Thromboxane Generation after Thrombin

Protective Effect of Thromboxane Synthetase Inhibition on Lung Fluid Balance


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SUMMARY. We examined the role of thromboxane in mediating the alterations in pulmonary hemodynamics and in lung fluid and protein exchange after thrombin. Studies were made in control sheep and in sheep pretreated with the thromboxane synthetase inhibitor, Dazoxiben (injection of 10 mg/kg followed by infusion of 4 mg/kg per hr). Thrombin infusion caused an increase in mixed venous and aortic concentrations of thromboxane B2, a stable degradation product of thromboxane A2, whereas the concentrations of 6-keto-PGF1α, a degradation product of prostacyclin, did not change significantly. In sheep pretreated with Dazoxiben, thromboxane B2 concentrations did not increase, indicating effectiveness of the thromboxane synthetase inhibitor. The blood concentrations of 6-keto-PGF1α after thrombin increased in the thromboxane synthetase-inhibited group, indicating shunting towards prostacyclin synthesis. Thrombin in untreated sheep increased pulmonary lymph flow (Qtym) and the lymph protein clearance (Qtym × lymph-to-plasma protein concentration ratio). The increases in lymph parameters were due to an increase in pulmonary vascular permeability to proteins because raising left atrial pressure further increased Qtym but did not change lymph-to-plasma ratio. Dazoxiben prevented the thrombin-induced increase in pulmonary vascular permeability because the increase in left atrial pressure resulted in an increase in Qtym and a decrease in lymph-to-plasma ratio, as was the case after left atrial hypertension in normal animals. Therefore, thrombin results in selective release of thromboxane A2 which precedes the increase in pulmonary vascular permeability. Thromboxane A2 may contribute to the increased permeability after thrombin, since inhibition of thromboxane synthesis prevents the permeability change. (Circ Res 53: 214-222, 1983)

THROMBIN-INDUCED pulmonary microembolism results in an increase in lung vascular permeability (Johnson et al., 1982; Malik et al., 1982; Tahamont and Malik, 1983). The increased permeability is dependent on the presence of the circulating granulocytes because it was preventable by granulocyte depletion with hydroxyurea (Malik et al., 1982; Tahamont and Malik, 1982). Platelet depletion with anti-platelet serum did not have the same protective effective, however (Malik et al., 1982; Tahamont and Malik, 1983).

Prostaglandins of the E and F series (Hyman et al., 1978), as well as thromboxane A2 (TXA2) and prostacyclin (PGI2) (Utsunomiya et al., 1982), are released after pulmonary microembolism induced by injecting preformed blood clots. The effect of thrombin on generation of TXA2 and PGI2, both potent vasoactive substances in the lung (Moncada and Vane, 1979), has not been examined. TXA2 and PGI2 may participate in mediating the pulmonary arterial pressure and pulmonary vascular resistance changes that occur after administration of thrombin (Johnson et al., 1982; Malik et al., 1982). Also, it is known that TXA2 promotes leukocyte margination and adherence (O'Flaherty et al. 1979; Spagnuolo et al., 1980), whereas PGI2 has opposite effects (Boyer et al., 1980; Jacobs et al., 1982), suggesting that TXA2 and PGI2 play a role in increasing lung vascular permeability.

The purpose of this study was to examine the release of endogenously formed thromboxane and prostacyclin after thrombin-induced pulmonary microembolism in sheep and to correlate any measured changes in concentrations of these eicosanoids with alterations in lung transvascular fluid and protein exchange. Since thromboxane may be involved in mediating the pulmonary hemodynamic effects of thrombin and the leukocyte-dependent increase in permeability, we also examined the effect of the selective thromboxane inhibitor, Dazoxiben (UK-37,248) (4-[2-(1H-imidazol-1-yl)ethoxy] benzoic acid hydrochloride) (Tyler et al., 1981).

