Inhibition of prostaglandin synthesis during polystyrene microsphere-induced pulmonary embolism in the rat

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Submitted 15 August 2002; accepted in final form 29 January 2003

Inhibition of prostaglandin synthesis during polystyrene microsphere-induced pulmonary embolism in the rat. Am J Physiol Lung Cell Mol Physiol 284: L1072–L1081, 2003. First published March 14, 2003; 10.1152/ajplung.00283.2002.—Our objective was to test the effect of inhibition of thromboxane synthase versus inhibition of cyclooxygenase (COX)-1/2 on pulmonary gas exchange and heart function during simulated pulmonary embolism (PE) in the rat. PE was induced in rats via intrajugular injection of polystyrene microspheres (25 µm). Rats were randomized to one of three posttreatments: 1) placebo (saline), 2) thromboxane synthase inhibition (furegrelate sodium), or 3) COX-1/2 inhibition (ketorolac tromethamine). Control rats received no PE. Compared with controls, placebo rats had increased thromboxane B2 (TxB2) in bronchoalveolar lavage fluid and increased urinary dinor TxB2. Furegrelate and ketorolac treatments reduced TxB2 and dinor TxB2 to control levels or lower. Both treatments significantly decreased the alveolar dead space fraction, but neither treatment altered arterial oxygenation compared with placebo. Keta-rolac increased in vivo mean arterial pressure and ex vivo left ventricular pressure (LVP) and right ventricular pressure (RVP). Furegrelate improved RVP but not LVP. Experimental PE increased lung and systemic production of TxB2. Inhibition at the COX-1/2 enzyme was equally as effective as inhibition of thromboxane synthase at reducing alveolar dead space and improving heart function after PE.

thromboembolism/treatment; cyclooxygenase; thromboxane; leukotriene; ketorolac; heart failure; Langendorff; animal model

PULMONARY EMBOLISM (PE) continues to be a major cause of morbidity and mortality in the United States. In one large autopsy-based study, massive PE was the second leading cause of sudden death in adults aged <65 yr (4). The primary treatment strategy for massive PE is the recanalization of occluded pulmonary vasculature by fibrinolytic agents (11), catheter fragmentation (32), or surgical removal of clot (16). However, up to one-half of patients with massive PE have contraindications to fibrinolysis (15), and few hospitals have facilities for invasive treatment of PE. Even under optimal conditions (e.g., immediate bolus infusion of a fibrinolytic agent), these interventions require >2 h to effect a significant reduction in pulmonary vascular resistance (20). PE may also cause pulmonary vasoconstriction through the liberation of vasoconstrictive agents, including PGE2α and thromboxane (Tx) A2 and B2 (10). PE can cause hypoxemia and increased pulmonary arterial pressure in previously healthy patients (19). Both hypoxemia and increased shear forces in the pulmonary vascular bed have been found to increase expression of the cyclooxygenase (COX)-2 gene (3). In humans with PE, blood concentrations of thromboxane B2 have been found to be elevated for up to 7 days after onset of symptoms (10). Both the mechanical vascular occlusion and release of vasoconstrictive agents from massive PE appear to produce a synergistic effect that causes acute pulmonary hypertension, worsened gas exchange, impaired right ventricular (RV) function that can culminate in acute cor pulmonale, circulatory shock, and even death (26, 30, 39).

In the present work, we inhibited two enzymes in the prostaglandin pathway shown in Fig. 1. Site A, the COX-1/2 enzyme, was inhibited with ketorolac, and site B, the thromboxane synthase enzyme, was inhibited with furegrelate. Figure 1 shows that one could rationalize that proximal inhibition at the COX-1/2 enzyme might result in decreased substrate that is available to produce vasodilatory prostaglandins (PGI2 and PGE2), resulting in worsened pulmonary hypertension (34) and ventilation-perfusion relationships (7, 33). Also, the location of COX-1/2 suggests that COX-1/2 inhibition might cause arachidonate to be shunted toward the lipoxygenase pathway, resulting in increased synthesis of vasoconstrictive and bronchoconstrictive leukotrienes (28, 38). We sought to test the hypothesis that thromboxane synthase inhibition would provide greater improvement than COX-1/2 inhibition in alveolar dead space (Vd/Vtalv) and RV function after pulmonary vascular occlusion.

METHODS

Experiments were performed in Sprague-Dawley rats weighing between 415 and 543 g. The study had three phases. 1) Assessment of treatment efficacy: four groups of...

