Pentoxifylline Rescue Preserves Lung Function in Isolated Canine Lungs Injured With Phorbol Myristate Acetate*

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Objective: We hypothesized that pentoxifylline, administered after phorbol myristate acetate (PMA), would diminish the severity of lung injury.

Setting: Animal research laboratory.

Design: Comparative study.

Subjects: Mongrel dogs (n = 33).

Interventions: Baseline measurements were obtained from the isolated blood-perfused dog lung lobes after 1 h of stable perfusion and ventilation. Four different measures of lung compliance were obtained along with WBC and neutrophil counts. Pulmonary vascular resistance (PVR) and capillary filtration coefficient (Kf) were calculated, and the ratio of a normalized maximal enzymatic conversion rate to the Michaelis-Menten constant (Amax/Km) was used to assess perfused capillary surface area. The control lobes (n = 8) were ventilated and perfused for an additional 40 min while the injured lobes (n = 17) received PMA (0.1 μg/mL of perfusate). The pentoxifylline-protected lobes (n = 8) were treated with pentoxifylline (1 mg/mL of perfusate) 10 min after injury with PMA. All measurements were then repeated.

Measurement and main results: The three groups did not differ significantly at baseline. The control lobes remained relatively stable over time. The injured lobes demonstrated marked deterioration in compliance: 8.79 ± 0.70 to 5.97 ± 0.59 mL/cm H2O (p < 0.05) vs 10.1 ± 1.0 to 8.07 ± 0.72 mL/cm H2O and 9.6 ± 1.1 to 9.9 ± 0.85 mL/cm H2O in the control and protected lobes, respectively. Both groups receiving PMA had similar drops in WBC and neutrophil counts, but the pentoxifylline-protected lobes had preservation of all four compliance measures. PVR increased from 37.8 ± 1.8 to 118.6 ± 12.7 cm H2O/L/min (p < 0.05) in the injured lobes vs 35.4 ± 0.5 to 36.3 ± 2.8 cm H2O/L/min and 40.4 ± 0.04 to 46.7 ± 2.8 cm H2O/L/min (p < 0.05) in the control and protected lobes, respectively. Kf increased <25% in the protected group but more than tripled in the injured group. Amax/Km dropped from 559 ± 36 to 441 ± 33 mL/min (p < 0.05) in the injured lobes vs 507 ± 14 to 490 ± 17 mL/min and 609 ± 34 to 616 ± 37 mL/min in the control and pentoxifylline-protected lobes, respectively.

Conclusions: The use of pentoxifylline as a rescue agent prevented the PMA-induced deterioration of lung compliance, vascular integrity, and endothelial metabolic function in this acute lung injury model, despite significant pulmonary neutrophil sequestration. (CHEST 2001; 119:1893–1900)

Key words: acute lung injury; pentoxifylline; phorbol myristate acetate; pulmonary function

Abbreviations: ACE = angiotensin-converting enzyme; ALI = acute lung injury; Amax/Km = ratio of a normalized maximal enzymatic conversion rate to the Michaelis-Menten constant; ANC = absolute neutrophil counts; 3H-BPAP = 3H-Benzoyl-Phe-Ala-Pro; Kf = capillary filtration coefficient; LLL = left lower lobe; PEEP = positive end-expiratory pressure; PMA = phorbol myristate acetate; PMN = polymorphonuclear leukocyte; PVR = pulmonary vascular resistance; TNF = tumor necrosis factor; Vt = tidal volume

ARDS remains a common cause of morbidity and mortality in patients with sepsis and trauma.1 Evidence2 points to the neutrophil as a key intermediary in acute lung injury (ALI), since ARDS results from a complex interplay of inflammatory mediators, including products of arachidonic acid metabolism, cytokines, and oxygen-free radicals.1 Models using artificially stimulated neutrophils (polymorphonucle-
ar leukocytes [PMNs]) have demonstrated PMN sequestration and activation in the pulmonary capillaries. This sequestration leads to endothelial cell damage, pulmonary hypertension, neutropenia, hypoxemia, and increased microvascular permeability. Phorbol myristate acetate (PMA) has been shown to induce ALI by activating PMNs, aggregating platelets, and by a neutrophil-independent production of oxygen-free radicals and pulmonary vasoconstriction.