Methods

Experiments were made in nonheparinized sheep weighing 19–25 kg. All animals received only water for 24 hours before the experiment. Anesthesia was induced by a single intravenous injection of thiopental sodium (25 mg/kg body weight). After endotracheal intubation, anesthesia was maintained with a mixture of 1% halothane in...
oxygen and nitrous oxide delivered by a Harvard respirator.

The caudal mediastinal node and its efferent duct were isolated through a right thoracotomy for collection of pulmonary lymph (Staub et al., 1975; Malik and van der Zee, 1978). The node was resected just caudal to the distal margin of the pulmonary ligament to minimize systemic contamination. All identifiable diaphragmatic, esophageal, and chest wall afferent lymphatics were cauterized (Roos et al., 1981). The efferent duct was cannulated with a heparin-coated Silastic catheter (C.R. Bard) and secured in position in the chest. Blood-free pulmonary lymph was successfully obtained in all sheep. Polyethylene catheters (10F) were placed in the aorta and jugular vein, and a 7F thermistor-tipped Swan-Ganz catheter was positioned in the right pulmonary artery. Another 10F catheter was positioned in the left atrium through a left thoracotomy. Aortic, pulmonary arterial, and left atrial pressures were monitored continuously using Statham P23Db pressure transducers referred to the left atrial appendage. The pressures were recorded on a Grass 7B polygraph. Pulmonary perfusion pressure was calculated as the difference between the mean pulmonary arterial and left atrial pressures. Pulmonary blood flow was determined in triplicate by the thermodilution technique (Edwards Laboratories, model 9520).

The pulmonary lymph and plasma samples were collected simultaneously at 15-minute intervals, with the sheep in the prone position. The steady state lymph flow was defined by at least four flow values differing by less than 0.25 ml/hour. Lymph and protein concentrations were determined by the biuret technique on an Autoanalyzer (Technicon Instruments).

Arterial counts of leukocytes and platelets were measured during baseline, at 15 minutes postembolization, and then at 30-minute intervals. The leukocyte and platelet counts were determined after staining with new methylene blue (Bjorkman, 1959).

Blood (arterial and mixed venous) and lymph samples were carefully collected and placed into tubes containing 0.05 ml of 15% EDTA and 24 µg/ml of indomethacin (whole blood) final concentration. Samples were centrifuged at 1000 g for 10 minutes at 5°C. Plasma and lymph samples were placed into polypropylene test tubes and stored at —70°C. Measurements of thromboxane B2 (TXB2), the stable metabolite of TxA2, and 6-keto prostaglandin F1α (6-keto-PGF1α), a circulating stable metabolite of PGI2, were made by double-antibody radioimmunoassay (Hales et al., 1981). Standards for TXB2 and 6-keto-PGF1α were provided by Dr. John Pike, Upjohn Co. Antisera were purchased from Immunalysis. Other serological reagents were purchased from Clinical Assays. Radioactivity was determined with a model 3320 Packard Tri-Carb liquid scintillation counter. Cross-reactivities of these antibodies have been reported previously (Hales et al., 1981).

Studies were made in three groups:

**Group I Control Thrombin (n = 5)**

The sheep received 55.0 ± 12.9 U/kg of human α-thrombin (supplied by Dr. John Fenton, New York State Department of Health, Albany, N.Y.) (Fenton, 1981) infused over 15 minutes. The amount of thrombin was selected to produce a 3-fold increase in pulmonary vascular resistance (PVR). The sheep were studied for 3 hours after the thrombin infusion. In three sheep of this group, a left atrial balloon catheter (Foley 12F) was positioned in the left atrium via the appendage, the purpose of which was to increase the left atrial pressure from 6.0 ± 1.0 mm Hg to 16.7 ± 2.4 mm Hg following the steady state increase in pulmonary lymph flow (Qlym). Lung vascular permeability was assessed determining the changes in Qlym and lymph-to-plasma protein concentration ratio (L:P) after left atrial hypertension (Taylor et al., 1981).