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animals were used to compare the efficacy of the drugs of interest. These groups include animals treated with placebo, furegrelate sodium, or ketorolac tromethamine and a control group. A sufficient number of animals were studied to allow n = 9 in each four groups. 2) Pulmonary angiography: four groups of rats (placebo, furegrelate, ketorolac, and control, n = 3 per group) were studied by pulmonary angiography. 3) Measurement of pleural effusion, lung water, and histology after PE: two groups (control and placebo, n = 6 per group) were designed to allow measurements of lung wet-to-dry weight and to measure pleural effusion volumes. Studies were conducted according to the National Institutes of Health guidelines on the use of experimental animals. The Institutional Animal Care and Use Committee (IACUC) of Carolinas Medical Center approved all methods. Before experimentation, rats had ad libitum access to standard Teklad rat diet (Harlan Teklad, Madison, WI).

**Pulmonary embolization protocol.** Experimental animals were anesthetized with an intramuscular injection of 100 mg/kg of ketamine and 3 mg/kg of xylazine. The neck was shaved and prepared with aseptic technique. The left jugular vein was dissected and cannulated with PE-90 tubing. Undiluted polystyrene microsphere beads (mean diameter 24 ± 1 μm, catalog no. 7525A; Duke Scientific, Palo Alto, CA) totaling 0.15 ml/100 g body wt with 0.1 ml/100 g body wt of saline flush were given at a rate of 0.1 ml/min to induce fixed pulmonary obstruction. We previously demonstrated in an acute model that this dose of microspheres produced approximately a peak reduction in mean arterial blood pressure (MAP) of 25% from basal measurements followed by partial recovery of arterial blood pressure to ~10% below basal level (5). This dose also causes the in vivo RV systolic blood pressure to increase from 30 ± 1 at baseline to 55 ± 1.8 mmHg measured 30 min after embolization, suggesting ~75% pulmonary vascular occlusion (19). In the present experiment, we extend the duration of the exposure of the PE to 16 h. After PE induction, the jugular vein was ligated with 2-0 silk ligature, and the incision was closed with 2-0 silk suture. Rats were placed back in cages to recover with ad libitum access to food and water.

**Measurement of treatment efficacy.** The treatments were placebo (1 ml saline), furegrelate sodium (15 mg/kg in 1 ml saline; Cayman Chemical, Ann Arbor, MI), and ketorolac tromethamine (10 mg/kg in 1 ml saline; Abbott Laboratories, Chicago, IL). A technician prepared fresh treatment solutions each day according to a computer-generated (Excel Visual Basic Macro; Microsoft, Seattle, WA) random schedule of treatments. This technician did not otherwise participate in the in vivo portion of the experiments but was aware of the outcome of each rat and had instructions to stop using a treatment when nine rats survived to completion of data collection. Two identical treatment injections were given intraperitoneally to awake rats, the first at 5 h and the second at 14 h after induction of PE. The first treatment was timed to correspond with the clinical observation that massive PE is usually diagnosed at least 5 h after symptom onset (4, 37). The investigators who performed the injections and physiologic and biochemical measurements were blinded to the treatment group. Animals were studied at 16 h, because in pilot studies, we recognized this as the time when rats usually began to develop visible evidence of respiratory distress. A timeline of the experimental protocol is shown in Fig. 2. Controls rats were not randomized and were studied after the treatment efficacy experiment was completed. Control rats were not subjected to any stress other than anesthesia for instrumentation.

At 16 h after PE induction, surviving rats were again anesthetized by blinded technicians in the above-described fashion. Rats were placed on a warming pad filled with recirculating water warmed to 105°F (Gaymar solid-state T-pump; Orchard Park, NY). The rat’s neck was shaved, and a tracheostomy was performed by cannulating the trachea with PE-240 tubing. Both the right carotid artery and right internal jugular vein were dissected and cannulated with Millar Mikro-Tip micromanometer catheter transducers (Millar Instruments, Houston, TX). A 2-French Millar catheter (SPR-249-A) monitored arterial blood pressure in the carotid artery. A 2-French bent Millar catheter (SPR-513) was advanced through the internal jugular vein to monitor right atrial pressure. The right femoral artery was dissected and cannulated with PE-50 tubing filled with saline for arterial blood sampling.

