Effects of a Thromboxane A2 Analogue and Prostacyclin on Lung Fluid Balance in Newborn Lambs

Kazuhiko Yoshimura, Mary L. Tod, Kristi G. Pier, and Lewis J. Rubin

We have previously shown that the pulmonary venoconstriction produced by a stable thromboxane A2 analogue (STA2) is attenuated by prostacyclin (PGI2), but PGI2 increases the STA2-induced edema. The present study was designed to determine the effects of STA2 and PGI2 on the fluid balance in isolated blood-perfused newborn lamb lungs. Vascular permeability was evaluated by use of the fluid filtration coefficient (Kf) and the osmotic reflection coefficient for total proteins (σ, hematocrit-protein double indicator technique), and pulmonary capillary pressure (Pc) was estimated by the double occlusion technique. All lungs had a period of hydrostatic stress induced by elevation of the left atrial pressure from 5 to 20 mm Hg to promote fluid filtration, and the rate of lung weight gain (ΔW/ΔT) during this period was determined. Studies were made in four groups; before the hydrostatic stress, lungs were given 1) STA2 (50 μg, n=6), 2) PGI2 (0.4 μg/kg/min, n=6), 3) both PGI2 and STA2 (n=6), or 4) vehicles (control, n=5). Measurements of Kf were made at the baseline period and after the hydrostatic stress. Kf was significantly increased by 76% with STA2, by 121% with PGI2, and by 157% with both PGI2 and STA2, but remained constant in controls. In comparison with control lungs, a similar ΔW/ΔT was observed with less of an increase in Pc during the hydrostatic stress in the STA2 group, and greater values of ΔW/ΔT were obtained with smaller elevations in Pc in the groups receiving PGI2 or both PGI2 and STA2. The σ of 0.66±0.07 in the control group was the highest in these experiments. Treatments with STA2 and/or PGI2 significantly decreased σ. These results suggest that both STA2 and PGI2 may increase pulmonary microvascular permeability to protein. Furthermore, PGI2 may increase fluid filtration by increasing vascular surface area.

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Thromboxane A2 (TxA2) is a potent pulmonary vasoconstrictor that may be involved in the early phase of endotoxin-induced pulmonary hypertension and edema. This pulmonary hypertensive response has been suggested to be the result of TxA2-mediated pulmonary venoconstriction since the protein-poor lung lymph flow is increased in this early phase.1,2 By use of the arterial and venous occlusion technique in isolated newborn lamb lungs, we have recently demonstrated that a stable TxA2 analogue, 9,11-epithio-11,12-methano-TxA2 (STA2), produced pulmonary hypertension primarily by causing pulmonary venoconstriction.3 Although exogenous prostacyclin (PGI2) attenuated this response to STA2 by decreasing the pulmonary venoconstriction, PGI2 administration significantly increased the STA2-induced pulmonary edema.3 These observations were interpreted as being consistent with an increase in microvascular permeability by PGI2 that potentiated the STA2-induced pulmonary edema, despite the attenuation of the elevation of the pulmonary microvascular pressure by PGI2. However, the possibility that PGI2 increased lung vascular surface area rather than permeability was not completely excluded in this study because vascular permeability was not directly evaluated.

Pulmonary microvascular membrane permeability may be estimated by changes in filtration coefficient (Kf) in isolated perfused lungs.4 However, when changes in the number of perfused microvessels occur, as with microvascular embolism, vasodilation, or vasoconstriction, Kf may not be a reliable parameter of changes in permeability because Kf depends on membrane surface area.5 In
contrast, the osmotic reflection coefficient is a measure of membrane permeability to proteins that is independent of vascular surface area. The purpose of this study was investigation of the effects of STA2 and PGI2 on transvascular fluid and protein exchange in isolated newborn lamb lungs by use of both the fluid filtration and the osmotic reflection coefficients as indicators of microvascular permeability change.

