenous TxB2 release and a fourfold decrease in PGE2 release after 2 h of injury. These findings could have great potential clinical significance because increased endogenous renal thromboxane release and decreased renal PGE2 release may be one of several unrecognized mechanisms contributing to acute renal failure after severe mesenteric I/R injury.

The increased ratio of the renal release of vasoconstrictor to vasodilator eicosanoids and the parallel decrease in renal function after severe intestinal I/R injury was prevented by Ptx, allopurinol, and 1-benzylmida-zolone pretreatment. Although severe intestinal I/R decreased total renal artery blood flow by 25%, these changes in renal artery blood flow were only prevented by pretreatment with 1-benzylimidazole and were not prevented by Ptx or allopurinol pretreatment. Although the mechanisms of this finding were not specifically examined in the present study, previous studies have provided some insight into the protective effects of Ptx treatment on visceral organ function after intestinal I/R injury (5, 16, 39, 51, 52). Myers et al. (39) examined the effects of Ptx on splanchnic PGI2 release after severe intestinal I/R injury. In that study, Ptx preserved splanchnic PGI2 release and significantly decreased intestinal histological injury (39). Flynn et al. (16) showed that Ptx preserved hepatic blood flow and function during resuscitation from hemorrhagic shock. The exact mechanisms of Ptx preservation of visceral organ protection after I/R injury are not known and are not the focus of this study. However, our study, when considered in the context of the previous studies mentioned above, suggests that Ptx prevents the renal injury after splanchnic I/R at the microvascular level and not at the level of total renal artery blood flow. Data from the allopurinol pretreatment group suggest that the renal microvascular injury after intestinal I/R is due in part to the production of toxic oxygen metabolites. The source of the oxygen-derived free radicals could be from circulating leukocytes activated by intestinal I/R or from within the kidney. The enzyme xanthine oxidase has been shown to be present in the intestine and the kidney (19, 20, 43). During ischemia, xanthine oxidase is transformed into xanthine dehydrogenase during ischemia, and concurrently ATP is converted in a series of steps into hypoxanthine. Hypoxanthine, in the presence of molecular oxygen, is converted by xanthine dehydrogenase into xanthine with concomitant release of the superoxide radical. The superoxide radical can then be converted by superoxide dismutase into hydrogen peroxide, which can be further metabolized into water and oxygen by the enzyme catalase.
(17–20, 28, 43). Allopurinol prevents the first series of reactions and thus prevents production of the superoxide radical. The site of action of allopurinol in our study could be the intestine (which would prevent leukocyte activation), the activated leukocytes, or the renal tissue. The 1-benzylimidazole data suggest that the increased endogenous renal release of thromboxane after intestinal I/R contributes to changes in renal blood flow. Although the site of action of increased endogenous thromboxane synthesis was not examined in this study, one could hypothesize that thromboxane contributed to vasocstriction at the level of the renal arterioles.

The present study represents the first group of experiments to suggest that severe intestinal I/R induces a downregulation of renal function. The eicosanoid data from sham, I/R, and I/R groups pretreated with Ptx, allopurinol, and 1-benzylimidazole provide insight into the mechanisms of the remote renal injury. The decrease in renal function is associated with a relative increase in the ratio of the renal vasoconstrictor to vasodilator eicosanoids and the exposure of the renal tissue to toxic oxygen metabolites. Although we cannot state the specific mechanisms of renal injury after intestinal I/R, we hypothesize that the renal tissue is exposed to toxic oxygen metabolites released from leukocytes activated by intestinal I/R. The exposure of renal tissue to toxic oxygen metabolites occurs in the milieu of altered renal eicosanoid release, which reflects both a fourfold decrease in PGE₂, the principal vasodepressor lipid of rabbit renal medulla. The allopurinol experiments further support this hypothesis, suggesting that toxic oxygen metabolites contribute to the altered renal eicosanoid release found in our experimental model (1, 12, 13, 45, 53).

The immunoblot data provide some insight into the mechanisms involved with renal eicosanoid release after intestinal I/R. The rise in renal TXB₂ release after intestinal I/R was not due to an increase in content of thromboxane synthase and occurred despite a 30% decrease in cyclooxygenase-1 content. We hypothesize that the increase in renal TXB₂ release after intestinal I/R could be secondary to an increased activity of thromboxane synthase. The decrease in renal PGE₂ after intestinal I/R could be due to a decrease in cyclooxygenase-1 content, a decrease in cyclooxygenase activity, or a combination of both.

In summary, the present study supports the hypothesis that severe SMA I/R injury decreases renal function, which is associated with altered renal eicosanoid release. The rat kidney responded to severe splanchic I/R injury by a relative increase in the ratio of the release of vasoconstrictor endogenous eicosanoids (TXA₂) to the release of endogenous vasodilator renal eicosanoids (PGE₂, PG₁₂), corresponding to a decrease in renal function. The prevention of these findings by Ptx, without reversal of the decrease in total renal artery blood flow, supports the hypothesis that Ptx exerted its protective effect on renal eicosanoid release and renal function at a microvascular level. The injury could be caused by leukocyte activation and adhesion with subsequent release of toxic substances such as oxygen-derived free radicals. The allopurinol experiments further support this hypothesis, suggesting that toxic oxygen metabolites contribute to the renal microvascular injury after severe intestinal I/R.

This study was supported by National Institute of General Medical Sciences Grants GM-38529 (S. I. Myers) and GM-38342 (S. I. Myers), Veterans Affairs Merit Grant (S. I. Myers), and American Heart Association Grant in Aid (R. H. Turnage).

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Received 6 April 1995; accepted in final form 15 May 1997.

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Splanchnic Ischemia Downregulates Renal Function