Thromboxane A$_2$ mediates increased pulmonary microvascular permeability after intestinal reperfusion

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1Department of Surgery, University of Texas Southwestern Medical School, Dallas 75235-9031; 2Dallas Veterans Administration Medical Center, Dallas, Texas 75216; and 3Department of Surgery, Temple University School of Medicine, Philadelphia, Pennsylvania 19140

Turnage, Richard H., John L. LaNoue, Kevin M. Kadesky, Yan Meng, and Stuart I. Myers. Thromboxane A$_2$ mediates increased pulmonary microvascular permeability after intestinal reperfusion. J. Appl. Physiol. 82(2): 592–598, 1997.—This study examines the hypothesis that intestinal reperfusion (IR)-induced pulmonary thromboxane A$_2$ (TxA$_2$) release increases local microvascular permeability and induces pulmonary vasoconstriction. Sprague-Dawley rats underwent 120 min of intestinal ischemia and 60 min of IR. Sham-operated animals (Sham) served as controls. After IR or Sham, the pulmonary vessels were cannulated, and the lungs were perfused in vitro with Krebs buffer. Microvascular permeability was quantitated by determining the filtration coefficient (K$_f$), and pulmonary arterial (Ppa), venous (Ppv), and capillary (Ppc) pressures were measured to calculate vascular resistance (Rt). After baseline measurements, imidazole (TxA$_2$ synthase inhibitor) or SQ-29,548 (TxA$_2$-receptor antagonist) was added to the perfusate; then K$_f$, Ppa, Ppv, and Ppc were again measured. The K$_f$ of lungs from IR animals was four times greater than that of Sham (P < 0.001), and Rt was 63% greater in the injured group (P = 0.01). Pc of IR lungs was twice that of controls (5.4 ± 1.0 vs. 2.83 ± 0.3 mmHg, IR vs. Sham, respectively; P < 0.05). Imidazole or SQ-29,548 returned K$_f$ to baseline measurements (P < 0.05) and reduced Rt by 23 and 17%, respectively (P < 0.05). IR-induced increases in Pc were only slightly reduced by 500 µg/ml imidazole (14% P = 0.05) but unaffected by lower doses of imidazole (5 or 50 µg/ml) or SQ-29,548. These data suggest that IR-induced pulmonary edema is caused by both increased microvascular permeability and increased hydrostatic pressure and that these changes are due, at least in part, to the ongoing release of TxA$_2$.

capillary filtration coefficient; hydrostatic pressure; pulmonary vascular resistance; pulmonary edema

INTESTINAL REPERFUSION (IR) injury induces pulmonary microvascular dysfunction manifested by fluid and protein extravasation, leukosequestration, and histological and ultrastructural evidence of pulmonary capillary endothelial cell injury (7, 15, 26). More recent studies have related IR-induced pulmonary edema to increased pulmonary artery pressure (Ppa; 24) and enhanced microvascular permeability to fluid (3). A variety of circulating inflammatory mediators have been incriminated in the pathogenesis of this injury, particularly neutrophils and complement (3, 9, 11, 15, 25, 26). Paracrine inflammatory and vasoactive substances released by the lung also appear to be important mediators of pulmonary injury in this model (10, 23, 24).

Thromboxane A$_2$ (TxA$_2$) is one such paracrine substance that likely contributes to IR-induced lung injury. Evidence for this includes the observations that pulmonary TxA$_2$ release is upregulated in this and similar injuries (10, 12, 24); inhibition of TxA$_2$ synthesis or TxA$_2$-receptor blockade prevents pulmonary injury in these models (12, 24, 29); inflammatory mediators active in IR [e.g., complement (14) and oxygen-derived free radicals (17)] upregulate pulmonary TxA$_2$ release; and TxA$_2$ induces many of the pathophysiological phenomena characteristic of IR-induced lung injury, including increased microvascular permeability, vasoconstriction, and neutrophil infiltration (12, 13).