After instrumentation, animals were ventilated with a small-animal, pressure-regulated, mechanical ventilator (model 2094; Kent Scientific, Litchfield, CT). Ventilator settings were as follows: respiratory rate 30–35, peak inspiratory pressure 10–14 cmH2O, flow 1.0 l per min, and partial pressure of oxygen was at room air. To measure ventilation parameters, we attached a gas flow transducer (model TSD 137C; Biopac Systems, Santa Barbara, CA) to the inspiratory limb of the ventilator circuit. End-tidal CO2 was measured by a side stream quantitative CO2 capnometer (model CO2,100A; Biopac Systems) attached to the expiratory limb. End-tidal arterial blood sampling.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>0</th>
<th>5</th>
<th>14</th>
<th>16</th>
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<tr>
<td>PE Induction</td>
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<td>Injection #2 Treatment</td>
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<td>Blood, urine and BAL samples taken; hearts removed for ex-vivo perfusion</td>
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**Fig. 2.** Timeline for the experimental protocol. See METHODS for description of interventions. PE, pulmonary embolism; BAL, bronchoalveolar lavage.
O₂ was measured by a side stream paramagnetic oxygen sensor attached to the expiratory limb (model O2100A; Biopac Systems). All pressure transducers as well as the flow transducer, oxygen sensor, and CO₂ capnometer supplied data that were visualized in real time and recorded via a commercially available data acquisition software program (AqQKnowledge, version 3.5.6; Biopac Systems). After instrumentation, animals in all groups were given succinylcholine (1 mg/kg iv) to relax breathing efforts, allowing end-tidal CO₂ measurements to be obtained from flat-topped, steady-state expirograms, during controlled, constant mechanical ventilation and without interference from interposed spontaneous breathing efforts. Injection of succinylcholine marked the beginning of data collection (time 0). Parameters measured included: end-tidal expired carbon dioxide (etCO₂), end-tidal expired oxygen (etO₂), minute ventilation (MV), MAP, right atrial pressure (RAP), pH, partial pressure of carbon dioxide in arterial blood (Paco2), partial pressure of oxygen in arterial blood, and lactate. Arterial blood gas and lactate results were obtained using a Med/Ultra II (Staf Profile Ultra; Nova Biomedical, Waltham, MA).

Pulmonary gas exchange was estimated primarily by the VD/Vt,calv, calculated using etCO₂ in the Severinghaus equation (1, 14, 25). Use of the etCO₂ to estimate the alveolar dead space fraction as opposed to the mixed-expired CO₂ was chosen because the end-tidal equation provides a more specific estimate of the alveolar dead space fraction compared with the physiological dead space fraction, which measures both airway and alveolar dead space (25). VD/Vt,calv correlates with pulmonary vascular resistance (22), and perfusion defects in humans with PE (17) decreases in parallel with reduction in perfusion defects during treatment (25) and correlates with the plasma concentration of TxB₂ in dogs with PE (39).

Measurements of MAP, RAP, MV, etCO₂, etO₂, and arterial blood gases were performed at 15 and 45 min after injection of succinylcholine. Total body CO₂ production (VCO₂) was calculated at body temperature, ambient pressure, and saturated with water vapor. In pilot work, we found that the side stream pumps that aspirate a portion of the expired breath volume for the oximeter and capnometer affected the MV, MAP, and arterial blood gas measurements. Therefore, the pumps were turned on only long enough to obtain steady-state measurements of etCO₂ and etO₂. After the 45-min gas exchange measurements were completed, bronchoalveolar lavage (BAL) was performed with 8 ml normal saline (23°C) using four washes via the tracheostomy fl bronchoalveolar lavage (BAL) was performed with 8 ml normal saline (23°C) using four washes via the tracheostomy fl bronchoalveolar lavage (BAL) was performed with 8 ml normal saline (23°C) using four washes via the tracheostomy fl bronchoalveolar lavage (BAL) was performed with 8 ml normal saline (23°C) using four washes via the tracheostomy fl bronchoalveolar lavage (BAL) was performed with 8 ml normal saline (23°C) using four washes via the tracheostomy.
ments were given with the same protocol described above. Sixteen hours after induction of PE, rats were anesthetized, and the right jugular vein was dissected and cannulated with PE-60 tubing. Spontaneously breathing, anesthetized rats were taken to the fluoroscopy suite. The tip of the PE-60 tubing was advanced under fluoroscopic guidance to touch but not traverse the tricuspid valve. One milliliter of iopamidol contrast agent (Isovue 250; Bracco Diagnostics, Princeton, NJ) was injected over 5 s while high resolution cineangiographic images were obtained in the anteroposterior views. The animal was repositioned, and repeat injections were performed to obtain lateral and then posterior-anterior views. Developed films were viewed on a cineangiographic projector (Vanguard XR-350; Vanguard Instrument, New York, NY) by a blinded observer to determine the single frame at which maximal opacification was observed and to obtain images for the manuscript and to grade the impairments in right heart function and lung perfusion. On the basis of pilot work, the observer graded the severity on a scale of 1–3 (1 = unchanged, 2 = moderately impaired, and 3 = severely impaired compared with images from healthy rats). The following parameters: 1) RV impairment (dilation, reduced filling, and hypokinesis), 2) degree of reversed flow (reflux) of contrast across the tricuspid valve into the inferior vena cava, 3) relative reduction in maximal lung opacification, and 4) delay in return of contrast to the LV after injection.