**Group II: Dazoxiben followed by Thrombin (n = 11)**

After the baseline steady state Qlym, sheep were treated with Dazoxiben (UK-37,248), (4-[2(1H-imidazol-1-yl)ethoxy] benzoic acid hydrochloride) (Pfizer Labs). Dazoxiben was dissolved in normal saline and sodium bicarbonate solution and was then injected at a dosage of 10 mg/kg, followed immediately by infusion of 4 mg/kg per hr for the duration of the study. The sheep received...
TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 5)</th>
<th>Thrombin†</th>
<th>Dazoxiben (n = 11)</th>
<th>Dazoxiben thrombin†</th>
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</thead>
<tbody>
<tr>
<td>Pulmonary lymph flow (ml/hr)</td>
<td>3.48 ± 0.73</td>
<td>8.84 ± 0.98*</td>
<td>4.65 ± 1.05</td>
<td>4.66 ± 0.80</td>
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<td>Lymph:plasma protein concentration ratio</td>
<td>0.67 ± 0.03</td>
<td>0.79 ± 0.06</td>
<td>0.77 ± 0.04</td>
<td>0.78 ± 0.04</td>
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<tr>
<td>Transvascular protein clearance (ml/hr)</td>
<td>2.26 ± 0.42</td>
<td>6.90 ± 0.87*</td>
<td>3.85 ± 0.70</td>
<td>3.43 ± 0.47</td>
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<tr>
<td>Pulmonary arterial pressure (mm Hg)</td>
<td>14.60 ± 1.47</td>
<td>23.20 ± 1.24*</td>
<td>16.55 ± 1.58</td>
<td>14.50 ± 0.95</td>
</tr>
<tr>
<td>Left atrial pressure (mm Hg)</td>
<td>4.00 ± 1.10</td>
<td>5.21 ± 0.73</td>
<td>6.0 ± 1.06</td>
<td>5.00 ± 0.84</td>
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<tr>
<td>Pulmonary vascular resistance (mm Hg/liter per min)</td>
<td>4.94 ± 0.65</td>
<td>9.30 ± 0.89*</td>
<td>7.18 ± 0.81</td>
<td>6.30 ± 0.7</td>
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<td>Pulmonary blood flow (liters/min)</td>
<td>2.27 ± 0.25</td>
<td>2.02 ± 0.24</td>
<td>1.55 ± 0.16</td>
<td>1.58 ± 0.12</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± 1 SEM.
* Different from Baseline (P < 0.05).
† Different from Dazoxiben Baseline (P < 0.05).
‡ Values at 3 hours after thrombin infusion.

thrombin (82.2 ± 2.8 U/kg) within 15 minutes to triple PVR, as in group I. In four sheep of this group, left atrial pressure was increased from 5.3 ± 0.7 mm Hg to 15.6 ± 1.0 mm Hg after the thrombin steady state Q_{lym} to assess the effect of Dazoxiben on the increase in lung vascular permeability (Taylor et al., 1981).

Group III: Thrombin Same Dosage as Group II (n = 5)

Since tripling the PVR necessitated a greater amount of thrombin in group II (82.2 ± 2.8 U/kg) than in group I (55.0 ± 12.9), in group III we infused 85.7 ± 5.9 U/kg to compare the response with that obtained in group II.
The significance of changes at time intervals from baseline was assessed by the two-way analysis of variance. The significance of steady state changes from baseline was tested by the paired t-test. Differences between the control-thrombin and the Dazoxiben-thrombin groups at time before and after thrombin infusion were assessed with the unpaired Student's t-test. Difference between slopes of lines was assessed by the analysis of covariance.