This study examines the pathophysiological mechanisms by which IR-induced TxA$_2$ release alters local pulmonary microvascular function in this model. Specifically, this study examines the hypothesis that IR-induced TxA$_2$ release enhances pulmonary microvascular permeability, as measured by the capillary filtration coefficient (K$_f$), and induces pulmonary vasoconstriction.

MATERIALS AND METHODS

Animal Model

The rat model of IR has been described in detail previously (10, 11, 15, 23–25). Briefly, pathogen-free male Sprague-Dawley rats (250–300 g) were weighed and then anesthetized with pentobarbital sodium (40 mg/kg ip). A microvascular clip was placed across the superior mesenteric artery (SMA) for 120 min. Removal of the clip allowed reperfusion for 60 min (IR). This degree of IR is associated with an intestinal, hepatic, and pulmonary injury and a 12-h mortality rate of 100% (22). Other parameters of generalized inflammation that have been associated with this model include endotoxemia (21), oxidant stress (20), and complement activation (25). Lesser periods of ischemia and reperfusion have been examined and are associated with less consistent remote organ injury. Time-matched, sham-operated animals undergoing dissection of the proximal SMA without occlusion served as controls (Sham).

Quantitation of Pulmonary Microvascular Dysfunction In Vitro

Isolated perfused lung model. After IR or sham operation, the pulmonary arterial trunk, left atrium, and trachea were cannulated, and the lungs and heart were excised en bloc. The lungs were weighed and then suspended by a ligature from a force transducer (FT 03, Statham, Oxnard, CA) for continu-
ous measurement of lung weight. The lungs were then perfused with Krebs-Henseleit buffer containing 3% bovine serum albumin at 0.04 ml·g body wt⁻¹·min⁻¹ and ventilated with room air at a rate of 60 strokes/min. Ppa and left atrial pressure were measured with pressure transducers (P23, Statham) with zero reference at the level of the apex of the lung. These measurements were continuously recorded with a four-channel polygraph (Grass Instruments, Quincy, MA).

The lungs were perfused for 15 min before initial measurements to eliminate circulating blood elements from the vascular space and to allow the lungs to reach an isogravimetric state with a stable perfusion pressure and temperature. Zone III conditions (arterial > venous > alveolar pressures) were maintained throughout all experiments. In these experiments, airway pressures were uniformly <2 mmHg, a value similar to that previously reported in this model (24).

Determination of capillary Kᵢ. Pulmonary microvascular permeability to fluid was quantitated by determining Kᵢ, as described by Drake et al. (4) and Zanaboni et al. (30). After a 15-min equilibration period during which the perfused lungs were isogravimetric, a baseline capillary pressure (Pcap) was measured by using the double occlusion technique as described and validated by Allison et al. (2) and Townsley et al. (19). After this measurement, pulmonary venous pressure (Ppv) was elevated to 8 mmHg by partial occlusion of the pulmonary microvascular bed (minutes 0–2), and the second slow constant weight increase is due to fluid filtration across the microvascular bed (minutes 2–5). After 5 min of partial venous occlusion, Ppc is again measured before releasing the partial outflow occlusion (Ppcpost; 2, 4, 6, 18, 19, 30). Calculation of Kᵢ is as follows

\[ K_i = \frac{\Delta Wf}{\Delta t} \]

where \( \Delta Wf \) is the change in lung weight between minutes 2 and 5 of partial venous outflow occlusion; \( \Delta t \) is the duration of partial outflow occlusion; and \( \Delta P \) is the difference between Ppcpost and Ppcpre. Kᵢ is normalized to body weight and expressed as grams·min⁻¹·mmHg⁻¹·100 gram body wt⁻¹. Kᵢ measured in this manner (2, 30) correlates well with the zero-time extrapolation technique described by Drake (4, 6, 18).