Measurement of pleural effusion, lung tissue water content, and pulmonary histology. Six rats were subjected to the PE procedure as described above, but no treatment was given. Six healthy rats were used for comparison. Animals were anesthetized, and a celiotomy was performed. We used a battery-operated electrocautery device to cauterize a 3-mm hole in the midline of the ventral diaphragm and took care not to touch the lung. A flexible polyvinyl catheter attached to a suction bulb was passed through the hole to aspirate fluid found in the thorax. The volume of the fluid was then measured. The chest was then opened via midline thoracotomy, and the lungs were freeze-clamped in situ with aluminum tongs cooled in liquid nitrogen. Powdered frozen wet lung tissue was weighed and dried overnight at 90°C and reweighed to calculate the wet-to-dry ratio from which the total lung water content was measured. For histology, six additional rats underwent the PE protocol, and after anesthesia, both lungs and the heart were removed and placed in 10% buffered formalin solution. The organs were sent to a commercial histology laboratory (Histoserve, Vienna, VA) for fixation and slide preparation with hematoxylin and eosin staining. Hearts were sectioned in two planes in the long axis to show a four-chamber view, and lungs were sectioned in two planes in the long axis.

Statistical analyses and sample size calculation. Data are presented as means with standard error. Data were compared for significance between the three groups using a one-way ANOVA with Tukey-Kramer post hoc test with $\alpha = 0.05$. All statistical tests were performed with StatsDirect software, version 1.2.2. In accordance with IACUC requirements, we calculated the minimum sample size required to test the hypothesis that pharmacological reduction in production of vasoconstrictor prostaglandins would improve $V_{T}/V_{T}^{\text{val}}$ and $R_{V}$ function following PE. In a pilot study, we found that rats subjected to PE (0.15 ml/100 g of 10% polyvinyl microspheres of mean diameter 24 μm) demonstrated a mean increase in $V_{P}/V_{T}^{\text{Val}}$ from 0.30 ± 0.15 (SD) compared with healthy rats 0.08 ± 0.06 (SD). Assuming that a 50% improvement in $V_{P}/V_{T}^{\text{Val}}$ represented an important change, at an 80% power to detect this difference with $\alpha = 0.05$, we estimated the sample size at $n = 9$. Rats were assigned to treatments based on a computer-generated random sequence. An independent monitor determined when nine surviving rats from each treatment group were studied to completion of data collection.

RESULTS

The survival rate 16 h after induction of PE in rats treated with saline was 9/15 (60%, 95% CI: 32–84%), and the survival rate after furegrelate and ketorolac treatment was identical, at 9/11 (82%, 95% CI: 48–98%).

Effect of PE and treatment on prostanoid production. BAL samples from surviving rats subjected to PE 16 h before demonstrated a significant increase in TxB2 and leukotriene C4, D4, and E4 concentrations compared with lavage samples from healthy controls (Fig. 3, A and B). Treatment with both furegrelate and ketorolac significantly reduced the TxB2 concentrations compared with placebo. However, neither treatment was
associated with a significant change in cysteinyl-leukotriene C₄, D₄, and E₄ concentrations, indicating that blockade of prostaglandin synthesis at either the COX-1/2 or the thromboxane synthase enzyme did not lead to a significant shunt in arachidonate toward the synthesis of leukotriene C₄, D₄, and E₄.

Figure 4, A–C, demonstrates the concentrations of 2,4-dinor TxB₂, PGE₂, and PGF₂α found in urine aspirated from the bladder 16 h after induction of PE. Rats subjected to PE demonstrated approximately a threefold increase in urinary 2,4-dinor TxB₂ concentration but no increase in PGE₂. With both furegrelate and ketorolac treatments, the urinary 2,4-dinor TxB₂ concentrations were decreased. Ketorolac, but not furegrelate, caused a decrease in urinary PGE₂ and PGF₂α concentration. These data reflect the fact that ketorolac causes proximal inhibition of prostaglandin synthesis. Furegrelate treatment also caused a decrease in 2,4-dinor TxB₂ concentration but did not lead to an increase in either PGE₂ or PGF₂α, as might be predicted based on blockade of thromboxane synthase (site B in Fig. 1).

Table 1 demonstrates in vivo hemodynamic data measured in surviving rats 16 h after induction of PE (n = 9 per group). Means from two 1-min sample periods (the first obtained at 15 min and the second at 45 min after injection of succinylcholine) were averaged to give one number for each parameter in Table 2. Compared with healthy controls, rats subjected to PE and treated with saline demonstrated significant arterial hypotension (P = 0.007), increased arterial lactate concentration, but no significant change in RAP. Treatment with both furegrelate and ketorolac increased the MAP to a level that was not different from control, but only rats treated with ketorolac demonstrated a significant improvement in MAP compared with placebo. Treatments did not significantly alter arterial lactate concentrations. These data suggest that neither treatment completely ameliorated the total-body perfusion defect from the PE insult, but ketorolac treatment was associated with a slightly higher peripheral vascular resistance, leading to the higher arterial pressures.