Results

Effects of Thrombin

Thrombin infusion (55.0 ± 12.9 U/kg) tripled pulmonary vascular resistance (PVR) from 4.95 ± 0.65 mm Hg/liters per min to 14.3 ± 3.64 mm Hg/liters per min within 15 minutes after the end of the thrombin infusion (Fig. 1). Thereafter, PVR declined steadily toward baseline, although remaining elevated above baseline at the end of the 3-hour study period (Fig. 1; Table 1). The mean pulmonary arterial pressure (Ppa) also increased immediately after thrombin infusion and, like the PVR, declined steadily during the study (Fig. 1). The mean left atrial pressure (Pia) and pulmonary blood flow (QL) did not change significantly from baseline after thrombin infusion (Fig. 1; Table 1).

The steady state baseline and post-thrombin lymph data are indicated in Table 1, and the time course of the changes in pulmonary lymph flow (Qlym), lymph-to-plasma protein concentration (L/P) ratio, and transvascular protein clearance are shown as absolute values in Figure 2a and as experimental/baseline values in Figure 2b. Qlym increased after thrombin infusion and the increase became significant within 30 minutes post-thrombin (Fig. 2); Qlym remained elevated for the duration of the study (Fig. 2). The increase in Qlym was associated with a gradual increase in L/P ratio, which was significantly elevated from baseline at 135 and 150 minutes post-thrombin (Fig. 2). Transvascular protein clearance (Qlym × L/P) increased significantly at 30 minutes post-thrombin and remained elevated during the study (Fig. 2). Platelet and leukocyte counts decreased within 30 minutes of thrombin infusion, and both values remained depressed for the study (Fig. 3).

Inflation of the left atrial balloon to increase Pia resulted in an increase in Qlym without a significant change in L/P (Fig. 4). On the other hand, increasing
P^ in normal lungs was associated with an increase in Qiym and a consistent decrease in L:P (Minnear et al., 1981) (Fig. 4).

Effects of Dazoxiben

Dazoxiben itself did not significantly affect Qiym, L:P, P^, P^, Q^, or PVR (Table 1). A greater amount of thrombin (82.2 ± 2.8 U/kg) was required after Dazoxiben to produce the 3-fold increase in PVR that occurred in control sheep (Fig. 1). Unlike the control animals, the increase in PVR was sustained for the duration of the study. The initial increase in P^ after thrombin was less than in controls, despite the increased thrombin dosage in the Dazoxiben group; moreover, P^ remained elevated during the study, unlike the decrease occurring in control animals (Fig. 1). P^ and Q^ did not change from baseline after thrombin in the Dazoxiben-treated group, and the values were not significantly different from those in the control-thrombin group.

Qiym increased gradually after thrombin in the Dazoxiben group, but the increase was much less than observed in the control group (Fig. 2, a and b). The increase in Qiym was significant only at 90 minutes after thrombin (Fig. 2b). Transvascular protein clearance did not change within 30 minutes post-thrombin, in contrast to the rapid increase at 30 minutes post-thrombin in the control group (Fig. 2, a and b). L:P did not change significantly from baseline after thrombin in the Dazoxiben-treated group (Fig. 2, a and b). The normalized L:P ratio values at 105, 120, 135, and 150 minutes post-thrombin in the Dazoxiben-treated group were less than in control animals at the same times (Fig. 2b). Transvascular protein clearance did not change from baseline after thrombin in Dazoxiben-treated sheep in contrast to the rapid increase occurring within 30 minutes post-thrombin in the control group (Fig. 2, a and b). The normalized clearance values in Dazoxiben-treated sheep were significantly different from the control group at all times after thrombin (Fig. 2b).

The platelet count decreased at 30 minutes after thrombin and remained depressed during the study, whereas the leukocyte count did not decrease significantly from baseline (Fig. 3). The leukocyte count was increased in the Dazoxiben-treated group from baseline at 60 minutes after thrombin and remained elevated for the duration of the study.

The increase in left atrial pressure following thrombin in the Dazoxiben-treated group produced an increase in Qiym and a concomitant decrease in L:P ratio (Fig. 4). The slope of the Qiym vs. L:P ratio line was similar to that obtained in normal lungs after raising left atrial pressure (Minnear et al., 1981) (Fig. 4).