Materials and Methods

Isolated Lung Preparation

Our method of isolated lung preparation has been previously described. Briefly, 23 newborn lambs of either sex, 0–4 days of age and weighing between 2.1 and 5.5 kg, were anesthetized with ketamine hydrochloride (50 mg/kg i.m.), and a catheter was placed in a femoral artery. After administration of heparin (3,000 units), the animals were exsanguinated through the catheter. A tracheotomy was performed and the pulmonary artery and left atrium were cannulated. The lungs were rapidly and carefully removed and weighed. After the lungs were placed on a weighing instrument suspended from a strain-gauge force transducer (model FT 10, Grass Instruments, Quincy, Massachusetts), the cannulas were connected to an extracorporeal perfusion system consisting of a reservoir that could be adjusted to set outflow pressure at any level, a roller pump (Varistaltic S-series, Manostat, New York) for constant flow, a heat exchanger (Pediatric Miniprime, Baxter Healthcare, Deerfield, Illinois), a blood filter, an electromagnetic flow probe (model EP 300 A 1/4, Carolina Medical Electronics, King, North Carolina), and a manually actuated bypass valve that allowed for the rapid diversion of flow from the lungs directly back to the reservoir. The lungs were perfused with heparinized autologous blood at a rate of 10–12 breaths/min, and an end-expiratory pressure of 3 mm Hg, and were periodically hyperinflated for prevention of atelectasis.

Mean pulmonary arterial (Ppa), mean left atrial (Pla), and tracheal pressures were measured with pressure transducers (Statham models P10EZ and P23ID; Spectramed, Oxnard, California). The zero reference for all transducers was the top of the lungs, and the outflow pressure was adjusted to 5 mm Hg so that all lungs remained in zone 3 conditions. The flow rate of the perfused blood was measured with an electromagnetic flowmeter (model FM 501D, Carolina Medical Electronics). Lung wet weight and the pressures were continuously recorded on a physiological recorder (model 7D, Grass Instruments).

Estimation of Pulmonary Capillary Pressure

Pulmonary capillary pressure (Pc) was estimated by the double occlusion technique, as previously described. The respirator was turned off at end-expiration before occlusion. Arterial inflow and venous outflow occlusions were performed in duplicate by simultaneous diversion of inflow from the lungs to the reservoir by means of the bypass valve and clamping of the outflow tubing, respectively. The time courses of Ppa and Pla were monitored on an X-Y-Y recorder (model 50,000 S, Gould, Cleveland, Ohio). During the occlusion period, Ppa and Pla rapidly equilibrated to the same or nearly the same pressure. The Pc was taken as the equilibrium pressure at 5 seconds after occlusion. However, in the cases of vasoconstriction induced by STA2, Ppa did not equilibrate with Pla after occlusion. Thus, in these conditions Pc was determined as the equilibrium pressure for Pla at 5 seconds after occlusion.

Determination of Pulmonary Capillary Filtration Coefficient

The capillary Kf was measured as previously described. After achievement of isogravimetric conditions, Pla was rapidly raised by 5–8 mm Hg by elevation of the reservoir for 7 minutes. The Pc was measured immediately before the increase in Pla and at the end of the 7-minute elevation of Pla to obtain the difference. The rapid lung weight increase during the first 2 minutes after Pla elevation corresponds to a vascular volume change, and the slower component represents transvascular fluid filtration. The rate of lung weight increase was measured for each minute after elevation of Pla and was expressed as a semilogarithmic function over time. The slower component of weight increase was extrapolated to time 0 for estimation of the fluid filtration rate. This value was then divided by the change in Pc for calculation of Kf.

The Kf was expressed in milliliters per minute per mm Hg per 100 g wet lung weight.
Estimation of Osmotic Reflection Coefficient

For estimation of the osmotic reflection coefficient ($\sigma$), red blood cells and the plasma proteins were considered as endogenous nondiffusible and diffusible indicators, respectively. The relative increases in hematocrit and plasma protein concentration resulting from movement of fluid from the vascular to the extravascular space can be calculated from the equation

$$\sigma = 1 - \frac{C_2}{C^*} \left[ 1 - \frac{(1 - H_2)}{(1 - H_1)} \right] \left[ 1 - \frac{(C_1/C_2)}{(1 - H_2/H_1)} \right]$$

where $C_1$, $C_2$, and $C^*$ are the initial, final, and mean protein concentrations, respectively, and $H_1$ and $H_2$ are initial and final hematocrits. The osmotic reflection coefficient was corrected for the degree of hemolysis produced by the perfusion pump and the filtration fraction, as previously described.