Table 2 presents arterial blood gas and end-tidal partial pressure data for the four groups. Rats subjected to PE demonstrated a significant decrease in peak partial pressure of carbon dioxide measured at end-tidal respiration (PetCO₂) and an increase in the nadir end-tidal partial pressure of oxygen measurement (PetO₂), coincident with increased V₀₂/VTalv (Fig. 5A) and significantly increased the difference between the PetO₂ and the arterial P O₂ (Fig. 5B). Rats treated with ketorolac and furegrelate after PE did not show significant change in these parameters when compared with control rats. All rats with PE tended to have a lower VCO₂, and this effect reached statistical significance in the placebo and furegrelate groups. This re-

<table>
<thead>
<tr>
<th>Table 1. In vivo hemodynamic data and arterial lactate concentrations</th>
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</tr>
<tr>
<td>Control</td>
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<tr>
<td>Placebo</td>
</tr>
<tr>
<td>Furegrelate</td>
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<tr>
<td>Ketorolac</td>
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*Controls were healthy rats with no pulmonary embolism (PE). Rats in treatment groups were studied 16 h after induction of PE. The mean (±SE) shown for each parameter is the average of 2 measurements made at 15 and 45 min after start of mechanical ventilation (MV). MAP, mean arterial pressure; RAP, right arterial pressure. *P < 0.05 by Tukey-Kramer post hoc test compared with control; †P < 0.05 by Tukey-Kramer post hoc test compared with placebo.
duction in V\textsubscript{CO\textsubscript{2}} may have offset any increase in V\textsubscript{D}/V\textsubscript{Talv}, preventing a significant increase in Pa\textsubscript{CO\textsubscript{2}}. Treatment with both furegrelate and ketorolac decreased the V\textsubscript{D}/V\textsubscript{Talv} significantly compared with placebo. Both treatments tended to improve the Pet\textsubscript{CO\textsubscript{2}}, Pet\textsubscript{O\textsubscript{2}}, and the Pet\textsubscript{O\textsubscript{2}}-to-arterial P\textsubscript{O\textsubscript{2}} gradient, although none of the changes were statistically significant when compared with placebo rats. Neither furegrelate nor ketorolac treatment significantly altered arterial blood P\textsubscript{O\textsubscript{2}} or Sa\textsubscript{O\textsubscript{2}}% compared with placebo.

Figure 6 shows the RV and LV systolic pressure data from ex vivo beating hearts. Hearts from rats treated with placebo demonstrated a significant reduction in both RV and LV systolic function. Both furegrelate and ketorolac treatments were associated with significantly improved RV systolic function. Ketorolac, but not furegrelate treatment, was associated with improved LV systolic function.

Table 2. Respiratory and arterial blood gas measurements

<table>
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<tr>
<th>Group</th>
<th>MV, ml/min</th>
<th>pH</th>
<th>P\textsubscript{aco2}, Torr</th>
<th>P\textsubscript{ao2}, Torr</th>
<th>Sa\textsubscript{o2}, %</th>
<th>Pet\textsubscript{co2}, Torr</th>
<th>Pet\textsubscript{o2}, Torr</th>
<th>V\textsubscript{CO2} ml/min at BTPS</th>
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<tr>
<td>Control</td>
<td>59 ± 4.2</td>
<td>7.53 ± 0.02</td>
<td>35 ± 1.7</td>
<td>87 ± 2.7</td>
<td>98 ± 0.2</td>
<td>33 ± 1.9</td>
<td>125 ± 2.8</td>
<td>0.197 ± 0.015</td>
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<tr>
<td>Placebo</td>
<td>50 ± 3.4</td>
<td>7.42 ± 0.03</td>
<td>36 ± 2.8</td>
<td>67 ± 5.6</td>
<td>91 ± 2.9</td>
<td>28 ± 2.4</td>
<td>131 ± 2.0</td>
<td>0.101 ± 0.008*</td>
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<tr>
<td>Ketorolac</td>
<td>50 ± 4.7</td>
<td>7.47 ± 0.03</td>
<td>32 ± 2.3</td>
<td>69 ± 5.2</td>
<td>91 ± 2.6</td>
<td>28 ± 1.7</td>
<td>131 ± 2.0</td>
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Values are means ± SE. P\textsubscript{aco2}, partial pressure of carbon dioxide in arterial blood; P\textsubscript{ao2}, partial pressure of oxygen in arterial blood; Sa\textsubscript{o2}, oxygen saturation in arterial blood; Pet\textsubscript{co2}, peak partial pressure of carbon dioxide at end-tidal respiration; Pet\textsubscript{o2}, nadir partial pressure of oxygen and end expiration; V\textsubscript{CO2}, total body carbon dioxide production; BTPS, body temperature. *P < 0.05 by Tukey-Kramer post hoc test compared with control, ambient pressure, saturated with water.