Effects of High-Dose Thrombin

Table 2 indicates the effects of infusion of thrombin at a dosage of 85.7 ± 5.9 U/kg (group III), which matched the dosage used in the Dazoxiben-pre-treated group. The increases in P^ and PVR at 30 minutes after thrombin in the high-dosage thrombin group were greater than the 30-minute values of 20.01 ± 1.70 mm Hg and 10.26 ± 1.60 mm Hg/liter per min in the Dazoxiben group (Table 2; Fig. 1). The increases in P^ and PVR at 3 hours after thrombin, however, were similar in both groups receiving the same thrombin dosage (Tables 1 and 2).

Qiym increased within 30 minutes after thrombin in the high-dosage thrombin group, and remained elevated for the duration of the study (Table 2). L:P ratio was not increased at 30 minutes after thrombin, but was increased at 3 hours (Table 2). Transvascular protein clearance increased within 30 minutes after thrombin and remained elevated for the 3-hour duration of the study. The increases in Qiym, L:P, and transvascular protein clearance in the high-dosage thrombin group were greater than in Dazoxiben group receiving the same amount of thrombin (Tables 1 and 2; Fig. 2a).

Effects on Thromboxane B2 (TxB2) and 6-Keto Prostaglandin F1α (6-Keto PGF1α) Concentrations

TxB2 concentrations in aortic (arterial) and pulmonary arterial (mixed venous) blood increased within 5 minutes after the end of the thrombin infusion (Fig. 5). Significant elevations were measured for up to 20 minutes after the end of thrombin infusion in both arterial and venous blood. The

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>30-min thrombin</th>
<th>3-hr thrombin</th>
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</thead>
<tbody>
<tr>
<td>Pulmonary lymph flow (ml/hr)</td>
<td>3.81 ± 1.04</td>
<td>13.39 ± 2.87*</td>
<td>13.36 ± 3.49*</td>
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<tr>
<td>Lymph:plasma protein concentration ratio</td>
<td>0.74 ± 0.07</td>
<td>0.74 ± 0.06</td>
<td>0.81 ± 0.04</td>
</tr>
<tr>
<td>Transvascular protein clearance (ml/hr)</td>
<td>3.03 ± 1.12</td>
<td>10.00 ± 2.37*</td>
<td>10.92 ± 2.85*</td>
</tr>
<tr>
<td>Pulmonary arterial pressure (mm Hg)</td>
<td>18.00 ± 1.61</td>
<td>28.8 ± 1.46*</td>
<td>24.01 ± 3.27*</td>
</tr>
<tr>
<td>Left atrial pressure (mm Hg)</td>
<td>6.20 ± 1.32</td>
<td>10.00 ± 0.84*</td>
<td>6.60 ± 1.44</td>
</tr>
<tr>
<td>Pulmonary vascular resistance (mm Hg/liter per min)</td>
<td>5.51 ± 0.62</td>
<td>13.18 ± 1.27*</td>
<td>15.42 ± 4.57*</td>
</tr>
<tr>
<td>Pulmonary blood flow (liters/min)</td>
<td>2.22 ± 0.27</td>
<td>1.76 ± 0.36</td>
<td>1.20 ± 0.21*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± 1 SEM. * Different from baseline (P < 0.05).
increase in arterial concentration of TxB₂ at 5 minutes was greater than the increase in mixed venous concentration (Fig. 5). Arterial and mixed venous TxB₂ concentrations gradually fell to control values by 120 minutes (Fig. 5). The initial increases in TxB₂ concentrations were coincident with the development of pulmonary hypertension (Fig. 1) and slightly preceded maximum changes in QLV (Fig. 2).

In contrast to increases in blood TxB₂ concentration, there was no significant change in lymph TxB₂ from baseline; however, there was an increasing trend (zero time 2422 pg/ml vs. maximum concentration 3325 pg/ml) (Fig. 5). The lymph TxB₂ concentration at 140 minutes was less than the baseline value (Fig. 5), but the lymph TxB₂ concentration remained above blood values, consistent with previous observations (Watkins et al., 1982).