Hematocrit was determined in quadruplicate by the microhematocrit technique, and the plasma protein and blood or plasma hemoglobin concentrations were measured by spectrophotometer (model DU-70, Beckman Instruments, Fullerton, California) in duplicate by the protein-dye binding technique (Bio-Rad Protein Assay Kit, Bio-Rad Laboratories, Richmond, California) and cyanmethemoglobin technique (Hemoglobin Assay Kit No. 525, Sigma Chemical, St. Louis, Missouri), respectively.

Experimental Protocol

We evaluated the effects of STA2 and PGI2 on pulmonary microvascular permeability as measured by the capillary filtration coefficient and the osmotic reflection coefficient in four experimental groups (Figure 1):

1) Vehicle control group ($n=5$). During the initial 60 minutes of equilibration, the lungs were allowed to reach an initial isogravimetric state. Baseline filtration coefficient ($K_{f1}$) was determined by elevation of the Pla. After the lungs achieved an isogravimetric state by the restoration of Pla to the baseline level, Pla was raised from 5 to 20 mm Hg by elevation of the height of the reservoir (hydrostatic stress). Under maintained hydrostatic stress, filtration was allowed to continue for 90 minutes or until the lung weight gain was approximately 200% of initial lung weight. Then Pla was decreased to the baseline level, and the lungs were allowed to reestablish a new isogravimetric state; the final filtration coefficient ($K_{f2}$) was determined by elevation of Pla to a level similar to that used for $K_{f1}$. These control lungs also received vehicles for both STA2 and PGI2.

2) STA2 group ($n=6$). After the equilibration period and $K_{f1}$ measurement, the lungs were given a bolus injection of STA2 (50 µg) into the pulmonary arterial cannula. After the lung weight was stabilized, the hydrostatic stress was performed 15 minutes after the STA2 injection and maintained as described above. Then $K_{f2}$ was determined by elevation of Pla to a level similar to that used for $K_{f1}$. This group also received the vehicle for PGI2.

3) PGI2 group ($n=6$). After the equilibration period and $K_{f1}$ measurement, the lungs received a continuous infusion of PGI2 (0.4 µg/kg/min) through the pulmonary arterial cannula by use of an infusion pump (model 935, Harvard Apparatus, South Natick, Massachusetts). The infusion was maintained for the duration of the experiment. After the lung weight was stabilized, the hydrostatic stress was performed and maintained as described above. Then $K_{f2}$ was determined by elevation of Pla to a level similar to that used for $K_{f1}$. This group also received the vehicle for STA2.

4) PGI2 + STA2 group ($n=6$). The protocol was the same as that for the PGI2 group, except that the lungs were also administered STA2 (50 µg) after the equilibration period and $K_{f1}$ measurement. After the lung weight was stabilized, the hydrostatic stress was performed as described above. Then $K_{f2}$ was determined by elevation of Pla to a level similar to that used for $K_{f1}$. This group also received the vehicle for STA2.
4) PGI₂ and STA₂ group (n=6). After the equilibration period and Kf₁ measurement, the lungs received a continuous infusion of PGI₂. A bolus injection of STA₂ was administered 20 minutes after the beginning of the PGI₂ infusion. After the lung weight was stabilized, the hydrostatic stress was performed and maintained as described above. Then Kf₂ was determined by elevation of Pla to a level similar to that used for Kf₁.

Blood samples (4 ml) for measurements of hematocrit and plasma protein or hemoglobin were drawn every 30 minutes in all groups.

