Prostaglandin E₁ Prevents Increased Lung Microvascular Permeability During Intravascular Complement Activation in Sheep

Marlys H. Gee, Maria V. Tahamont, John T. Flynn, Jeffrey W. Cox, Robert H. Pullen, and Nicholas A. Andreadis

Prostaglandin E₁ (PGE₁) inhibits a variety of functions of activated neutrophils including respiratory burst, release of leukotriene B₄, and adherence to endothelial cells. To determine if PGE₁ alters the pathophysiology of complement-induced lung vascular injury, experiments were conducted in anesthetized sheep with lung lymph fistulas given a 1-hour infusion of zymosan-activated plasma. PGE₁ (30 ng/min/kg) or its saline vehicle was infused intravenously for 90 minutes beginning 30 minutes before the infusion of activated plasma. PGE₁ had no effect on leukocyte count, the initial hypoxemia and thromboxane A₂ release, or the development of acute pulmonary hypertension. However, PGE₁ prevented steady-state increases in lung lymph flow that in vehicle-treated sheep signaled an increase in lung microvascular permeability. Furthermore, extraction of PGE₁ by pulmonary endothelial cells was unaffected by the infusion of activated plasma. We propose that PGE₁ prevented the increase in lung vascular permeability by inhibiting adherence of activated neutrophils to endothelial cells. (Circulation Research 1987;61:420-428)

Intravascular activation of neutrophils produces an acute lung injury characterized by hypoxemia, increased lung vascular permeability, and morphologic evidence of endothelial and epithelial cell injury. Several in vivo and in vitro studies have been made that support the hypothesis that some forms of vascular injury occur because of synthesis and release of oxygen-derived free radicals from activated neutrophils adherent to endothelial cells. Prostaglandins of the E series (PGE₁ and PGE₂) and prostacyclin alter neutrophil responses to specific stimuli. Neutrophils treated with PGE₁, and subsequently stimulated with a synthetic chemotactic peptide (fMLP) or zymosan show a dose-dependent inhibition of free radical release. The mechanism of inhibition is related to an increase in cyclic adenosine 3',5' monophosphate concentration that occurs as a consequence of prostaglandin E₁ treatment. These experiments also demonstrated that free radical release stimulated by phorbol myristate acetate (PMA) or zymosan-treated serum is not affected by treatment with PGE₁. The effect of PGE₁ on fMLP-stimulated cells is also observed in neutrophils isolated from rats treated systemically with the drug, suggesting that PGE₁ may react with a neutrophil receptor to prolong the effects of treatment.

The ability of activated neutrophils to adhere to endothelial cells appears to be an obligatory step in the genesis of immune vascular injury. Boxer and coworkers showed that treating neutrophils with prostaglandins inhibits fMLP-induced adherence to endothelial cells. PGE₁ inhibits release of leukotriene B₄ (LTB₄) from neutrophils stimulated with fMLP. Since LTB₄ stimulates adherence of neutrophils to endothelial cells, the inhibitory effect of E series prostaglandins on neutrophil adherence may be the result of inhibition of LTB₄ release.

The anti-inflammatory actions of PGE₁ are also apparent in vivo. Systemic administration of the drug suppresses acute inflammatory responses associated with intradermal administration of histamine, bradykinin, serotonin, or immune complexes. However, PGE₁ is a potent vasodilator and as such can reduce net transvascular fluid flux and edema formation independent of anti-inflammatory actions of the drug. The present study was made to determine the effects of moderate doses of PGE₁ on lung injury produced by intravenous infusion of zymosan-activated plasma (ZAP).

Materials and Methods

Animal Preparation

Anesthesia was induced in 18 adult sheep of either sex by sodium thiamylal (1g i.v.) and was maintained by administration of 30–60 mg sodium pentobarbital every 30 minutes throughout the experiment. A tracheostomy was made, and sheep were ventilated with a constant volume pump to deliver 10 ml/kg of room air at a frequency needed to achieve a systemic arterial
CO₂ tension of 35–40 mm Hg. A positive end-expiratory pressure of 10 cm H₂O was applied. The lungs were hyperinflated every 30 minutes to minimize atelectasis.