In contrast in the untreated group, animals treated with Dazoxiben had lower baseline TxB₂ values in blood and lymph than the control group, and these values did not increase significantly from baseline in either blood or lymph (Fig. 5).

In the control-thrombin animals, venous and arterial 6-keto-PGF₁α concentrations did not change significantly from baseline after thrombin at any time point (Fig. 6). However, the lymph 6-keto-PGF₁α concentrations in control-thrombin sheep at 10 and 15 minutes after the end of thrombin infusion were significantly increased (Fig. 6). As with TxB₂, the lymph 6-keto-PGF₁α values were greater than the blood values, as has been previously reported (Watkins et al., 1982).

In Dazoxiben-treated animals, the arterial and venous 6-keto-PGF₁α concentrations were increased significantly at 5 and 10 minutes after the end of thrombin infusion, and both values remained elevated at more than twice the baseline values for up
to 35 minutes (Fig. 6). The increase in arterial 6-keto-PGF1α concentration from baseline at 5 minutes after thrombin in the Dazoxiben group was greater than the increase in venous 6-keto-PGF1α concentration. There was also an upward trend in the lymph 6-keto-PGF1α concentrations at these times, although this increase was not significant (Fig. 6).

The venous, arterial, and lymph 6-keto-PGF1α/TxB2 ratios in the control group after thrombin are shown in Table 3. There were decreases in the venous and arterial ratios after thrombin, although only the 5-minute post-thrombin arterial ratio was significantly less than the baseline value (P < 0.05). The decrease in the arterial ratio at 5 minutes was the result of greater generation of TxB2 in arterial blood (Fig. 5). The lymph 6-keto-PGF1α/TxB2 ratio increased to any extent only at 10 minutes after thrombin (P < 0.07) reflecting the greater generation of 6-keto-PGF1α than TxB2 in lymph at this time (Figs. 5 and 6).

### Discussion

In the present study, we have examined the effects of thrombin-induced pulmonary microembolism on lung fluid and protein exchange and the role of thromboxane A2 (TXA2) and prostacyclin (PGI2) in mediating the response. We observed that thrombin resulted in an increase in pulmonary lymph flow (Qlym), and an increase in the lymph-to-plasma protein concentration ratio (L:P). These results indicate that pulmonary microvascular permeability to protein increases after thrombin-induced pulmonary microembolization. A similar observation has been made using other methods of inducing embolism, including air (Ohkuda et al., 1978), glass beads (van der Zee and Malik, 1978), and bone marrow (Barie and Malik, 1982).

In animals pretreated with the thromboxane synthetase inhibitor, Dazoxiben (Tyler et al., 1981), the increase in Qlym after thrombin was greatly attenuated and the increase in transvascular protein clearance (Qlym × L:P) was altogether prevented, suggesting that thromboxane synthetase inhibition prevented the increase in permeability. When pulmonary vascular permeability was assessed in the Dazoxiben-pretreated animals by producing a small rise in left atrial pressure with a left atrial balloon, the further increase in Qlym was associated with a decrease in L:P, whereas the increase in Qlym in the control-thrombin group was associated with an unchanged L:P. These results support the notion that thromboxane synthetase inhibition prevented the increase in permeability.

TXA2 was released as a result of thrombin because the arterial and mixed venous plasma concentrations of TxB2, a degradation product of TXA2 (Moncada and Vane, 1979), increased within 5 minutes of thrombin infusion. The vasculature appeared to be the origin of TxB2 because the increase in arterial concentration occurred immediately after the thrombin and was greater than the increase in mixed venous concentration. The lymph TxB2 concentration also increased after thrombin, but this change was not significant because the baseline lymph TxB2 concentration was elevated, possibly as a result of manipulation of the lungs during surgery. The finding that the lymph TxB2 concentration at 120 minutes after thrombin was lower than the baseline value supports this idea.