The effects of hemolysis, resulting from pumping blood through the perfusion system, on hematocrit and plasma protein concentrations were evaluated in four separate studies. Approximately 750 ml of heparinized blood was circulated in the same system used in the perfused lung study at a constant flow rate of 180 ml/min for 180 minutes. The blood volume and flow rate were chosen to approximate those of mean values in perfused lung experiments. Blood samples (4 ml) were drawn every 30 minutes for 180 minutes. The relationships between hematocrit and plasma hemoglobin concentration and between plasma protein and plasma hemoglobin concentrations were analyzed by linear regression analysis. The average slopes of the regression equations were used to correct the hematocrit and plasma protein concentration to a state of no hemolysis, as described by Maron et al.12 In addition, the final plasma hemoglobin concentration was corrected for filtration-related loss of plasma in each experiment.12

A stock solution of STA₂ (100 μg, Ono Pharmaceutical, Osaka, Japan) was prepared by dissolving in 95% ethanol (1 ml) and diluting to 10 ml in 0.06 M monobasic-dibasic phosphate buffer (pH 8.0) and stored at -20° C. PGI₂ (Burroughs-Wellcome, Research Triangle Park, North Carolina) was dissolved in glycine buffer (pH 10.5) immediately before use.

At the end of the experiment, a final blood sample was taken, and the lungs were rapidly removed from the perfusion system. The lungs were then homogenized, and extravascular lung water was determined and expressed as the ratio of extravascular lung water to dry bloodfree lung, according to the method of Pearce and coworkers17 and Selinger and associates.18

Statistics

The data are expressed as mean±SEM. Regression lines were obtained using least-squares linear regression. The changes induced by the treatments with STA₂ and PGI₂ within and between groups were evaluated by analysis of variance and least significant difference. Comparisons between these treatments and the control group were assessed using Dunnett's t statistic. Significance was determined when p<0.05 was obtained.19

Results

Hemodynamics

Figure 2 summarizes the hemodynamic data from the four groups. The original baseline values (a) of Ppa and Pc were similar in all groups. There were tendencies in the control group for Ppa and Pc to increase during (b and c) or after (d) the administration of vehicles, but these changes were not statistically significant. The hydrostatic stress resulting from the elevation of the Pla by 15 mm Hg (e) increased Ppa and Pc from 19.9±2.8 to 43.4±1.4 mm Hg and from 8.5±0.6 to 24.6±0.6 mm Hg, respectively. The restoration of the Pla to the baseline level (f) resulted in a return of Pc to a level similar to the baseline value, but Ppa remained elevated for the duration of the study.

Injection of STA₂ (50 μg bolus, at c) produced considerable increases in Ppa from 25.8±2.5 to 51.8±3.2 mm Hg, and in Pc from 9.1±0.2 to 11.7±0.4 mm Hg. Although these pressures gradually declined, they remained elevated at 15 minutes (d), before the hydrostatic stress was performed. Elevation of Pla by 15 mm Hg (e) produced an
TABLE 1. Effects of STA 2 and PGI2 on Transvascular Fluid Filtration in Isolated Newborn Lamb Lungs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$K_f 1$ (ml/min/mm Hg/100 g wet lung wt)</th>
<th>$K_f 2$ (ml/min/mm Hg/100 g wet lung wt)</th>
<th>$\sigma$</th>
<th>EVLW/BFDLW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.18±0.02</td>
<td>0.20±0.02</td>
<td>0.66±0.07</td>
<td>8.25±0.58</td>
</tr>
<tr>
<td>STA 2</td>
<td>0.17±0.01</td>
<td>0.30±0.02††</td>
<td>0.31±0.02*</td>
<td>8.88±0.66</td>
</tr>
<tr>
<td>PGI2</td>
<td>0.19±0.02</td>
<td>0.42±0.03†††‡‡</td>
<td>0.32±0.05*</td>
<td>8.43±0.98</td>
</tr>
<tr>
<td>PGI2+STA 2</td>
<td>0.14±0.01</td>
<td>0.36±0.02†††‡‡</td>
<td>0.40±0.05*</td>
<td>10.28±1.30</td>
</tr>
</tbody>
</table>

Data are mean±SEM. STA 2, thromboxane A 2 analogue; PGI2, prostacyclin; $n$, number of experiments; $K_f 1$, $K_f 2$, baseline and final fluid filtration coefficients, respectively; $\sigma$, osmotic reflection coefficient; EVLW, extravascular lung water; BFDLW, bloodfree dry lung weight.

* $p<0.05$ vs. control.
† $p<0.05$ vs. $K_f 1$.
‡‡ $p<0.05$ vs. STA 2.
§§ $p<0.05$ vs. PGI2.