Modification of the neutrophil-endothelial interaction stimulated by proinflammatory events has been the focus of much research in the past several years. One approach is to attempt to interrupt the inflammatory process with drugs. Pentoxifylline, a methylxanthine-derived phosphodiesterase inhibitor, has been used experimentally for this purpose. Pentoxifylline increases both RBC and neutrophil deformability, increases intracellular cyclic adenosine monophosphate, and decreases pulmonary PMN sequestration in ALI models. Pentoxifylline may exert protective effects by increasing cyclic adenosine monophosphate, acting as an antioxidant, or by interfering with the elaboration of inflammatory mediators.

Pentoxifylline pretreatment prior to PMA injury has prevented many of the manifestations of ALI in isolated blood-perfused dog lungs (L.L. McCloud; unpublished data; 1998). This evaluation of ALI included changes in pulmonary vascular resistance (PVR), capillary filtration, neutrophil trapping, and estimates of endothelial angiotensin-converting enzyme (ACE) activity. ACE is a pulmonary endothelial ectoenzyme that is homogeneously distributed along the luminal pulmonary endothelial surface. This ACE distribution allows the use of ACE activity as an assessment of the metabolic function of the entire pulmonary vasculature. Since pulmonary ACE dysfunction can occur before morphologic changes, the measurement of altered enzyme function can serve as a sensitive marker of endothelial injury.

In addition to chemical interruption of the inflammatory ALI, we were interested in research that focused on limiting ventilator-associated lung injury. Mechanical ventilation strategies, which use positive end-expiratory pressure (PEEP), have been pivotal in the treat of patients with ARDS. PEEP can recruit collapsed alveoli and help avoid cyclic alveolar collapse/reopening, thereby improving functional residual capacity and lung compliance. Inappropriately low PEEP can also lead to a marked increase in inflammatory cytokine release. We postulated that PEEP might also alter PMN flux by maintaining appropriate capillary architecture through the recruitment of atelectatic segments.

Since most of the previous work done with ALI models has involved pretreatment prior to an inflammatory event, we wanted to evaluate a rescue strategy. We hypothesized that pentoxifylline administered as a rescue agent after PMA would diminish the severity of lung injury. In addition, we hypothesized that using PEEP higher than the pressure at closing volume, to maintain normal end-expiratory lung volume, would diminish the manifestations of PMA-induced ALI. Using our isolated blood-perfused canine lung model, we could assess lung compliance, endothelial metabolic function, vascular integrity, and neutrophil trapping to determine the impact of our interventions.

**Materials and Methods**

**Isolated Lung Lobe Preparation**

We used mongrel dogs weighing 20.7 ± 0.33 kg (mean ± SD). Investigators complied with national standards regarding care and use of laboratory animals. The facilities were fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. They were anesthetized with 30 mg/kg of IV pentobarbital sodium (Abbott Laboratories; North Chicago IL). They were then intubated and received mechanical ventilation (rate, 10 to 15 breaths/min; tidal volume [VT], 15 mL/kg) with a Harvard ventilator (model 607; Harvard Apparatus; Holliston, MA). The rate and VT were adjusted to achieve adequate chest rise and normal arterial blood gas levels. Central arterial and venous access was obtained from the right groin via cutdowns. The left side of the chest was opened through the fifth intercostal space; the upper and cardiac lobes were tied and removed. The dogs were then anticoagulated with 10,000 U of heparin sodium through a femoral venous line. After 10 min, they were exsanguinated from a femoral arterial cannula into a graduated cylinder that contained another 10,000 U of heparin sodium. After phlebotomy and before cardiac arrest, the left lower lobe (LLL) artery was tied and the LLL bronchus was clamped during full inflation. The lobes were then removed and weighed. The perfusion circuit was filled with 400 mL of the shed blood and diluted with 100 mL of normal saline solution with a resulting hematocrit of 35.2 ± 0.77. The LLL bronchus, vein, and artery were then cannulated. Care was taken to avoid both the introduction of air bubbles into the artery and deflation of the lobe. The LLL was placed in a nonrestrictive plastic bag, perforated to allow drainage of any condensation or fluid, and placed on a platform. The flexible airway and perfusion catheters were suspended and their positions fixed by clamping for the course of the experiment. The lobe was then perfused at constant flow, 600 mL/min, via the lobar artery using a roller head pump (Sarns Model P/N 206944; Sarns 3M Health Care; Ann Arbor, MI). The perfusion circuit consisted of an acrylic (Plexiglas) venous reservoir in which the blood was continuously stirred and recirculated to the LLL after passage through a blood filter, a water-jacketed heat exchanger maintained at 38 ± 0.5°C, and a bubble trap. Blood temperature was maintained at 37°C, and the blood was returned to the reservoir via a large-diameter venous catheter. A screw clamp was placed on the venous catheter to keep the lobar vein pressure constant at 5 cm H2O. Pump blood flow was calibrated daily by timed collections from the pulmonary arterial catheter. During the experiment, pulmonary artery pressures, pulmonary vein pressures, and bronchial pressures were monitored continuously via pressure transducers. Lobe weight
change was monitored using a force transducer (Grass FT03C; Grass Instrument; West Warwick, RI) attached to a trip balance. Transducers were zero referenced to the midpoint of the lobe hilum with electrically meaned pressures continuously recorded on a polygraph (Grass model 7D; Grass Instruments).