Dazoxiben was effective in inhibiting thromboxane production, since it prevented the thrombin-induced increases in TxB2 in plasma, as well as in lymph. Dazoxiben is an imidazole derivative, and it is known to selectively inhibit thromboxane synthetase (Tyler et al., 1981). Production of TXA2 from isolated-perfused rabbit lungs is abolished, and there is a 144% increase in PGI2 output (Pfizer Investigators Reference Manual).

The arterial and venous concentrations of 6-keto prostaglandin F1α (6-keto-PGF1α), a degradation product of PGI2 (Moncada and Vane, 1979), after thrombin were not elevated coincidentally with the increases in TxB2 after thrombin; therefore, there was a significant decrease in arterial 6-keto-PGF1α/TxB2 ratio immediately after thrombin in the control group. However, the lymph 6-keto-PGF1α concentration increased after thrombin in the control group, suggesting that the lung tissue rather than the blood was the site of its origin (Hsueh, 1979). Previous experiments using cultured endothelial cells have indicated that PGI2 is liberated when cultured cells are exposed to thrombin (Weksler et al., 1978). The reason for the lack of increase in plasma 6-keto-PGF1α concentrations in the intact sheep is not clear. It may reflect (1) release in immeasurably small quantities in the pulmonary circulation or (2) rapid enzymatic degradation of 6-keto-PGF1α to 6-keto-PGE1.

In animals pretreated with Dazoxiben, both the arterial and venous plasma concentrations of 6-keto PGF1α increased immediately after thrombin, suggesting shunting toward the PGI2 pathway (Moncada and Vane, 1979). Another possibility is that TXA2 is a feedback inhibitor of PGI2, and that the PGI2 concentration increases when the inhibitor is removed by thromboxane synthetase inhibition. The lung vasculature appeared to be the origin of 6-keto-

### Table 3

<table>
<thead>
<tr>
<th>Time after thrombin (min)</th>
<th>Venous 6-keto-PGF1α/TxB2</th>
<th>Arterial 6-keto-PGF1α/TxB2</th>
<th>Lymph 6-keto-PGF1α/TxB2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1.02 ± 0.41</td>
<td>0.92 ± 0.23</td>
<td>1.79 ± 0.63</td>
</tr>
<tr>
<td>5</td>
<td>0.57 ± 0.21</td>
<td>0.33 ± 0.07</td>
<td>2.58 ± 0.88</td>
</tr>
<tr>
<td>10</td>
<td>0.66 ± 0.28</td>
<td>0.46 ± 0.13</td>
<td>3.25 ± 1.43</td>
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<td>15</td>
<td>0.63 ± 0.17</td>
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<td>20</td>
<td>0.70 ± 0.24</td>
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<td>120</td>
<td>1.42 ± 0.36</td>
<td>1.01 ± 0.29</td>
<td>2.12 ± 0.63</td>
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</table>

Results are expressed as mean ± 1 SEM.
PGF$_{2\alpha}$, since the 250% increase in arterial concentration at 5 minutes after the end of thrombin infusion was greater than the 100% increase in mixed venous concentration; however, this does not rule out peripheral sites in contributing to the increase in 6-keto-PGF$_{1\alpha}$ concentrations.

In other models of lung injury, such as endotoxin infusion (Hales et al., 1981) and veno-veno extracorporeal bypass (Peterson et al., 1982), the initial increases in the plasma concentrations of TxB$_2$ were followed by increases in 6-keto-PGF$_{1\alpha}$ concentration, which have been interpreted as cause and effect (Hales et al., 1981; Peterson et al., 1982). However, in the present study, the plasma TxB$_2$ concentration increased after thrombin infusion, whereas there was no increase in 6-keto-PGF$_{1\alpha}$ concentration, indicating a dissociation between TxA$_2$ and PGI$_2$ production in this model. The findings that the plasma 6-keto-PGF$_{1\alpha}$/TxB$_2$ ratio decreased in association with the increase in lung vascular permeability and that thromboxane synthetase inhibition prevented the increase in permeability suggest an important role for TxA$_2$ in mediating thrombin-induced lung vascular injury.