Ppv and the Calculation of Vascular Resistance

Total pulmonary vascular resistance (Rt) was calculated as the total pressure drop across the lung as expressed in Eq. 2

\[ Rt = \frac{(Ppa - Ppv)}{Q} \]  

where Q is the flow through the isolated perfused lung. Rt is normalized for body weight and expressed as mmHg·ml⁻¹·min⁻¹·100 g body wt⁻¹.

As described by Allison et al. (2), the pulmonary circulation can be represented as a simple linear model in the isogravimetric state. In this model, Ppa is separated from Ppc by a precapillary resistance (Ra) and Ppc is separated from Ppv by a postcapillary resistance (Rv). Therefore, where Rt is determined in the isogravimetric state, Ra and Rv can be calculated as follows

\[ Ra = \frac{(Ppa - Ppc)}{Q} \]  

\[ Rv = \frac{(Ppc - Ppv)}{Q} \]  

\[ Rt = Ra + Rv \]

All resistance calculations (Rt, Ra, and Rv) were normalized for body weight and expressed as mmHg·ml⁻¹·min⁻¹·100 g body wt⁻¹.

In these determinations of Kᵢ and vascular resistance, Ppc was measured by using the double occlusion method as described by Allison et al. (2) and Townsley et al. (19). This methodology has been demonstrated to correlate closely with measurements of isogravimetric capillary pressure (Ppc; 6, 18) in both normal (19) and acutely injured lungs (2).

TXA₂ antagonists. The effect of TXA₂ on IR-induced pulmonary microvascular dysfunction was assessed in vitro by adding an inhibitor of thromboxane synthetase (5, 50, and 500 µg/ml imidazole; Sigma Chemical, St. Louis, MO) or a thromboxane-receptor antagonist (10⁻⁴ M SQ-29,548; Cayman Chemical, Ann Arbor, MI) to the perfusate of the isolated perfused lung. The experimental design is illustrated in

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**Fig. 1.** Protocol for experiments performed in the isolated, perfused rat lung model. Lungs of animals sustaining intestinal reperfusion injury (IR; n > 5) or sham operation (Sham; n > 5) were excised and perfused in vitro with modified Krebs buffer. After 15-min equilibration period, baseline measurements of capillary filtration coefficient (Kᵢ) and pulmonary arterial (Pₐ), pulmonary venous (Pᵥ) and pulmonary capillary pressure (Pₖ) were obtained. Perfusate was also sampled for determination of thromboxane B₂ (TXB₂), a stable metabolite of thromboxane A₂ (TXA₂). The TXA₂ synthetase inhibitor imidazole (5, 50, 500 µg/ml) or the TXA₂-receptor antagonist SQ-29,548 (10⁻⁴ M) was then added to perfusate. Kᵢ and pulmonary hemodynamic measurements were determined 20 min later. Perfusate and bronchoalveolar lavage fluid were then collected for measurement of TXB₂.
In these experiments, the lungs of animals sustaining IR or Sham (n > 5 per group) were perfused in vitro as described above. After a 15-min equilibration period, baseline measurement of $K_f$, Ppa, Ppv, and Ppc was performed. Imidazole or SQ-29548 was then added to the perfusate and allowed to circulate for 20 min; then these parameters were again measured.

Measurement of Pulmonary TxA₂ Release

Pulmonary TxA₂ release was quantitated by measuring the concentration of thromboxane B₂ (TXB₂; stable metabolite of TxA₂) within the bronchoalveolar lavage (BAL) fluid of isolated perfused lungs from animals sustaining IR or Sham (n > 5 per group). On completion of in vitro perfusion, BAL was performed by infusing 3 ml of normal saline into the trachea. This was repeated three times, and the lavage fluid was collected and frozen at −70°C for later measurement of TXB₂ by a commercially available enzyme immunoassay (Cayman Chemical).

Pulmonary TxA₂ release was also quantitated by measuring TXB₂ in perfusate obtained from both the arterial (afferent) and venous (efferent) sides of the isolated perfused lung. This strategy allowed the detection of a TXB₂ gradient across the lung. The concentration of TXB₂ in BAL fluid and in perfusate was expressed as picograms per milliliter.