The sheep were prepared for hemodynamic measurements, blood sampling, and collection of lung lymph by procedures previously described in detail. Briefly, central venous catheters were placed for administration of drugs and plasma. A thermodilution balloon-tipped catheter was placed in a pulmonary artery to measure arterial and arterial wedge pressures, sample mixed venous blood, and measure cardiac output by injection of 5 ml of room temperature 0.9% NaCl. A systemic arterial catheter was placed for pressure measurement and blood sampling. Catheters were connected to transducers (P23DC, Statham, Hato Rey, P.R.) positioned at the level of the left atrium, and pressures were continuously recorded on a direct-writing oscillograph (model 7, Grass, Quincy, Mass.). An efferent duct of the caudal mediastinal lymph node was cannulated through a right thoracotomy. The tail of the lymph node was ligated, and visible afferent lymphatics from the esophagus or diaphragm were eliminated to minimize systemic contamination of lung lymph. After completing the preparation, sheep were given sodium heparin (100 U/kg i.v.). Lymph was collected in 30-minute intervals in graduated centrifuge tubes pretreated with heparin. These samples were centrifuged, and an aliquot was frozen for subsequent measurement of protein concentrations. Blood pressures and cardiac output were measured midway in each lymph collection. Cardiac output was the average of measurements made with three consecutive injections (cardiac output computer, Sorenson, Salt Lake City, Utah).

Two samples of systemic arterial blood were also drawn. The first was used for measurement of blood gases (model 16, Corning, Corning, N.Y.). The second was placed in a centrifuge tube pretreated with heparin and 5 ng phenyl pyrazolidone to inhibit coagulation and ex vivo eicosanoid synthesis. Leukocyte cell counts were measured using an automated counter calibrated for sheep leukocytes (model 3, Clay-Adams hematology analyzer, Parsippany, N.J.). The blood was centrifuged and plasma aliquots were frozen for measurement of protein concentration and concentrations of thromboxane A₂ (TXA₂) and prostacyclin metabolites. Additional simultaneous arterial and mixed venous blood samples (4 ml) were drawn periodically for measurement of plasma PGE₂ concentration. The measurement was made in 5 sheep receiving PGE₂ and in 1 sheep receiving its vehicle. The samples were placed in precooled centrifuge tubes pretreated as described above, and duplicate hematocrit measurements were made. The blood was kept on ice until centrifuged, after which plasma aliquots were frozen.

Protocol
Each experiment began by establishing a baseline steady state that is defined as three consecutive, 30-minute lymph collections during which the lymph flow rate, pulmonary blood pressures, and cardiac output change by 10% or less. An intravenous infusion was begun in which the sheep received PGE₂, (30 ng/min/kg) or its saline vehicle at a flow rate of 0.38 ml/min for 90 minutes. Thirty minutes after the PGE₂ infusion was initiated, a second intravenous infusion of either ZAP or plasma was begun at 3.8 ml/min. This infusion began with a 5 ml bolus injection of ZAP or plasma and continued for 60 minutes. The infusions ended simultaneously, and the experiment was continued for a 3-hour postinfusion period. Sheep were randomly assigned to one of three experimental groups: vehicle + ZAP (n = 6, body weight = 38.3 ± 1.5 kg), PGE₂ + ZAP (n = 6, body weight = 40.2 ± 2.1 kg), or PGE₂/vehicle + plasma (n = 6, body weight 37.7 ± 1.3 kg). In the latter group, 3 sheep received PGE₂, and 3 received the vehicle. The data in this group were pooled since no differences were observed between treated and untreated sheep.

Assays
Protein concentrations in plasma and lung lymph were measured using an automated biuret method (autoanalyzer, Technicon, Tarrytown, N.Y.). Variability of repeated measures was less than 5%.

Plasma concentrations of stable metabolites of TXA₂, thromboxane B₂ (TXB₂), and prostacyclin, 6-keto PGF₁α, were measured using specific radioimmunoassays as described previously. The linear working range of both antibodies is 10–2,500 pg of their specific eicosanoid. Cross-reactivities of the antibodies with other eicosanoids have been reported. Samples were analyzed in duplicate with standards for an eight-point standard curve.