Granulocytes play a central role in increasing lung vascular permeability after pulmonary embolism induced by air (Ohkuda et al., 1978), glass bead (Malik et al., 1982), bone marrow (Barie and Malik, 1982), as well as thrombin (Tahamont and Malik, 1983). TxA$_2$ may contribute to the leukocyte-dependent lung vascular injury because TxA$_2$ promotes leukocyte aggregation and adherence (Spagnuolo et al., 1980). In the present study, we observed an immediate decrease in the leukocyte count in control animals after thrombin, but no change in the thromboxane synthetase-inhibited group, which is consistent with the hypothesis that TxA$_2$ contributes to leukocyte sequestration. The increase in leukocyte count above baseline in the Dazoxiben-treated group may be due to inhibition of TxA$_2$ production in this group. The lack of TxA$_2$ may have prevented the leukocyte aggregation and adherence, and thus resulted in high circulating levels.

If TxA$_2$ is an important mediator of the increase in lung vascular permeability after thrombin, then the question arises why infusion of U46619 (a stable endoperoxide analog) or arachidonic acid do not increase permeability in normal lungs (Ogletree, 1982). The role of TxA$_2$ may be to amplify the response by prolonging leukocyte sequestration in pulmonary vessels, rather than being the initiator of the sequestration. Other mechanisms, such as fibrinolytic pathway activation and complement activation, may also be required for the initial leukocyte aggregation (Kaplan et al., 1982; Malik et al., 1982).

The platelet count decreased comparably in both groups. The decrease in platelet count after thrombin, even in the absence of TxA$_2$ generation, indicates that release of other factors after thrombin such as platelet-activating factor (Hanahan et al., 1980) and leukotrienes (Rosenblum and Ellis, 1982) are responsible for platelet aggregation. Since the platelet count decreased similarly and rapidly after thrombin in both control and thromboxane synthetase-inhibited groups, it is doubtful that platelet aggregation per se is responsible for the increase in the lung vascular permeability. This agrees with our previous observation that platelet depletion induced by anti-platelet serum failed to prevent the increase in permeability following thrombin (Malik et al., 1982; Tahamont and Malik, 1983).

Another reason for the protective effect of thromboxane synthetase inhibition is the compensatory increase in the 6-keto-PGF$_{1\alpha}$ concentration that occurred after thrombin in the Dazoxiben-pretreated animals. An increase in PGI$_2$ could prevent the increase in lung vascular permeability by disaggregating leukocytes (Boxer et al., 1980) lodged in the pulmonary microcirculation.

PVR was increased to comparable levels in control and thromboxane synthesis-inhibited groups to allow comparison of lung fluid balance between the two groups. Since it was necessary to inject 82 U/kg thrombin in the Dazoxiben group vs. 55 U/kg required in the control group, a part of the initial increase in PVR may be the result of the pulmonary vasoconstrictor effect of TxA$_2$ (Ogletree, 1982). This was confirmed in a third group which received the same amount of thrombin (87 U/kg) as the Dazoxiben-pretreated group. The increases in both P$_{aw}$ and PVR at 30 minutes after thrombin were greater than in the Dazoxiben-treated group, supporting the notion that the observed release of TxA$_2$ immediately after thrombin mediates pulmonary vasoconstriction and contributes to the increase in PVR.

In summary, pulmonary microembolism induced with thrombin increased lung vascular permeability to proteins. The increase in permeability was associated with intravascular generation of TxA$_2$ but not PGI$_2$. Selective inhibition of TxA$_2$ synthesis prevented the thrombin-induced increase in transvascular protein clearance and the thrombin-induced leukopenia. We postulate that TxA$_2$ generation plays an important role in increasing lung vascular permeability after thrombin.

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