The Effect of Pentoxifylline on Acid-induced Alveolar Epithelial Injury

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Background: Acid instillation into one lung is known to cause an increase in the permeability of the endothelium to protein in both the instilled and the contralateral lungs. Activated neutrophils are believed to be involved in causing this increased permeability. Pentoxifylline, a drug used in clinical practice, has multiple effects on neutrophils, including inhibition of phagocytosis, degranulation, and superoxide generation. This study investigated whether pretreatment with pentoxifylline would protect the alveolar epithelium or lung endothelium from injury.

Methods: The effect of acid instillation into one lung of anesthetized rabbits using several quantitative parameters was investigated. The quantification of the bidirectional movement of the alveolar (^{125}I-albumin) and the circulating protein tracers (^{131}I-albumin) was used as a measurement of the permeabilities of the lung epithelium and the lung endothelium in the acid-instilled lung. Bronchoalveolar lavage and measurement of the entry of the circulating protein tracer were used to assess the permeabilities of these barriers in the noninstilled lung.

Results: The instillation of HCl (pH 1.25, 1.2 ml/kg) into the right lung resulted in an increase in the protein permeability of the right lung's alveolar epithelium and endothelium as well as an increase in the permeability to protein of the left lung's endothelium. Pentoxifylline pretreatment attenuated the increase in the endothelial permeability of both lungs by 50% and restored the $Pa_{O_2}/F_{I_{O_2}}$ to normal in the pretreated animals exposed to acid injury.

Conclusions: Acid aspiration causes a dramatic increase in the alveolar epithelial permeability of the acid-instilled lung, but the permeability of the alveolar epithelium of the contralateral lung remains normal. In contrast, unilateral acid instillation causes an increase in the permeability of the endothelium of both lungs. The increase in endothelial permeability can be attenuated by pretreatment with pentoxifylline administration, and this leads to restoration of normal gas exchange. (Key words: Lung, acid aspiration: endothelium; epithelium; lung injury; pentoxifylline; tumor necrosis factor.)

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THE effects of acid instillation into the lung have been extensively studied.[1–5] Acid instillation of one lung, unlike the instillation of bacteria,[6,7] causes an increase in permeability of the endothelium to protein in the instilled lung, in the contralateral lung, and even in other organs.[3,8] Activated neutrophils are believed to be important in causing this increase in protein permeability of the lung endothelium and of the distant organs.[1,8] The extent to which acid instillation injures the epithelium and whether the epithelium of the contralateral lung is affected by this injury are unknown.

Several experimental studies have demonstrated that pentoxifylline decreases the hemodynamic perturbations seen in hypovolemic shock,[9,10] endotoxin shock,[11] and tumor necrosis factor (TNF) induced-lung injury.[12,13] Pentoxifylline is a methylxanthine derivative and an inhibitor of phosphodiesterase. Pentoxifylline is known to inhibit phagocytosis,[14] degranulation,[15] and the superoxide generation[15–17] from neutrophils. Therefore, we hypothesized that pentoxifylline might attenuate the increase in endothelial permeability caused by HCl instillation through these effects on neutrophils and we wondered whether it would have any effect on the alveolar epithelial injury. Therefore, the objectives of this study were to use quantitative methods to establish the amount of alveolar epithelial and lung endothelial injury caused by acid instillation,

to compare this injury to other lung injuries that have been measured, and to determine whether acid instillation causes alveolar epithelial injury of the contralateral lung. Finally, we wanted to determine whether a clinically used drug, pentoxifylline, can modify the alveolar epithelial or endothelial injuries in the instilled or contralateral lung after acid instillation.

Methods

Surgical Preparation and Ventilation

In specific pathogen-free Japanese White Rabbits (weight, range 3–4 kg) anesthesia was induced with pentobarbital (25 mg/kg) and maintained with 1% halothane. A 20-G intravenous catheter was inserted into an ear vein for administering fluid and drugs. A catheter was inserted into the carotid artery to monitor blood pressure and for blood sampling. Pancuronium (0.3 mg/kg) was given for neuromuscular blockade. An endotracheal tube was inserted through a tracheotomy. The rabbits were supine and their lungs ventilated with a constant-volume pump (Harvard, Millis, MA) with an inspired oxygen fraction of 1.0, and positive end-expiratory pressure of 3 cmH_2O was applied. The tidal volume was 3 ml/kg, and the respiratory rate was adjusted to maintain an arterial P_{CO_2} between 35 and 45 mmHg.

All animal experiments were done in conformity with the "Guiding Principles for Research Involving Animals and Human Beings" of Yokohama City University School of Medicine.

Preparation of the Intraalveolar Instillate

A 5% isosmolar rabbit albumin solution was made using Ringer's lactate; 2 mg of Evan's blue and 3 μCi of ^{125}I-human albumin were added to the instillate, as we have done before.[6] For the rabbits receiving HCl, HCl (*p*H 1.25, vol 1.2 ml/kg) was instilled 15 min before the instillation of the albumin solution. This amount of acid was chosen because it caused significant alveolar epithelial damage but did not lead to the death of the animals, and several other investigators have used this dose.[18,19] The acid instillate, 0.1N HCl, was diluted to *p*H 1.25 by adding sterile normal saline. A sample of the protein instillate was saved for measurement of radioactivity and protein concentration.