Statistical Analysis

Data are expressed as means ± SE. Analysis of data from more than two groups was performed by using analysis of variance and Fisher’s post hoc test. Analysis of unpaired data from two groups was performed using a two-tailed, unpaired Student’s $t$-test. Analysis of paired measurements was performed by using a two-tailed, paired Student’s $t$-test. Statistical significance was considered for a type I error of < 0.05.

All experiments were approved by the Committee on the Care and Use of Animals at the University of Texas Southwestern Medical School and Dallas Veterans Administration Medical Center.

RESULTS

In Vitro Measurement of IR-Induced Pulmonary Microvascular Dysfunction

Capillary $K_f$. The $K_f$ of lungs from animals sustaining IR was 0.019 ± 0.002 g·min⁻¹·mmHg⁻¹·100 g body wt⁻¹. This was four times greater than that of animals sustaining Sham (P < 0.001). The addition of imidazole (500 µg/ml) or the TxA₂-receptor antagonist SQ-29,548 (10⁻⁴ M) to the perfusate of the isolated perfused lung model prevented the increase in $K_f$ associated with IR. These data are shown in Fig. 2. A dose-response curve with 5, 50, and 500 µg/ml imidazole is shown in Fig. 3. In paired experiments in which $K_f$ was measured before and then 20 min after the addition of imidazole, there was a 25% reduction in $K_f$ on addition of 50 µg/ml (P = 0.08, paired Students $t$-test) and a 40% reduction with 500 µg/ml (P = 0.03, paired Student’s $t$-test). The $K_f$ of lungs of sham-operated animals treated with imidazole or SQ-29,548 was not different from that of lungs exposed to perfusate alone (data not shown).

Pulmonary hemodynamic measurements. The results of the hemodynamic measurements in the lungs of animals sustaining IR and Sham are shown in Fig. 4 and Table 1. $R_t$ was significantly greater in the lungs of animals sustaining IR when compared with Sham (P = 0.01). This was principally due to an increase in the postcapillary resistance as quantitated by $R_v$. $R_v$ was four times greater in the lungs of animals sustaining IR when compared with controls (P < 0.001). Ra was 20% greater in the lungs of IR animals when compared with Sham (P < 0.05). Ppc as measured by the double occlusion technique was twofold greater in the lungs of animals sustaining IR compared with sham-operated controls (P < 0.05).
The addition of 10 and 50 µg/ml did not significantly affect pulmonary Rv. The isolated perfused IR lung reduced Rt by 17% (P = 0.05). Lesser doses of imidazole (5 and 50 µg/ml) had no significant effect on IR-induced increases in Ppc. Similarly, the addition of the TXA2-receptor antagonist SQ-29,548 did not attenuate IR-induced increases in Ppc.

Measurement of Pulmonary TxB2 Release

There was twice as much TxB2 released by the lungs of animals sustaining IR compared with those of sham-operated controls. The concentration of TxB2 within the BAL fluid of sham-operated animals was 1,964.2 ± 185.3 pg/ml. This was significantly less than that of lungs from injured animals (4,151 ± 925 pg/ml; P < 0.01).

The concentration of TxB2 in the perfusate by lungs from IR animals was nearly 50% greater than that of sham-operated controls (87.8 ± 7.2 vs. 59.7 ± 9.0 pg/ml, respectively; P = 0.05). There was no difference in the concentration of TxB2 in perfusate sampled from the arterial and venous sides of the lungs from the Sham group (46.5 ± 7.3 vs. 59.7 ± 9.0 pg/ml, arterial vs. venous ports, respectively; P = 0.1), whereas the concentration of TxB2 in perfusate distal to the IR lungs was 33% greater than that obtained from the arterial port (71.5 ± 14 vs. 94.4 ± 14 pg/ml, arterial vs. venous ports, respectively; P = 0.006).