**Measures of Lobe Compliance**

Once the isolated lobes were perfused, the bronchial clamp was released and the lobes were allowed to completely deflate. A syringe filled with room air was then attached to the bronchial catheter, and inflation was initiated from complete collapse to total lobe capacity, 240 mL, in 20-mL aliquots.\(^{14,15}\) The lobes were given time for airway pressures to stabilize between each stepwise increase or decrease. All the lobes underwent inflation and deflation to construct pressure-volume curves. Each lobe had the opening pressure, the static compliance, and the closing pressure determined from its individual pressure-volume curve. The opening pressure is the pressure at the lower inflection point of the inflation limb and represents alveolar recruitment. Opening pressure has been used clinically\(^{21}\) to estimate lung stiffness and to help determine optimum PEEP. The static compliance used in this set of experiments is determined from the slope of deflation limb between the upper deflection point and closing volume. Closing pressure is the pressure at closing volume and is demarcated at the deflation limb lower inflection point. Closing volume closely correlates with phase IV of \(N_2\) washout, which represents alveolar and small airway collapse.\(^{17,18}\) By measuring these three estimates of overall lung compliance before and after the experimental protocols, we were able to determine their relative effects on respiratory function.

**Ventilation and Perfusion**

All lobes were perfused with blood at 600 mL/min and ventilated with a Siemens 900c ventilator (Siemens Corporation; Munich, Germany). Ventilation was performed with PEEP just above closing pressure and \(V_t\) equivalent to 10 mL/kg of dog weight at a rate of 10 breaths/min. Fraction of inspired oxygen was set at 21%, and \(CO_2\) was added to maintain a normal blood gas level. Blood samples were obtained from the venous cannula for blood gases and pH measurements (AVL 945; AVL Scientific; Roswell, GA). Sodium bicarbonate was added to the venous reservoir as needed to maintain pH at normal levels. After 1 h of stable perfusion and ventilation, baseline measurements were obtained.

**Spot Compliance**

Testing was performed with an inline Collins 1-L spirometer (Warren E. Collins, Inc; Braintree, MA) using exhaled \(V_t\), plateau pressure, and PEEP to calculate compliance at various time intervals throughout the protocol. Plateau was obtained by measuring inspiratory pressure at end inspiration and applying an inspiratory pause hold. The best spot compliance obtained for each lobe was then compared to the last spot compliance to determine relative changes over time.

**WBC and Neutrophil Counts**

WBC and neutrophil counts were obtained serially throughout the protocol, beginning with a jugular vein sample. The specimens for comparison were obtained after 60 min of stable perfusion and ventilation prior to any group-specific intervention and then again 40 min later in all groups. The CBC counts were done by either Unopette manual determination method (Becton Dickinson; Franklin Lakes, NJ) and counted on a Reichert hemocytometer (Hauser Scientific; Horsham, PA) or collected in purple-top specimen (Vacutainer) tubes with ethylenediaminetetra-acetic acid and counted on a Coulter counter (Beckman Coulter; Fullerton, CA). All differentials for neutrophil determination were counted manually. This was accomplished by placing 1 mL of blood in each of two Wintrobe tubes, and then centrifuging at 3,000 revolutions/min for 10 min. Slides were then made from the resulting buffy coats. The slides were stained using the Yeti-stain technique (Kacey; Hendersonville, NC), which is similar to a Wright-Giemsa stain. Differential counts were based on 100 cells read on a Diapan microscope (type 020-437.033; Diapan Leitz; Wetzlar, Germany). Absolute neutrophil counts (ANCs) were calculated by multiplying the percentage of PMNs by the total number of WBCs.