PGE₂ was determined in plasma (n = 5) and lung lymph (n = 1) by high-performance liquid chromatography (HPLC) as described previously. Briefly, PGE₂ was isolated by double antibody precipitation and centrifugation. Acetonitrile extracts of the pellet were derivatized with panacyl bromide and analyzed by column-switching HPLC with fluorescence detection. Assay results were corrected for extraction efficiency, which was determined for individual samples by fortifying 0.5 ml of plasma or lymph with 70 pg of (3H) PGE₂, extracting in parallel with samples for HPLC analysis. The extraction efficiency was 64 ± 2% for arterial and venous plasma and 70 ± 1% for lymph. The assay sensitivity was 50 pg/ml (signal-to-noise ratio 3:1), at which the interday assay relative standard deviation was 18%. No significant assay bias was observed for fortified plasma controls included with each assay set (actual concentration: 100, 510, and 993 pg/ml; assay result: 104 ± 4, 545 ± 33, and 999 ± 111 pg/ml, respectively).

Leukocyte Aggregation Measured in Whole Blood
Three-milliliter samples of systemic arterial blood were obtained periodically to measure the aggregation response to homologous zymosan-activated plasma. Three hundred microliters of blood were added to 700
μl of N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid (HEPES) buffer (pH 7.4, NaCl 140 mM, HEPES 10 mM, KCl 10 mM, CaCl₂ 0.1 mM, MgCl₂ 0.2 mM, NaHCO₃ 11.9 mM, glucose 5 mM, albumin 14 μM) maintained at 37°C in a siliconized glass cuvette. Electrodes were placed in the stirred solution, and a stable recording of electrical impedance was made (whole blood aggregometer, Chrono-Log, Havertown, Penn.). The gain was adjusted to provide 80% of the full scale deflection as impedance increases during aggregation. Cells were stimulated with 70 μl of ZAP, and the recording continued until the aggregation wave was complete (about 3 minutes). The response was quantitated by measuring the half-time of the aggregation wave. Three consecutive aggregation waves were produced from each sample and the average half-time reported.

Statistical Analyses

The data are presented as mean ± SEM. Within group data were compared to baseline steady-state values using a one-way analysis of variance with multiple comparisons (Dunnett’s test). Between-group data were compared using a two-way analysis of variance for data obtained during and after the infusion of zymosan-activated plasma or plasma. Statistical significance was determined as p < 0.01.

Preparation of Zymosan-Activated Plasma

Blood was removed in 100-ml aliquots with 300 U heparin. After centrifugation, plasma was removed, and 2.5 mg/ml zymosan (Sigma Chemical Co., St. Louis, Mo.) was added and mixed by gentle agitation. The samples were incubated at 37°C for 30 minutes. Control plasma was incubated without zymosan. The samples were centrifuged to pellet the zymosan, and the plasma was decanted and either frozen or maintained at 37°C until infused. All samples were filtered through 4 layers of surgical gauze prior to infusion.

Of the nearly 240 ml of activated or control plasma infused, 60 ml was autologous. The 5-ml bolus injection and the first 50-ml volume infused were autologous plasma. The remainder was taken from frozen stores of homologous activated or control plasma.

In Vitro Studies of Neutrophil Adherence

To test the hypothesis that PGE₂ may alter adherence of stimulated neutrophils, cells were isolated from sheep blood according to a previously described method.²⁴ Briefly, blood was drawn in 0.1 M ethylenediaminetetraacetic acid (EDTA), 9 parts blood to 1 part EDTA. Red cells were removed by hypotonic lysis. The washed buffy coat cells were resuspended in HEPES buffer and layered on a preformed continuous density gradient composed of 60% Percoll (Pharmacia Biotechnology Group, Piscataway, N.J.) and 40% autologous plasma. After centrifugation, neutrophils were located at the 1.097 g/ml density interface. The cells were removed, washed, and resuspended in HEPES buffer. The cells were greater than 95% viable and pure as assessed by trypan blue dye exclusion and differential count.