Despite the relatively brief duration of treatment (20 min), imidazole resulted in a significant reduction in TxB2 concentration within the BAL of animals sustaining IR. The addition of 50 µg/ml of imidazole to the perfusate of lungs isolated from IR animals resulted in a 63% reduction in TxB2 concentration within the BAL fluid (4,151 ± 925 vs. 1,536.9 ± 221.4 pg/ml, untreated and 50 µg/ml imidazole, respectively; P < 0.05). The addition of 500 µg/ml imidazole resulted in a nearly 70% reduction in TxB2 concentration within the BAL fluid (4,151 ± 925 vs. 1,331 ± 186.9 pg/ml in untreated and 500 µg/ml imidazole, respectively; P = 0.007).

Treatment with SQ-29,548 or 5 µg/ml imidazole had no effect on TxB2 release into the BAL of IR animals (data not shown).

Table 1. Hemodynamic parameters in lungs of animals sustaining intestinal reperfusion before and 20 min after addition of thromboxane synthetase inhibitor imidazole or thromboxane receptor antagonist SQ-29,548

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pulmonary Capillary Pressure, mmHg</th>
<th>Total Vascular Resistance, mmHg·ml⁻¹·min⁻¹·100 g body wt</th>
<th>Pre-capillary Resistance, mmHg·ml⁻¹·min⁻¹·100 g body wt</th>
<th>Post-capillary Resistance, mmHg·ml⁻¹·min⁻¹·100 g body wt</th>
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<tbody>
<tr>
<td>Sham</td>
<td>2.83 ± 0.3</td>
<td>0.49 ± 0.06</td>
<td>0.41 ± 0.05</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>IR</td>
<td>5.4 ± 1.0a</td>
<td>0.80 ± 0.07a</td>
<td>0.51 ± 0.06a</td>
<td>0.30 ± 0.04a</td>
</tr>
<tr>
<td>IR + imidazole</td>
<td>9.6 ± 1.1</td>
<td>0.65 ± 0.07</td>
<td>0.46 ± 0.07a</td>
<td>0.36 ± 0.03a</td>
</tr>
<tr>
<td>IR + imidazole</td>
<td>5.4 ± 1.0</td>
<td>0.72 ± 0.09</td>
<td>0.5 ± 0.06</td>
<td>0.27 ± 0.04</td>
</tr>
<tr>
<td>IR + imidazole</td>
<td>8.5 ± 1.3</td>
<td>0.63 ± 0.06</td>
<td>0.47 ± 0.1</td>
<td>0.38 ± 0.04</td>
</tr>
<tr>
<td>IR + SQ-29,548</td>
<td>7.71 ± 0.92</td>
<td>0.57 ± 0.04</td>
<td>0.46 ± 0.06</td>
<td>0.32 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 per group. Data in first 2 rows are measurements from lungs of animals sustaining intestinal reperfusion (IR) or sham operation (Sham). Following rows, measurements from lungs of animals sustaining IR before (Baseline) and 20 min after (posttreatment) addition of imidazole or SQ-29,548. *P < 0.05 vs. sham, analyzed with 2-tailed, unpaired Student's t-test. †P ≤ 0.05 vs. baseline measurements, analyzed with 2-tailed, paired Student's t-test.
DISCUSSION

Reperfusion of ischemic intestine induces pulmonary microvascular dysfunction characterized by interstitial edema and ultrastructural evidence of endothelial cell injury (3, 7, 15, 26). The most commonly utilized parameter of IR-induced pulmonary microvascular dysfunction has been the transvascular leakage of protein quantitated by assays utilizing radiolabeled albumin (15) or vital dyes such as Evans blue (23). In the present study, the isolated perfused lung preparation was employed to examine the physiological determinants of pulmonary edema, i.e., altered microvascular permeability and hydrostatic pressure. Determination of the capillary Kᵣ quantitates microvascular permeability to fluid (2–4, 6, 18, 19, 30), whereas measurement of pulmonary Rv and Ppc quantitates changes in intravascular hydrostatic pressure (1, 2, 6, 18, 19).