Fig. 5. Indexes of gas exchange; n = 9 per group. A: the alveolar dead space fraction (V\textsubscript{D}/V\textsubscript{Talv}). Both furegrelate and ketorolac significantly decreased this measurement. B: the Pet\textsubscript{O\textsubscript{2}}-arterial P\textsubscript{O\textsubscript{2}} gradient. *P < 0.05 vs. control; **P < 0.05 vs. placebo; 1-way ANOVA, Tukey-Kramer post hoc.

Fig. 6. Measurements of intrinsic cardiac function in hearts that were removed from rats and perfused ex vivo; n = 9 per group. A: right ventricular systolic pressure (RVSP). B: left ventricular systolic pressure (LVSP). *P < 0.05 vs. control; **P < 0.05 vs. placebo; 1-way ANOVA, Tukey-Kramer post hoc.
showed significant impairment in all parameters graded in reference to controls. The images from placebo rats demonstrated severe RV hypokinesis with severe regurgitation of contrast into the vena cavae and slowed forward flow of contrast into the lungs. As a result of the dilution of the contrast from the tricuspid regurgitation as well as the slow forward flow, the maximal opacification of the lungs in placebo rats was reduced when compared with controls. Rats treated with furegrelate demonstrated a tendency to have greater lung opacification, mostly observed in the "return" or capillary phase during the period when contrast was returning to the LV. However, furegrelate-treated rats demonstrated moderate-to-severe reflux of contrast into the inferior vena cava and delayed LV opacification. Rats treated with ketorolac demonstrated mild RV dilation, slightly reduced peripheral lung opacification, no visible reflux of contrast into the inferior vena cava, and normal return of contrast to the LV.

Assessment of pleural effusion, lung water content, and histology. In six control rats, no measurable pleural effusion volume was recovered. In six rats subjected to the PE protocol and no treatment, the volume of pleural effusion after 16-h duration was 8.5 ± 0.9 ml. Lung water content was not different in control rats (left lungs 79.4 ± 0.34%, right lungs 77.9 ± 0.70%) vs.

Table 3. Pulmonary angiography data

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<th>Placebo</th>
<th>Furegrelate</th>
<th>Ketorolac</th>
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<tr>
<td>RV dilation</td>
<td>3.0</td>
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<tr>
<td>Lung opacification</td>
<td>2.0</td>
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<tr>
<td>Tricuspid regurgitation</td>
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<td>2.7</td>
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<tr>
<td>LV filling delay</td>
<td>2.0</td>
<td>2.7</td>
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Data were evaluated on a 1–3 score relative to controls (1 = no change, 2 = moderate impairment, 3 = severe impairment) for four parameters: 1) Right ventricle (RV) dilation and hypokinesis observed during contrast injection into the right atrium, 2) opacification of the periphery of both lungs, 3) regurgitation of the contrast media through the tricuspid valve and into the inferior vena cava, and 4) time delay for filling of the left ventricle (LV). Data are shown for the means of $n = 3$ in each group.

Fig. 7. Images are digitized single frames of anteroposterior views photographed from real-time cineangiofluoroscopy of rats that received 1.0 ml of iodinated contrast injected into the right atrium. Each image corresponds to the frame of maximal lung opacification within 60 s after contrast injection. A: from a healthy, anesthetized rat. The right ventricle is fully opacified and appears crescent-shaped. B: from a placebo rat that received pulmonary vascular occlusion 16 h previously with polystyrene microspheres as described in METHODS. Photograph B demonstrates 3 of 4 key abnormalities induced by untreated PE, including regurgitation of contrast into the inferior vena cava (arrow), impaired right ventricular filling, with a globular appearance to the right ventricle (arrowheads), and slightly decreased peripheral lung opacification (bracket). The photograph does not adequately demonstrate the abnormality of delayed opacification of the left ventricle. C: effect of furegrelate treatment, which produced bright opacification that was best observed during the return of contrast to the left ventricle, during the so-called venous return phase. D: effect of ketorolac treatment, including a relatively normal right ventricle and the absence of tricuspid regurgitation but with slightly diminished peripheral lung opacification.