Adherence was measured by layering neutrophils on plastic plates (35 x 10 mm) precoated with fMLP, PMA, or ZAP. One-milliliter aliquots of neutrophils, 5-15 x 10⁶ cells, in HEPES buffer were incubated with PGE₂ or its vehicle for 10 minutes. During incubation, the plates were wetted with HEPES and 100 μl of the stimulus was applied to the plate; fMLP and PMA were diluted in HEPES and applied in a concentration of 5 x 10⁻⁹ M. Excess fluid was drained from the plates. Using a tuberculin syringe, neutrophils were applied in a circular motion covering the entire surface of the plate. The plates were covered and left undisturbed for 15 minutes, then they were rinsed in HEPES and air dried. Cells were stained with Wright’s stain and counted through a microscope equipped with a grid (wipple disk). Grids were counted in 4 fields, 1 in each quarter of the plate. From the field counts, the total number of cells on the plate was calculated and expressed as a percentage of the number applied. For each stimulus, plates were run in pairs using PGE₂- and vehicle-treated cells and were analyzed using a paired t test.

Results

Infusion of plasma had no effect on the number of circulating leukocytes in either the presence or absence of PGE₂ (Figure 1). In animals receiving activated plasma, leukocyte counts in systemic arterial blood decreased to about 50% of the baseline cell count. The results were similar for both treatment groups, indicating that PGE₂ had no effect on the decrease in circulating leukocytes elicited by infusion of zymosan activated plasma.

Synthesis and release of TXA₂ accompanied the
initial decrease in leukocyte count in animals given ZAP and was not observed in animals given plasma (Figure 2). The release was transient in that the plasma TXB₂ concentration rapidly decreased even as the infusion of activated plasma proceeded. The plasma thromboxane concentration tended to decrease more slowly during the ZAP infusion in PGE₂-treated sheep than in the vehicle-treated group. The plasma concentration of 6-keto PGF₁₀ was unchanged during the experiment by bolus injection or infusion of activated plasma.

In animals given the plasma infusion, the systemic arterial oxygen tension did not change during the experiment (Figure 3). The initial decrease in circulating leukocytes during infusion of ZAP was accompanied by development of severe hypoxemia that reversed within one hour in vehicle-treated animals. In contrast, recovery to the preinfusion oxygen tension was delayed in the PGE₂-treated sheep.

Hemodynamic data are illustrated in Figures 4 and 5. The infusion of plasma had no effect on blood pressures. However, in response to the volume load presented during infusion, cardiac output increased to a new postinfusion steady state level that averaged 25 ± 3% greater than the baseline flow. Using the criterion for a steady state, the postinfusion steady state was established during the last 90 minutes of the experiment from 1.5–3 hours after termination of the infusions. The bolus injection of zymosan-activated plasma produced a brisk, but transient pulmonary hypertension that was associated with no change in the

**Figure 2.** Concentrations of thromboxane B₂ and 6-keto PGF₁₀ in plasma from systemic arterial blood for 6 sheep in each group. Mean ± SEM are illustrated where appropriate. BL, baseline steady state; 8, duration of infusion of PGE₂ or its vehicle; ■, infusion time of plasma or activated plasma; *, within-group difference from baseline value at p<0.01.

**Figure 3.** Partial pressure of oxygen (mean ± SEM) measured in systemic arterial blood in 6 sheep per group. BL, baseline steady state; 8, duration of the infusion of drug or vehicle; ■, infusion of plasma or activated plasma; *, within group difference from the baseline value at p<0.01.

**Figure 4.** Mean blood pressure (mean ± SEM) measured in 6 sheep per group. Ppa, pulmonary artery; Pw, a wedged pulmonary artery; and Pa, a systemic artery. BL, baseline steady state; 8, infusion of drug or vehicle; ■, infusion of activated or control plasma; *, within-group difference from the baseline value at p<0.01.
groups. Treatment with 30 ng/kg/min PGE₁ did not produce measurable pulmonary or systemic hypotension. There was no postinfusion steady-state increase in cardiac output in the vehicle-treated sheep given ZAP (average increase 9 ± 8%) although the volume load was the same in this group as in the group receiving plasma. In contrast, the PGE₁ + ZAP group increased cardiac output by 23 ± 4%, which was the same increase measured in the plasma group. Therefore, in the postinfusion steady state, cardiac output was significantly less in the vehicle + ZAP group than in either of the other two groups.