The Kᵣ of lungs of animals sustaining IR was four times greater than that of sham-operated controls (Fig. 2), a finding consistent with that reported by Carden et al. (3) in a similar model of IR injury. These data suggest that a change in pulmonary microvascular permeability to fluid is one mechanism in the pathogenesis of pulmonary edema in this model. This finding is consistent with the experience of other investigators utilizing analogous models in the rat and other animals (2, 28, 30).

IR injury had a profound effect on pulmonary Rv. Rt was 63% greater in the lungs of animals sustaining IR when compared with controls (P = 0.01; Table 1). This was primarily due to a fourfold greater Rv in the lungs of animals sustaining IR when compared with controls (P = 0.001). In contrast, there was no difference in the Ra between these two groups. The effect of this increase in Rv was a doubling of hydrostatic pressure in the capillaries of injured animals compared with controls, a change that may have exacerbated the movement of fluid from the intravascular space into the pulmonary interstitium (5). These findings are consistent with previously reported experience with this model (24) and other models of acute lung injury (12, 17, 29, 30).

The measurement of Ppc by using the double occlusion technique correlates very well with classical isogravimetric techniques in both normal (19) and injured (2) lungs. Baseline Ppc measurements in the experiments reported in this manuscript ranged from 2.83 ± 0.3 in sham-operated controls to 9.6 ± 1.0 in one group of animals sustaining IR. The measurements in the sham-operated animals appeared particularly low, and more recent experiments in which Ppc was measured in Sham and IR animals yielded values of 4.6 ± 0.5 and 10.8 ± 1.5 for these two groups, respectively (n = 7 per group; P = 0.002). Review of the literature demonstrates significant variability in the measurement of Ppc in normal (or control) animals with values ranging from 4.6 to 7.5 (1, 2, 19, 30). The paired nature of the observations reported in these experiments (i.e., Ppc was measured before and after treatment with either imidazole or SQ-29,548) reduces the impact of this variability on the interpretation of these data.

The inflammatory mediators responsible for this alteration in pulmonary microvascular function have not been definitively defined. Previous in vivo (10) and in vitro (24) studies have demonstrated that pulmonary TxA₂ release is increased during IR. The present study demonstrated a >100% increase in TxB₂ in the BAL of animals sustaining IR (P < 0.01), 50% greater TxB₂ concentration in the perfusate of IR lungs when compared with Sham (P = 0.05), and in paired experiments a 50% increase in TxB₂ in perfusate collected from the efferent side of isolated, perfused IR lungs when compared with that from the afferent side (P = 0.006). The generation of TxA₂ by the lung has been described previously after a variety of inflammatory injuries including pulmonary ischemia-reperfusion (29), aspiration (8), and skeletal muscle reperfusion (12). Thus the endogenous release of TxA₂ by the lung appears to be a common mechanism by which this organ responds to a heterogeneous group of local and remote injuries.

Recent studies in our laboratory have demonstrated that one source of pulmonary TxA₂ generation may be alveolar macrophages. Alveolar macrophages isolated from the BAL fluid from animals sustaining IR generated greater than twice as much TxB₂ as did those from sham-operated controls (preliminary data). Furthermore, this increase in TxA₂ release is due, at least in part, to an increase in the content of the synthetic enzyme thromboxane synthase within the lungs of animals sustaining IR (10).