Infection of PGI2 (0.4 $\mu$g/kg/min, at b) significantly decreased the baseline values of Ppa and Pc. Elevation of Ppa (e) increased Pc by 14.9±0.1 mm Hg which was not significantly different from that in the control group.

The peak pressor responses of Ppa and Pc to STA 2 (c) during PGI2 infusion did not differ from those without PGI2. Although Ppa was significantly lower at 15 minutes after the STA 2 injection (d) in the group receiving the PGI2 infusion than in the group without PGI2, the values for Pc were not significantly different. Elevation of Ppa (e) produced an increase in Pc of 12.1±0.9 mm Hg, which was significantly less than the values in the control and PGI2 groups, but was not different from the STA 2 group.

**Fluid Filtration and Osmotic Reflection Coefficients**

The fluid $K_f$ and $\sigma$ measured in the four groups are shown in Table 1. There were no differences in $K_f 1$ between groups. The $K_f 2$ in the control group did not differ from the original baseline value. However, $K_f 2$ was increased from baseline by 76% with STA 2, by 121% with PGI2, and by 157% with both PGI2 and STA 2. In addition, the value for $K_f 2$ in the group receiving both PGI2 and STA 2 was significantly higher than that in the STA 2 group. Furthermore, the value for $K_f 2$ in the group receiving PGI2 alone was significantly greater than that in the group receiving both PGI2 and STA 2. Treatments with STA 2 and/or PGI2 significantly decreased the reflection coefficients; however, there were no statistical differences among these three treatment groups.

**Weight Gain by Left Atrial Pressure Elevation**

Elevation of Ppa produced a progressive gain in the lung weight with the pattern of increase exhibiting two components: 1) a rapid component attributed to vascular volume change (the first 2 minutes) and 2) a slower component representing fluid filtration (Figure 3). The slope of the line during the slower phase indicates the rate of lung weight gain ($\Delta W/\Delta T$) induced by the hydrostatic stress. In the group receiving STA 2, $\Delta W/\Delta T$ did not differ from the control group. On the other hand, there were marked increases in $\Delta W/\Delta T$ during the hydrostatic stress in the groups receiving PGI2 (either with or
without STA2). Because of higher filtration rates, the hydrostatic stress was maintained for approximately 45 minutes (mean values) in the groups receiving PGI2, as compared with 70–80 minutes in the other two groups. Restoration of Pla to the baseline level resulted in a rapid reduction of lung weight by an amount equal to the initial vascular volume increase seen with the Pla elevation, and the isogravimetric condition was reestablished (but at a higher level of lung weight) after 30 minutes. At the end of the experiment, the PGI2-treated lungs (either with or without STA2) had macroscopically apparent diffuse hemorrhagic edema.

The relationships between ΔW/ΔT and the increase in Pc produced by elevation of Pla are shown in Figure 4. Compared with control lungs, the hydrostatic stress produced a similar ΔW/ΔT with a smaller increase in Pc in the STA2 group, but greater values of ΔW/ΔT with smaller or comparable increases in Pc were observed in the groups receiving PGI2 or both PGI2 and STA2. Lungs receiving both PGI2 and STA2 had a higher value of ΔW/ΔT than in the STA2 group, although a similar increase in Pc was observed.

The ratios of extravascular lung water to dry bloodfree lung obtained at the end of the experiment did not differ among the four groups (Table 1).

Discussion

In the present study, we observed increases in vascular permeability in neonatal lungs receiving either STA2 and/or PGI2 compared with control lungs, as indicated by increases in Kf and decreases in σ. Furthermore, Kf was significantly increased in both groups receiving PGI2 as compared with the group receiving STA2 alone, despite comparable values of σ. In addition, the rates of weight gain with PGI2, either alone versus control or with STA2 versus STA2 alone, were increased despite smaller increases in Pc. Taken together, these findings suggest that, in addition to increasing vascular permeability, PGI2 increases vascular surface area as well.