During the baseline steady state, lung lymph flow rates were similar in the three groups: 4.8 ± 1.1 ml/hr in the plasma group, 3.1 ± 0.6 ml/hr in the vehicle + ZAP group, and 5.6 ± 1.4 ml/hr in the PGE₁ + ZAP group. In the plasma group, lung lymph flow increased slowly to a steady-state level that averaged 1.7 ± 0.1 times the preinfusion steady-state value (Figures 6 and 7). The pulmonary hypertension triggered a more rapid and larger increase in lymph flow in the two groups receiving activated plasma (Figure 6). In the vehicle + ZAP group, lymph flow initially increased to about 4 times the baseline flow and achieved a steady-state increase that averaged 3.1 ± 0.2 times the baseline flow (Figure 7). Neither the rapid nor the sustained increase in lymph flow was associated with a change in the lymph/plasma protein concentration ratio. These results are consistent with a sustained increase in lung microvascular permeability in the sheep receiving vehicle + ZAP. In the PGE₁ + ZAP sheep, the lymph flow initially increased about threefold and achieved a steady-state increase that averaged 1.9 ± 0.2 times the baseline flow. The steady-state increase in lymph flow in the PGE₁ + ZAP sheep was the same as that in the plasma group and was significantly less than that in the vehicle + ZAP sheep (Figure 7). In addition, lymph flows measured during and after the infusion of ZAP were significantly less in PGE₁-treated sheep than in vehicle-treated sheep using a two-way analysis of variance (Figure 6). Moreover, the rapid increase in lymph flow in the PGE₁ + ZAP group was associated with a decreased lymph/plasma protein concentration ratio indicating that the pulmonary endothelium retained normal selectivity in restricting net protein flux. The changes in lung lymph flow and protein concentration in the PGE₁ + ZAP sheep were those attributable to acute pulmonary hypertension and volume load.

PGE₁ was not detectable (<50 pg/ml) in the mixed venous or arterial plasma samples from sheep given vehicle infusions with control or activated plasma. During PGE₁ infusion, however, measurable concentrations were achieved in both mixed venous and arterial plasma samples; PGE₁ concentrations averaged 2.5 ± 1.0 pg/ml in the mixed venous plasma and 0.7 ± 0.2 pg/ml in the arterial plasma.
arterial plasma. The concentrations were inversely dependent on the calculated plasma flow because PGE₁ does not distribute significantly into red blood cells and, at the fixed infusion rate, PGE₁ is diluted by plasma volume. Plasma flow varied considerably both within and among sheep, and mixed venous plasma PGE₁ concentrations consequently ranged from 600–1,230 pg/ml measured in 3 PGE₁ + ZAP sheep and 2 PGE₁ + plasma sheep (Figure 8). Systemic arterial plasma concentrations varied similarly but over a smaller range (<50–117 pg/ml). PGE₁ was not detectable in pulmonary lymph during PGE₁ infusion, nor was it detectable in either mixed venous or arterial plasma 15 minutes after terminating the infusion.

The mean pulmonary extraction ratio for PGE₁, 15 minutes after beginning the infusion was 0.92 ± 0.03 (n = 5, Table 1). Regardless of whether the sheep received activated or control plasma, the pulmonary extraction efficiency for PGE₁ was maintained at the pretreatment level. The mean PGE₁ extraction ratio recorded in 3 sheep during ZAP infusion was 0.92 ± 0.02 (n = 6), compared with 0.94 ± 0.01 (n = 4) in 2 sheep during plasma infusion. There was also no difference between extraction ratios recorded at 15 or 45 minutes after starting the ZAP infusion. Both results indicate that infusion of ZAP had no effect on pulmonary PGE₁ extraction efficiency.

Addition of ZAP to whole blood produced an aggregation wave that previous studies indicate represents leukocyte rather than platelet aggregation.