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rats subjected to PE (left lungs 79.1 ± 0.2%, right lungs 79.2 ± 0.32%). Lung sections demonstrated homogenous distribution of the polystyrene microspheres in the prealveolar arterioles in both lungs. No polystyrene microspheres were observed in the coronary vasculature.

DISCUSSION

This study was conducted to determine whether inhibition of prostaglandin synthesis at either site A or B in Fig. 1 would have a beneficial effect on pulmonary gas exchange and cardiac function during fixed, prolonged pulmonary vascular occlusion in the absence of infused autologous clot.

We tested the hypothesis that inhibition of the thromboxane synthase enzyme would be more beneficial than inhibition of the COX-1/2 enzyme during pulmonary vascular occlusion.

The urine and BAL prostaglandin concentrations indicate that both treatments successfully inhibited their pharmacological targets. Ketorolac treatment produced significant reduction in PGF2α, PGE2, and TxB2 in the urine but did not cause leukotriene C4, D4, and E4 concentrations to increase significantly in BAL samples. Furegrelate also decreased TxB2 concentrations in BAL but did not increase PGF2α concentrations in urine. Our hypothesis was that thromboxane synthase inhibition (see Fig. 1) might produce a beneficial pattern of decreased thromboxane concentrations and increased concentrations of vasodilatory prostaglandins PGE2 and PGI, whereas ketorolac might uniformly decrease all prostaglandin concentrations and, therefore, be less efficacious. This hypothesis was formed primarily on theoretical grounds, because prior experimental evidence in a prolonged model of pulmonary vascular occlusion was lacking (9, 26, 39). On the basis of analysis of the physiological and angiographic data in this study, it appears that COX-1/2 inhibition demonstrated at least equal efficacy to thromboxane synthase inhibition comparing the end points of pulmonary gas exchange and RV function and possibly better efficacy on LV function and MAP.

The second question was whether inhibition of prostaglandin synthesis at either site A or B (Fig. 1) could exert a beneficial effect in the setting of nonthrombotic, fixed pulmonary vascular occlusion. Prior experimental models of PE that have either measured or pharmacologically altered prostaglandin concentrations included autologous clot infusion (9, 26, 30, 39). These animal models have found short-lived increase in plasma thromboxane concentrations (30, 39) and conflicting results as to whether pretreatment with COX-1/2 inhibition is beneficial (26, 30) or detrimental (9) to gas exchange and pulmonary vascular resistance (9). The use of autologous clot introduces two variables that are difficult to control. First, fresh clots, per se, produce vasoactive metabolites (13). Thus with fresh clots, it is difficult to determine if pharmacological inhibition of prostaglandin synthesis is affecting production of prostaglandins from the clot or production from the lung tissue. Second, in the rat, fresh clots are dissolved within a few hours by endogenous fibrinolysis (21). This effect could account for the observation of an ephemeral increase in plasma thromboxane concentrations with autologous clot models. In humans, thromboembolism to the lung almost always occurs from mature, organized thromboses that produce more persistent pulmonary vascular occlusion (40). In humans with major pulmonary thromboembolism (mean baseline perfusion defect ≥40%), anticoagulation with heparin relieves only 0–5% of the perfusion defect after 24 h, whereas fibrinolytic treatment relieves only 5–15% of the perfusion defect after 24 h (6, 12, 18, 27, 31). Moreover, the diagnosis of PE is often not discovered until 24 h after symptom onset (37). These data may help explain why humans diagnosed with PE maintain a significant increase in plasma TxB2 and PGF2α concentrations for up to 7 days after symptom onset (10).

For these reasons, we used polystyrene microspheres rather than infusing autologous clots. The concentration and size of the polystyrene microspheres were constant, whereas in prior work, we found that the concentration and size of blood-derived clots were difficult to standardize (36). Microscopic examination revealed that the microspheres were uniformly distributed in precapillary lung arterioles 16 h after dosing and that no microspheres were seen in the heart vasculature, suggesting the absence of embolization to the coronary arteries (as could be speculated if a patent foramen ovale were present). Microscopic examination of lungs containing the microspheres demonstrated that the pulmonary arteries were free of thrombotic material. The present model allows us to conclude that inhibition of COX-1/2 and thromboxane synthase after the induction of PE improved VD/VTalv and RV function by action on nonembolized vasculature, as opposed to recanalization of embolized vasculature.