Selective loss of red blood cells from the perfusate could lead to an overestimation of σ, and indeed we noted a diffuse hemorrhagic edema in lungs treated with PGI2. It is unlikely, however, that this condition led to an underestimation of permeability with PGI2 since the efflux of red blood cells should have been accompanied by a proportionate movement of fluid and proteins.

The pulmonary hypertension induced by STA2 in isolated non-blood-perfused lamb lungs is primarily the result of pulmonary venoconstriction.3 The present study demonstrates that the pulmonary pressor response to STA2 in blood-perfused lungs is greater than that observed with a non-blood-perfused system. Although we found a significant increase in Pc by STA2 injection, suggesting venoconstriction, the elevation of the Ppa was due mainly to vasoconstriction upstream from Pc (Figure 2). These results may be due to differences in the perfusates, since a variety of mediators derived from white blood cells or platelets may contribute to these pressor effects.

Garcia-Szabo et al20 demonstrated that a selective inhibitor of thromboxane synthesis prevented the thrombin-induced increases in pulmonary lymph flow and the lymph protein clearance in intact sheep, suggesting that TXA2 contributes to the increase in lung vascular permeability after thrombin infusion. However, while administration of arachidonic acid produced pulmonary venoconstriction associated with TXA2 production in lungs, the microvascular permeability was not increased.21-22 Our study demonstrated that STA2 injection causes an increase in Kf, a similar rate of lung weight gain during hydrostatic stress despite a smaller elevation of Pc, and a decreased σ as compared with control lungs, suggesting that TXA2 may also increase pulmonary microvascular permeability in isolated newborn lamb lungs. The disparity in these results may be due to differences in 1) the manner in which pulmonary edema was produced, 2) the parameters used to assess pulmonary vascular permeability, 3) species, and 4) age. Indeed, hypoxia has been shown to adversely affect lung fluid balance in newborn, but not adult, sheep.24

Consistent with the observations made by others in isolated adult lungs,25,26 we noted that PGI2 (either with or without STA2) produced a greater rate of lung weight gain in spite of a smaller increase in Pc induced by the hydrostatic stress, and a decrease in σ. However, Gunther et al27 and Ogletree28 reported that PGI2 did not alter the pulmonary vascular permeability in intact or unanesthetized sheep, and Demling et al29 showed that the lung injury caused by endotoxemia was prevented by PGI2. These observations suggest that PGI2 contributes to the increased microvascular permeability to protein in isolated perfused lungs, but not in intact animals. Since the adverse effects
of PGI₂ were also observed in non-blood-perfused lungs, the permeability increase may be independent of the presence of platelets.

The increase in vascular permeability to protein associated with elevation of outflow pressure has been attributed to the "stretched pore phenomenon." However, this phenomenon may not occur with every elevation of microvascular pressure, or may require either very high distending pressures or an inappropriate arterial relaxation resulting in greater transmission of pressure to the capillary bed. In control lungs we observed that a moderate increase in outflow pressure did not result in an increase in Kf from baseline, and was associated with a normal value of σ. In a preliminary report, Ehrhart et al have also shown that σ remained unchanged at venous pressures as high as 77 mm Hg in isolated dog lung lobes. In contrast, Rippe et al demonstrated that Kf was increased when Pla exceeded 41 mm Hg in isolated dog lungs; however, papaverine (1.3x10⁻⁴ to 2.7x10⁻⁴ M) was used to produce maximal vasodilation and surface area. Maron and Pilati also found that venous pressures elevated to only 18 mm Hg reduced the value of σ in lobes exposed to 10⁻¹ M papaverine. It is possible that vasodilation by papaverine or PGI₂ coupled with increased capillary hydrostatic pressure augments the stretched pore phenomenon.

In conclusion, the administration of STA₂ and/or PGI₂ to blood-perfused isolated newborn lamb lungs resulted in an increase in the fluid filtration coefficient and a decrease in the osmotic reflection coefficient. Our findings suggest that both STA₂ and PGI₂ increase pulmonary microvascular permeability to protein, and that PGI₂ additionally results in a greater amount of fluid filtration.

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**KEY WORDS** • pulmonary microvascular permeability • fluid filtration coefficient • osmotic reflection coefficient • hematocrit-protein double indicator technique • isolated perfused lungs
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