**Vascular Integrity Measures**

The capillary filtration coefficient \((K_f)\) is defined as change in the amount of fluid (millifilters per minute) filtering across the pulmonary capillary wall for a unit change in pressure, per unit of time, per 100 g of tissue.\(^{19,20}\) During baseline conditions, the lobe is maintained in an isogravimetric state by holding venous pressure at 5 cm \(H_2O\). \(K_f\) was determined by using a finely adjusted clamp on the venous cannula to increase the pulmonary venous pressure. This in turn led to an elevated capillary pressure with a resultant fluid filtration. The rate of weight gain was obtained by dividing the change in weight after the first 3 min by the change in time. The capillary pressure measurement required for the calculation of \(K_f\) was obtained by the double-occlusion technique before and during venous pressure elevation. This double-occlusion technique allows an accurate estimate of average microvascular pressure at the filtering midpoint of the lung.\(^{21}\) PVR was determined by subtracting the pulmonary vein pressure from the pulmonary arterial pressure and dividing the difference by the set flow of 600 mL/min. We measured the \(K_f\) and PVR after 60 min of stable perfusion and ventilation prior to any group-specific intervention and then again 40 min later in all groups. The lobes were weighed before and after the experimental protocols.

**Endothelial Metabolic Function Measures**

For determination of ACE activity, we injected trace amounts of \(^{3}H\)-Benzyol-Phe-Ala-Pro \((^{3}H\text{-BPAP})\), a synthetic ACE substrate under first-order reaction conditions in the pulmonary circulation. \(^{3}H\text{-BPAP}, 2 \mu\text{Ci} (20 \text{ Ci/mmol}),\) was injected into the pulmonary arterial cannula and flushed with 1 mL of saline solution. Simultaneously, a peristaltic pump withdrew blood (30 mL/min) from the lobe catheter into an automated fraction collector containing 13 \(\times\) 75-mm borosilicate tubes advancing at the rate of one tube per 0.6 s (0.3 mL/blood/tube). Blood was collected into normal saline solution containing 1 mM captopril to prevent further metabolism of \(^{3}H\text{-BPAP}\) by ACE. After collection, each tube was mixed by inversion and centrifuged at 3,000 revolutions/min for 10 min to separate the RBCs. A supernatant aliquot (0.5 mL) was transferred to 7-mL polyethylene scintillation vials, and “total” \(3H\) radioactivity was measured in a liquid scintillation spectrophotometer in the presence of 6 mL of Ecoscint A (National Diagnostics; Atlanta, GA). Another 0.5-mL supernatant aliquot was transferred to 7-mL scintillation vials containing 2.5 mL 0.11 N HCl, 3 mL 0.4% Omnifluor (Dupont, Boston, MA) in toluene was added, and radioactivity was measured in a scintillation spectrophotometer. This resulted in the extraction of 61% of the \(^{3}H\text{-BPAP}\) metabolite, \(^{3}H\text{-Benzyol-Phe,}\) and < 8% of the parent compound, \(^{3}H\text{-BPAP,}\) into
the organic (counting) phase. In addition to these samples, five standard tubes were prepared containing 1 mM captopril, 0.02 mL of the radiolabeled indicator mixture, and 0.3 mL of blood that was drawn before isotope administration. These standards were used to calculate the amount of radioactivity administered.

Percent metabolism of \(^3\)H-BPAP in each sample as well as total metabolism during a single pass were calculated as the ratio of product (\(^3\)H-Benzoyl-Phe) to total \(^3\)H with appropriate corrections for recovery, parent spill, and instrument efficiency. Each determination provides 7 to 11 usable sample points in the arterial effluent curve representing 7 to 11 different substrate concentrations. These substrate concentrations are used to calculate apparent Michaelis-Menten kinetic constants of lung ACE for \(^3\)H-BPAP during isolated perfusion. Thus, there are approximately 7 to 11 points employed in each estimation of constants. Only points representing > 10% substrate metabolism and radioactivity at least 10 times greater than background were used.