Addition of plasma produces no response, and leukocyte aggregation in whole blood can be used to screen plasma samples for the presence of complement anaphylatoxins. Aggregation half-times were constant during the experiment in the plasma and PGE₁ + ZAP groups (Figure 9). In contrast, the half-time of the aggregation response was significantly greater in untreated sheep receiving activated plasma during the infusion and postinfusion periods than in either of the other two groups. These results indicate a desensitization to activated plasma in animals receiving vehicle and zymosan-activated plasma that was not observed in animals treated with PGE₁.

For the in vitro neutrophil adherence assay, 10–14 pairs of plates were used for each stimulus. With fMLP, 34.4 ± 5.4% of vehicle-treated neutrophils adhered to the plate. PGE₁ in the highest dose tested, 1,500 pg/ml, had no effect on the adherence (30.3 ± 2.6%). With PMA as the stimulus, 20.3 ± 1.3% of untreated neutrophils adhered; these results were similar to the 24.2 ± 2.2% adherence using cells incubated with 1,500 pg/ml PGE₁. Coating the plates with zymosan-activated plasma resulted in about 10% adherence of untreated neutrophils. The reduced adherence is due to the presence of plasma proteins. However, with ZAP

![Figure 8](http://circres.ahajournals.org/)

**Figure 8.** Plasma PGE₁ concentrations in individual sheep measured during and immediately after drug infusion — samples obtained from mixed-venous blood; --- samples obtained from systemic arterial blood, —, infusion during and immediately after drug; ••, infusion of activated or control plasma.

![Figure 9](http://circres.ahajournals.org/)

**Figure 9.** Half-times (mean ± SEM) of complement-stimulated aggregation waves measured in systemic arterial blood for 6 sheep per group. BL, blood sample obtained during baseline steady state period; ■, infusion of drug or vehicle; ●, activated or control plasma infusion.

### Table 1. Pulmonary Extraction Efficiencies of PGE₁ During Constant Infusion in Sheep Given Control or Zymosan-activated Plasma (ZAP)

<table>
<thead>
<tr>
<th>Timepoint</th>
<th>Sheep 56–85</th>
<th>Sheep 53–85</th>
<th>Sheep 52–85</th>
<th>Sheep 6–85</th>
<th>Sheep 2–86</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 minutes (PGE₁)</td>
<td>0.94</td>
<td>0.89</td>
<td>0.93</td>
<td>&gt;0.95</td>
<td>0.90</td>
</tr>
<tr>
<td>15 minutes (PGE₁ + Plasma)</td>
<td>&gt;0.95</td>
<td>&gt;0.93</td>
<td>0.92</td>
<td>0.92</td>
<td>0.91</td>
</tr>
<tr>
<td>45 minutes (PGE₁ + Plasma)</td>
<td>0.94</td>
<td>&gt;0.92</td>
<td>0.88</td>
<td>0.95</td>
<td>0.94</td>
</tr>
</tbody>
</table>

*Calculated as Ep = (Cv - Ca)/Cv, where Ep is the pulmonary extraction ratio, and Cv and Ca are the PGE₁ concentrations in simultaneously drawn mixed venous (pulmonary arterial) and systemic arterial plasma samples, respectively. Inequality signs are used in some cases because the systemic arterial plasma PGE₁ concentration was below the detection limit of the assay.
as the stimulus, PGE, inhibited neutrophil adherence in a dose-dependent manner (Figure 10). The doses of PGE, reflect the range of values measured in vivo during drug infusion. Therefore, PGE, in doses similar to those measured in mixed venous blood inhibited complement-induced neutrophil adherence.

Discussion

Prostaglandin E₂ is an unusual anti-inflammatory drug for three reasons. First, it has no reported inhibitory effect on the activity of the cyclooxygenase complex, an observation confirmed by the present study. Second, PGE, is cleared during transit through the lung by carrier-mediated transport from plasma into cells, presumably pulmonary endothelial cells, followed by intracellular enzymatic catabolism. Both processes have a high Vₘₐₛ/Kₘ ratio so that, even at the 30 ng/kg/min infusion rate employed in these experiments, the pulmonary extraction efficiency was relatively independent of the PGE, concentration and clearance was flow limited. The 92 ± 3% extraction efficiency observed in sheep due to changes in steady state agrees with values reported for man, dog, and rat using constant infusion methods. Third, PGE, is a direct vasodilator. Because of its efficient pulmonary extraction, systemic PGE, concentrations were less than 0.2 ng/ml, and no evidence of systemic arterial hypotension was observed. Therefore, it is likely that any potential vasoactive effect of the drug would have been confined to the pulmonary circulation.