The data show that both COX-1/2 inhibition and thromboxane synthase inhibition reduced VD/VTalv without a significant change in arterial blood oxygenation compared with placebo-treated rats. This finding has mechanistic and therapeutic implications specific to PE. The present model produces primarily pulmonary vascular occlusion without an increase in lung water content, suggesting the absence of pulmonary edema. In contrast, most inflammatory models of acute lung injury are designed to produce increased transcapillary leak, resulting in alveolar edema, and increased shunt fraction. In an edematous model of lung injury produced by infusion of oleic acid, Schulman et al. (33) showed that meclofenamate improved oxygenation by promoting redistribution of lung perfusion away from injured lung toward normal lung. By enhancing perfusion redistribution, meclofenamate improved hypoxemia by decreasing the fraction of the cardiac output that perfused the poorly ventilated, edematous lung (i.e., decreased the shunt fraction). In contrast, Dantzker et al. (7) demonstrated that induction of PE in dogs causes arterial hypoxemia by redistribution of pulmonary blood flow away from the oc-
cluded vasculature, producing regions of nonoccluded lung with low ventilation-perfusion relationships, leading to increased venous admixture and hypoxemia. With this in mind, we were concerned that either treatment might reduce the effect of TxB2 in nonoccluded lung regions, thus producing a trade-off of increased flow through nonoccluded vasculature, but at the expense of increased venous admixture and worsened hypoxemia. In fact, neither treatment worsened blood oxygenation. Both treatments reduced Vp/Vtval and the degree of RV dilation (and tricuspid regurgitation was observed on pulmonary angiography) and improved RV function during ex vivo perfusion. Both treatments ameliorated the increase in TxB2 concentration found in the BAL. Together, these findings suggest that, after pulmonary vascular occlusion in our model, TxB2 produced mostly a detrimental effect on the balance of pulmonary vasoconstriction and dilation and that inhibition of its synthesis did not cause worsened hypoxemia.

Several authors have suggested that a controlled trial of a COX inhibitor to treat humans with PE can be justified on the basis of previously published literature (23, 29, 35). We believed that a randomized, blinded treatment study of treatment efficacy in animals was needed before further clinical testing. Because bias can occur in drug testing even in animal models (2), we used a study protocol that employed blinding and randomization to reduce this potential bias. We interpret the present findings as firm evidence that neither ketorolac nor furegrelate treatment was harmful to rats subjected to fixed, pulmonary vascular occlusion.

Several aspects of this model warrant critical consideration. The size of the microspheres was relatively small, causing occlusion of precapillary arterioles, rather than segmental or lobar vascular occlusion, which is the usual circumstance in humans (8). We were also unable to provide any direct evidence of pulmonary vasospasm in nonoccluded vasculature. In pilot work, we attempted to measure pulmonary arterial pressure and pulmonary vascular resistance but found that these measurements were extremely labile, and the rats with PE developed arterial hypotension with introduction of a micromanometer into the RV. For this reason, we used pulmonary angiography to demonstrate visual evidence of increased pulmonary vascular resistance. The finding that RAP 16 h after induction of PE did not increase in vivo in placebo rats might appear somewhat contrary, given that severe tricuspid regurgitation was observed on pulmonary angiography. When measured immediately after the infusion of polystyrene beads, the RAP increased by ~50% (5). In view of the severe depression in ex vivo function in placebo rats studied 16 h after PE induction, together with the observation of RV hypokinesis during angiography, we speculate that the RAP data in the placebo rats represent pseudonormalization secondary to the inability of the damaged myocardium to generate enough systolic contraction to compress the right atrium in dehydrated animals. Animals in all treatment groups were also pretreated with succinylcholine to facilitate accurate collection of physiological parameters. The effect of succinylcholine on prostaglandin metabolism in this model remains uncertain, but we can find no evidence to suggest that succinylcholine affects the production of eicosanoids. Finally, this model produced large pleural effusions that could have compromised ventilation. The etiology of the pleural effusions and the role of treatment in reducing pleural effusion as a possible mechanism of efficacy in this model are currently under study.

In summary, this experiment presents data from a randomized, blinded animal study that demonstrates the presence of increased concentrations of TxB2 in the BAL and 2,3-dinor TxB2 in the urine 16 h after induction of pulmonary vascular occlusion with polystyrene microspheres. Posttreatment with a nonselective COX-1/2 inhibitor, ketorolac, decreased the concentrations of TxB2 in BAL and PGF2α, and PGE2 in urine; it was associated with a significant decrease in measured Vp/Vtval and improved in vivo MAP and ex vivo RV function. Selective inhibition of thromboxane synthase with furegrelate decreased BAL TxB2 concentrations and significantly decreased the Vp/Vtval and improved ex vivo RV function. These data show that neither treatment was detrimental to the treatment of PE and that COX-1/2 inhibition was at least as effective as thromboxane synthase inhibition in a rat model of fixed pulmonary vascular occlusion.

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AJP-Lung Cell Mol Physiol • VOL 284 • JUNE 2003 • www.ajplung.org