This method can be used to calculate the modified first-order kinetic parameter corresponding to the ratio of a normalized maximal enzymatic conversion rate to the Michaelis-Menten constant (Amax/Km). When pulmonary blood flow remains constant, the perfused capillary surface area, or Amax/Km, serves as a measure of vascular injury.\(^{22}\) The usage of the form of the equation is chosen because of the high degree of substrate utilization (10 to 80%) necessary in our studies. Previous work\(^{23–25} \)has shown that Amax/Km expressed as milliliters per minute is decreased in response to lung injury.

**Groups**

The lobes were separated into three groups. Prior to the hour of stable ventilation and perfusion, all groups were treated similarly. After baseline measurements, the control group (n = 8) had 40 min more of perfusion and ventilation without receiving either injury or rescue agent. After the 40 min, all measurements were repeated, including full pressure-volume compliance curves and a total weight gain. The injured group lobes (n = 17) were administered PMA (0.1 \(\mu\)g/mL) (Sigma Chemical; St. Louis, MO), as a bolus into the venous reservoir after the baseline measurements were obtained. After 40 min of circulation, all measurements were repeated. A subgroup (n = 8) of the injured lobes had PEEP elevated 10 min after the instillation of PMA. The PEEP chosen was based on the closing pressure determined from the final pressure-volumes curves of the first subset of injured lobes. This subgroup (high PEEP) was otherwise treated and followed up like the rest of the injured lobes. The third group, the protected group (n = 8), received PMA (0.1 \(\mu\)g/mL) like the injured group after baseline measurements. In addition, pentoxifylline (1 mg/mL of perfusate) (Sigma Chemical) was given 10 min after the PMA. Again all measurements were repeated after 40 min of observation.

**Statistics**

Baseline intergroup differences were compared with analysis of variance. Paired t tests were used to compare baseline to final measurements within groups, and all other data were compared using t tests. Data are expressed as means ± SEM. Significance was accepted at p < 0.05.

**RESULTS**

The groups were not different from each other in baseline dog weight (20.7 ± 0.33 kg), lobe weight (39.9 ± 1.23 g), Vr used (55.5 ± 0.72 mL), or PEEP (4.4 ± 0.11 cm H\(_2\)O) settings. Initial measures of compliance were also similar, with opening pressure (10.9 ± 0.36 cm H\(_2\)O), closing pressure (2.8 ± 0.29 cm H\(_2\)O), static compliance (19.1 ± 0.51 mL/cm H\(_2\)O), and spot compliance (9.2 ± 0.52 mL/cm H\(_2\)O) not significantly differing between groups.

Vascular integrity measured by PVR and Kf were also similar at baseline, with a PVR mean of 37.8 ± 1.27 cm H\(_2\)O/L/min, and Kf of 0.14 ± 0.02 mL/min/mm Hg/100g. Endothelial metabolic function measured by Amax/Km and percentage of ACE metabolism were not significantly different between groups either, with a mean Amax/Km of 558.1 ± 20.8 mL/min, and percentage of ACE metabolism of 75.6 ± 0.96%.

There was clear deterioration in measures of compliance in the injured group when compared to the control and protected groups. Figure 1 depicts the changes in static and spot compliance, as well as opening and closing pressures over time. The injured group worsened in every measure of lung compliance in relation to both control and protected lobes. The protected lobes, which received PMA then pentoxifylline, had significantly better compliance measures in all categories when compared to the injured lobes. The pentoxifylline-protected lobes also had significantly better preservation of static compliance and opening pressure than the control lobes.

Endothelial integrity measures, including PVR, Kf, and weight gain, are shown in Figure 2. Large differences between the injured group and the lobes of the control and protected groups are also demonstrated. Both the PVR and Kf remained stable in the control group, while the injured lobes demonstrated at least a threefold increase in both, as well as significantly larger weight gain. The dramatic 1,242% rise in Kf in the injured lobes did not reach significance value (p = 0.07). Although the PVR in the protected lobes demonstrated a small but significant increase vs baseline and control, it remained significantly lower than the PVR in the injured lobes. The Kf remained stable in the protected lobes, and the average weight gain demonstrated in the protected lobes was even less than in the control lobes.