The initial severity of acute responses to intravascular complement activation was unaffected by treatment with PGE,. The development of hypoxemia is a thromboxane-mediated event that normally reverses soon after the plasma thromboxane concentration returns to baseline. With PGE, treatment, both the increase in plasma thromboxane concentration and the recovery time of the hypoxemia appeared to be prolonged. The slow recovery may be a function of the prolonged increase in the thromboxane concentration although another mechanism may contribute to this response. Weir and coworkers showed that infusion of PGE, inhibits the pulmonary pressor response to hypoxia. PGE, may have inhibited local hypoxic vasoconstriction, a normal compensatory response to ventilation/perfusion imbalance, hence prolonging the initial response to TXA₂.

Measurement of pulmonary vascular pressures produced no evidence of drug induced vasodilation, either before or during the infusion of ZAP. The acute pulmonary hypertension that follows a bolus injection of activated plasma can be produced by mediators other than TXA₂. Ogletree and Brigham in experiments with sheep reported significant decreases in pulmonary and systemic arterial pressures during intravenous infusion of PGE, and systemic arterial pressures during intravenous infusion of PGE,. However, these investigators infused 7.5 mg PGE, in 30 minutes compared with the total of about 80 μg given over 90 minutes in the present study. This difference in the dose of PGE, is the most likely explanation for the different results obtained in the two studies.

Treatment with PGE, prevented an apparent increase in lung microvascular permeability following intravascular complement activation. Factors that might alter net transvascular fluid and protein flux other than changes in permeability include changes in precapillary and postcapillary hydrostatic pressures and perfused surface area. To decrease the lymph flow rate with no change in mean pulmonary artery pressure would require that PGE, decrease pulmonary venous pressure. Yet, in the present study, pulmonary venous pressure, as estimated by the arterial wedge pressure, was unchanged by drug treatment. In the normal lung, recruitment and derecruitment of surface area occurs as cardiac output increases and decreases. The effect of perfused surface area on the net fluid filtration rate is apparent in the steady-state increases in cardiac output and lung lymph flow in the sheep given plasma (Figure 11). PGE,-treated sheep receiving activated plasma had the same increases in cardiac output and lymph flow as the control sheep, indicating that an increase in perfused surface area caused the increase in net transvascular fluid flux in both groups. There was no steady-state increase in cardiac output in the sheep given ZAP and only the PGE, vehicle, yet this group had the largest increase in lung lymph flow.

**Figure 10.** Adherence of neutrophils to plastic coated with zymosan-activated plasma. Ten or twelve paired aliquots of neutrophils were incubated with PGE, or its vehicle prior to plating. The percent (mean ± SEM) of the applied cells that adhered is illustrated.

**Figure 11.** Postinfusion steady-state increases in lung lymph flow (mean ± SEM) as a function of steady-state increases in cardiac output. The postinfusion steady state was obtained over the last 90 minutes of the experiment. 1.5–3 hours after both infusions were terminated. ▲, vehicle + ZAP; △, PGE, + ZAP; ○, vehicle/PGE, + Plasma.
Therefore, hemodynamic factors operating alone produced the steady-state increase in the net fluid filtration rate in the control and in PGE, + ZAP groups. In contrast, the steady-state increases in net fluid and protein flux in the vehicle + ZAP group cannot be explained by hemodynamic changes alone and are the result of an increase in endothelial permeability.