The role of TxA₂ release in the pathogenesis of IR-induced pulmonary microvascular dysfunction is suggested by experiments in which the TxA₂ synthetase inhibitor imidazole or the TxA₂-receptor antagonist SQ-29,548 was added to the perfusate of the isolated perfused lung. In paired experiments, the addition of 500 µg/ml imidazole reduced the Kᵣ of lungs of injured animals by 40% when compared with baseline measurements (P = 0.03). Treatment with SQ-29,548 had similar effects on the microvascular permeability of lungs from animals sustaining IR. In these experiments, the addition of SQ-29,548 to the perfusate of an IR lung resulted in a 50% reduction in Kᵣ from pretreatment values (P < 0.05). Despite reducing BAL TxB₂ concentration to nearly the same degree as the 500 µg/ml dose (i.e., 63% reduction), the addition of 50 µg/ml of imidazole to the perfusate of the isolated perfused lung in paired experiments, the addition of 50 µg/ml of imidazole to the perfusate of the isolated perfused IR lung reduced Kᵣ by 25% when compared with baseline values, a reduction that approached statistical significance (P = 0.08). This possibly represents a type II error because the sample size was only five animals. The implications of these experiments are that TxA₂, at least in part, mediates the increase in microvascular permeability associated with IR and that this increase in permeability is due to an ongoing process that is at least partially reversible.

Potential mechanisms by which TxA₂ alters pulmonary microvascular permeability have been postulated by a variety of investigators. In other injury models, TxA₂ has been shown to directly increase microvascular...
permeability by altering the endothelial cell cytoskeleton with microfilament disassembly and widening of interendothelial tight junctions (27, 29). In an in vitro perfused lung model of pulmonary ischemia-reperfusion, endogenous pulmonary TxA2 release was associated with ultrastructural evidence of abnormal endothelial cell tight junctions, changes ameliorated by TxA2-receptor blockade (29).

The influence of local TxA2 release on pulmonary Rv and Ppc appears to be less than its influence on microvascular permeability. The addition of imidazole (500 µg/ml) or SQ-29,548 to the perfusate of the isolated perfused lung resulted in a modest reduction in total pulmonary Rv in the lungs of animals sustaining IR (23 and 17% for imidazole and SQ-29,548, respectively; P < 0.05). Treatment with these agents reduced IR-induced increases in Ra by 19% (P = 0.15) and 30% (P = 0.03), respectively, but had no apparent effect on Rv. It is interesting that neither of these agents significantly altered Rv, since it is at this site that TxA2 exerts its greatest effect of vascular tone. These data suggest that other paracrine mediators are likely to be involved in the regulation of pulmonary vascular tone in this model. A reduction in the constitutive release of nitric oxide or prostacyclin by the pulmonary endothelium would be expected to significantly increase pulmonary Rv. Furthermore, the generation of potent vasoconstrictors, such as endothelin, in response to the systemic inflammatory state would also contribute to the vasocostriction associated with this and similar injury models.

The complexity of injury-induced pulmonary microvascular dysfunction is suggested by the present study as well as that of other investigators utilizing a variety of injury models. Stephenson et al. (16) found that inhibition of thromboxane synthetase with OKY-046 or TxA2-receptor blockade with ONO-3708 attenuated but did not prevent phorbol myristate acetate (PMA)-induced pulmonary hypertension in vivo, results that were similar to that reported by Allison et al. (1) in a blood-perfused isolated lung model. In this latter study, PMA-induced changes in pulmonary microvascular permeability were dependent on the presence of cellular components of blood (perhaps platelets or leukocytes), whereas PMA-induced pulmonary vasoconstriction occurred in a cell-free perfusate (1). In this model, inhibition of TxA2 release prevented PMA-induced increases in Kf and resulted in a 78% reduction in pulmonary Rv (1). Other investigators have related endogenous pulmonary TxA2 release to increased Rv after pulmonary ischemia-reperfusion injury (29), skeletal muscle reperfusion injury (12), and oxidant exposure (17).

In conclusion, this study suggests that, during IR, pulmonary microvascular permeability and hydrostatic pressure are both significantly increased when compared with controls. Inhibition of TxA2 synthesis or blockade of TxA2 receptors reduces IR-induced changes in microvascular permeability with relatively minimal changes in pulmonary Rv or Ppc. The observation that in vitro inhibition of TxA2 synthesis or TxA2-receptor blockade reduces Kf suggests that the increase in pulmonary microvascular permeability associated with IR is due to the ongoing release of TxA2 and not due to an irreversible microvascular injury.