The results of PMA-induced changes were similar when endothelial metabolic functions were assessed. Figure 3 highlights the relative changes in percent-
age of ACE metabolism and Amax/Km. The control lobes remained stable over time, with only a 0.7% drop in percentage of ACE metabolism and 3.3% decrement in Amax/Km. The injured lobes again demonstrated significantly larger decreases, with percentage of ACE metabolism and Amax/Km falling 8.8% and 21%, respectively. Despite the dramatic changes brought about by the PMA in the injured lobes, the addition of pentoxifylline in the protected lobes prevented this deterioration of metabolic function. The protected lobes actually demonstrated slight increases in both percentage of ACE metabolism (0.7%) and Amax/Km (1.1%).

When WBC and neutrophil counts were analyzed, the injured and protected groups behaved distinctly different from the lobes of the control group. The mean WBC in the control group dropped only 5.9%, from 967 ± 138/µL to 909 ± 122/µL, with a corre-

Figure 1. Compliance measures. The four measures of pulmonary compliance are compared before and after any group-specific intervention: spot compliance (top, A), static compliance (middle, B), opening pressure (middle, C), and closing pressure (bottom, D). The control group is represented by the solid bars, and the injured and protected groups are depicted as open and checkered bars, respectively. * = p < 0.05 vs its own baseline measurement; @ = p < 0.05 vs the control group.

Figure 2. Endothelial integrity measures. The three measures of vascular integrity are compared before and after any group-specific intervention: PVR (top, A), Kf (middle, B), and lobar weight gain (bottom, C). The control group is represented by the solid bars, and the injured and protected groups are depicted as open and checkered bars, respectively. * = p < 0.05 vs its own baseline measurement; @ = p < 0.05 vs the control group.
sponding decrease of 25% in ANC, from 261 ± 46/μL to 196 ± 44/μL. The injured-group lobes demonstrated decreases of > 80% in both counts, with WBC dropping from 1,369 ± 160/µL to 208 ± 35/µL and ANC falling from 195 ± 40/µL to 32 ± 7/µL. Similarly, the protected-group lobes also showed precipitous drops in these counts. Both WBC and ANC fell 80% in the protected lobes, from 2,340 ± 746/µL to 483 ± 116/µL and from 684 ± 229/µL to 102 ± 27/µL, respectively. When the differences in baseline numbers were taken into account, the relative decrements seen in circulating WBC and ANC counts in the injured and protected groups remained significant.

When the subsets of the injured group were compared to see the impact of a high PEEP strategy, few differences were detected. The lobes of the two subgroups were similar in terms of baseline measures, Vt, and PEEP. Ten minutes after the instillation of PMA, the high PEEP subgroup had PEEP elevated from a baseline PEEP of 4.6 ± 0.2 cm H2O to a mean PEEP of 10.1 ± 0.4 cm H2O. In every other respect, the two subgroups were treated the same. The only impact that the high PEEP subgroup demonstrated was a relative preservation of spot compliance. The injured subgroup with normal PEEP had spot compliance fall 43%, from 9.6 ± 1.1 to 5.5 ± 0.6 mL/cm H2O (p < 0.002) after PMA. The spot compliance of the high PEEP subgroup dropped only 17%, from 7.9 ± 0.9 to 6.6 ± 1.0 mL/cm H2O (p = 0.11). In other respects, the two subgroups behaved similarly and there were neither positive nor negative trends associated with the use of a higher PEEP.

**Discussion**

Using the isolated blood-perfused lung preparation allowed us to eliminate many confounding systemic influences. We were able to study endothelial metabolic function in its natural milieu as well as follow WBC trapping since the neutrophils were either in circulation or sequestered in the lung. The simplicity of the system also allowed for easy measure of Kf and lobar weight change. With the addition of compliance measures and estimates of ACE activity, we were able to follow the PMA-induced lung injury and the protection afforded by pentoxifylline across a wide spectrum of lung functions. We believe the stability of the control group lobes and the rapidity of the PMA-induced injury allowed for a valid testing of our hypothesis.

Despite impressive increases in Kf in the injured lobes (1,242%), there was only a trend toward significance (p = 0.07) when compared to the control lobes. A major contributing factor was that we were unable to obtain paired Kf values on 1 of the control lobes due to technical difficulties and on 3 of the 17 injured lobes. Those three lobes demonstrated such dramatic edema that there was insufficient circulating blood remaining to complete the data collection. The huge increases in permeability and edema prevented us from demonstrating the significance of the difference. We believe that the significant increases in weight gain seen in the injured lobes highlight the increases in capillary permeability.