Intravascular complement activation produces a sustained increase in lung microvascular permeability that is prevented by simultaneous administration of PGE,+, a conclusion supported by the data on pulmonary clearance of PGE,. Extraction of PGE, is sensitive to selective inhibitors of cellular uptake and metabolism or to conditions that produce endothelial injury. For example, the extraction efficiency is decreased by inhibitors of the mediated transport step by or by lung injury induced by hyperbaric oxygen or nitrogen dioxide.35 cardiopulmonary bypass surgery.36-37 or the adult respiratory distress syndrome.37 The depression in PGE, extraction in both man and dog following cardiopulmonary bypass surgery was 10%, and in man with the adult respiratory distress syndrome, it was about 20%. In the present experiments, there was no change in the PGE, extraction ratio in sheep given drug + plasma compared with those given drug + ZAP. This result is consistent with the normal functional status of pulmonary endothelial cells as indicated by the lack of change in vascular permeability in the PGE, + ZAP sheep.

Based on previous results with models of immune vascular injury, PGE, may prevent the injury by inhibiting free radical release from complement-stimulated neutrophils or by inhibiting neutrophil adherence to pulmonary endothelial cells. PGE, has no effect on free radical release from complement stimulated neutrophils isolated from sheep blood, a result that confirms studies with human neutrophils.4,11 PGE, may prevent neutrophil adherence to endothelial cells, thus negating the toxic effects of free radicals released to the surface of the activated cells. This mechanism is supported by in vitro data showing a selective, dose-dependent inhibition of complement-induced neutrophil adherence. The decrease in the numbers of circulating leukocytes was similar in treated and untreated sheep during the infusion of ZAP, a result that would appear to refute this hypothesis. However, the sequestration of leukocytes involves at least two mechanisms, aggregation and adherence, that cannot be distinguished on the basis of cell counts. PGE, had no effect on complement-stimulated leukocyte aggregation in whole blood. The characteristics of the aggregation response were similar in sheep receiving PGE, + ZAP and those given plasma. Aggregation of complement-stimulated neutrophils is a reversible phenomenon.39-40 However, in sheep given vehicle + ZAP, the aggregation responses were prolonged during and after the infusion of complement anaphylatoxins, suggesting a desensitization of leukocytes to the same stimulus. When PMA was used as the stimulus, infusion of ZAP had no effect on the aggregation response to PMA, illustrating that the desensitization was stimulus specific (unpublished observation).

Stimulated neutrophils in suspension release oxygen radicals with aggregation and terminate release when the cells deaggregate.41 Adherence of the cells, however, prolongs the respiratory burst.41 The present results are consistent with the following proposed mechanisms. In vehicle + ZAP sheep, activated neutrophils adhered to pulmonary endothelial cells, released free radicals, and injured the endothelial plasma membrane. The reaction is terminated by detachment of the neutrophil,42 which is subsequently less responsive to a second application of the same stimulus. With PGE, the activated neutrophils aggregate, but do not adhere, and display a brief respiratory burst in relatively large pulmonary vessels. The burst may signal deaggregation and the toxic oxygen metabolites are dispersed into the bloodstream with no direct effect on pulmonary endothelial cells. The neutrophils then respond normally to an in vitro application of the same stimulus. This scheme assumes that PGE, acts primarily on neutrophils to alter the immune response. Although ample evidence supports this view, an effect of the drug on pulmonary endothelial cell function cannot be ruled out. Such an effect may be indirect through a mediator such as leukotriene B, or direct through activation of PGE, transport and metabolism. Further studies are required to determine those factors that regulate the interaction between neutrophils and endothelial cells in vivo.

Acknowledgment

Prostaglandin E, was supplied by The Upjohn Company.

References

1. Gee MH, Margiotta M, Tahamont MV, Flynn JT: Role of free radical generation in complement induced pulmonary dysfunc-

2. Gee MH, Perkowski SZ, Tahamont MV, Flynn JT: Arachi-


4. Johnson KJ, Ward PA: Acute immunologic pulmonary alveo-


Key Words: lung microvascular permeability, lung lymph, thromboxane, neutrophils
Prostaglandin E1 prevents increased lung microvascular permeability during intravascular complement activation in sheep.

M H Gee, M V Tahamont, J T Flynn, J W Cox, R H Pullen and N A Andreadis

Circ Res. 1987;61:420-428
doi: 10.1161/01.RES.61.3.420

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/61/3/420

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at: http://circres.ahajournals.org/subscriptions/