The differences in WBC counts and ANCs between the groups throughout the experiment were most likely accounted for by minor changes in the perfusion circuit. The WBC counts directly obtained from the animals were not significantly different. A change in the type of filter that trapped clots resulted in less WBC trapping by the system. Lobes from all three groups were affected, but the majority were in the protected group. We believe the impact on our results except for the WBC and neutrophil counts is negligible since the more neutrophils available would likely increase the injury measured. Despite the change
in filters, a similar precipitous drop in WBC counts was seen in both groups receiving PMA.

To our knowledge, this is the first time that pentoxifylline administered as a rescue agent has been shown to prevent or blunt compliance changes, as well as the vascular integrity and metabolic function deterioration seen in this kind of ALI model. Pentoxifylline prevented this deterioration without preventing neutrophil sequestration in the lung. These findings differ from the findings of Welsh et al in pentoxifylline-pretreated dogs that were injured with endotoxin. In that model, the pentoxifylline pretreatment significantly reduced the neutrophil trapping. Our postinjury addition of pentoxifylline did not prevent this sequestration, yet it did diminish the negative impact of those trapped neutrophils.

Since our goal was to demonstrate organ-level protection by pentoxifylline, we were not able to focus on the cellular-level mechanisms by which pentoxifylline protected the lung. However, the review of the anti-inflammatory effects of pentoxifylline by Mandel highlights two major actions of the drug. Pentoxifylline reduces production of inflammatory cytokines by stimulated neutrophils and blocks the effect of these cytokines on other phagocytes. Therefore, pentoxifylline decreases neutrophil adherence, rigidity, oxidative burst, degranulation, and chemotactic movement. Pentoxifylline has also been shown to block the reduction in surfactant production caused by tumor necrosis factor (TNF)-α. This can account for early preservation of pulmonary compliance, while pentoxifylline inhibition of TNF-α–stimulated smooth-muscle cell migration helps to prevent late-stage fibrosis and subsequent compliance deterioration.

Our study highlights the protection afforded by pentoxifylline to the full spectrum of lung functions. Pentoxifylline preserved compliance and minimized increases in opening pressure while blunting other nonrespiratory manifestations of lung injury. Capillary fluid filtration, endothelial metabolic health, and vascular resistance all were preserved by pentoxifylline. These findings are contrasted by the lack of protection afforded by the high PEEP strategy. Although high PEEP maintained spot compliance, it failed to preserve other important lung functions. Perhaps a distinction should be made regarding what lung functions are preserved by which protective strategies. We speculate that all lung functions including compliance, vascular integrity, and endothelial metabolic health must be preserved to improve outcome in ALI/ARDS. Pentoxifylline may have a role in improving outcomes clinically.

Preliminary human trials have already begun to clarify pentoxifylline effects in the settings of ALI, sepsis, and postcardiopulmonary bypass. Some studies simply show no hemodynamic deterioration or slight trends toward improvement of oxygen consumption, while other studies demonstrate significant improvement. Pentoxifylline treatment improved both cardiac index and PVR index in patients with sepsis when compared to control subjects without sepsis. Pretreatment with pentoxifylline before and during cardiopulmonary bypass inhibited postoperative increases in PVR and blunted pulmonary leukocyte sequestration. In a randomized, double-blinded, placebo-controlled study of 51 surgical patients with severe sepsis, pentoxifylline administration helped to improve oxygenation and a multiple-organ dysfunction score. Perhaps the most provocative study to date has been the use of pentoxifylline in advanced cancer patients. The 15 patients randomized to receive pentoxifylline demonstrated not only decreased TNF levels and clinical improvement, but also had significant improvement in 7-day survival.

Many questions remained unanswered regarding the utility of pentoxifylline in the treatment of ARDS. What is the appropriate dosing? Should it be given continuously or intermittently? Will rescue dosing be as helpful as pretreatment? Until we can improve our ability predict which patients will develop ARDS, then we must rely on some agent to interrupt the inflammatory process. Pentoxifylline may be that agent.

In summary, in this isolated blood-perfused lung preparation, we conclude that pentoxifylline given as a rescue agent prevents the PMA-induced deterioration of lung compliance, vascular integrity, and endothelial metabolic function in this ALI model. This preservation of function occurred despite significant pulmonary neutrophil sequestration.

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