Preparation of Pentoxifylline

Pentoxifylline (Hoechst Japan, Tokyo, Japan) was dissolved in sterile normal saline to a concentration of 10 mg/ml and filtered through a 0.45-μm filter (Millipore, MA) before injection. One hour before the HCl instillation, 20 mg/kg pentoxifylline was injected intravenously and then 6 mg $\cdot$ kg^{-1} $\cdot$ h^{-1} pentoxifylline was continuously infused throughout the experiment. This dose of pentoxifylline has been used successfully by investigators of other forms of lung injury.[11,12]

Preliminary Test of Neutralization of Acid in the Lung

The interval necessary for the neutralization of the instilled HCl was documented in three additional anesthetized rabbits, the lungs of which were mechanically ventilated. The lungs were exposed by transecting the sternum. A *p*H indicator (methyl orange xylene cyanol) was mixed with the HCl and instilled into the right lung. The color change of the indicator was observed, and within 10 min of instillation, the lungs were rapidly frozen by pouring liquid nitrogen into the thoracic cavity. The frozen lungs were cut into 3-mm slices, and the color of the surface of the lung was compared with the color standards of the *p*H indicator. Only the right lung demonstrated a *p*H change, suggesting the acid remained in the instilled lung.
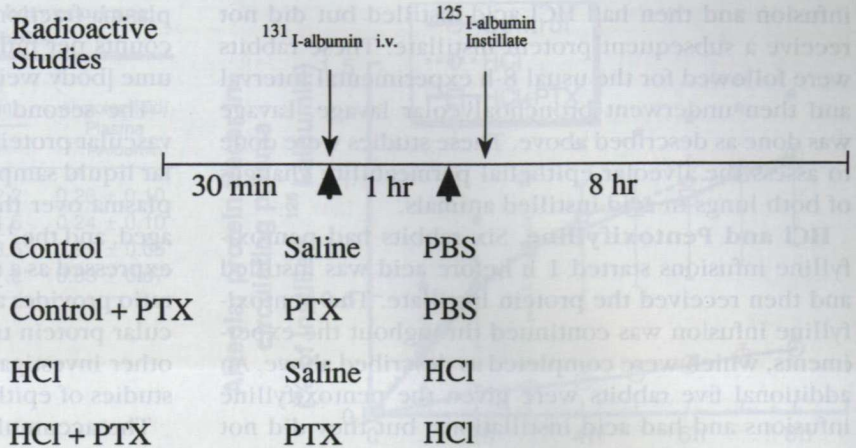
General Protocol

Two similar protocols were necessary (fig. 1) to investigate the effect of acid instillation on the epithelial and endothelial barriers of both the acid-instilled lung (right lung) and the contralateral, noninstilled lung (left lung). Radioactive studies were done as we have done previously[6,7]; an isosmolar 5% rabbit albumin solution was instilled into the right lung 15 min after the acid had been instilled. Nonradioactive studies were done to measure the effect of the acid instillation on the contralateral lung and to determine the effect of pentoxifylline pretreatment on gas exchange. In these experiments, a protein-containing solution was not instilled after the acid instillation.

In all experiments, 30 min after the surgical preparation, 2 ml/kg of normal saline with or without pentoxifylline was infused. Blood was sampled every 15 min for 1 h. Throughout the experiment, 2 ml $\cdot$ kg^{-1} $\cdot$ h^{-1} of lactated Ringer's solution with pancuronium (2 mg/h) was intravenously administered. Before the alveolar instillation, the rabbits were placed in the right lateral decubitus position to facilitate liquid deposition into the right lung. Using a 12-ml syringe and a pediatric feeding tube, 1.2 ml/kg of phosphate buffered saline (PBS, *p*H 7.4) for the control groups or

Fig. 1. Experimental design. The first arrowhead signifies when either saline or pentoxifylline (PTX) was intravenously infused. The second arrowhead signifies when either PBS or HCl was instilled into the right lung. Radioactive studies included additional procedures, such as the administration of intravenous ^{131}I-albumin (shown by the first arrow) and the intraaleolar instillation of ^{125}I-albumin (shown by the second arrow).

Radioactive Studies

	^{131}I-albumin i.v.	^{125}I-albumin Instillate
	30 min ▲ 1 hr ▲	8 hr
Control	Saline	PBS
Control + PTX	PTX	PBS
HCl	Saline	HCl
HCl + PTX	PTX	HCl

the same volume of HCl (*p*H 1.25) for the HCl groups was delivered into the right lungs (primarily the right lower lobe). Confirmation of liquid deposition was achieved by the use of Evan's blue dye in the protein instillates, the use of radioactivity in the protein instillates (see above), and the use of *p*H indicators (see above).

Radioactive Studies

The intravenous protein tracer (^{131}I-albumin) was injected 30 min after the surgical preparation (fig. 1). Three μCi was administered. Fifteen minutes after instillation of either the PBS or HCl, 1.8 ml/kg of the 5% isosmolar rabbit albumin protein-solution was instilled (see *Preparation of Instillate*) using the same feeding tube. Blood samples were obtained for ^{131}I-albumin and ^{125}I-albumin activity every hour after the instillation in these rabbits. After 8 h, the abdomen was opened and the rabbits were exsanguinated. Urine and right and left pleural fluids were sampled for radioactivity and for total protein measurement. The lungs were removed through a sternotomy, and the remaining alveolar liquid was aspirated using a 3-ml syringe and a Silastic tube (0.75 mm ID) that was passed into a wedged position in the right lower lobe. We previously reported that the liquid aspirated with a catheter wedged in the distal airways is a good reflection of alveolar fluid protein concentration.[20] The total protein and radioactivity of the alveolar samples was measured. Right and left lungs were homogenized separately for wet-to-dry ratio measurement and for radioactivity counts.[21]

Leukocyte and polymorphonuclear neutrophil counts were done on blood, pleural fluid, and on the final alveolar fluid obtained from the instilled lungs.

Nonradioactive Studies

These animals neither received the intravenous protein tracer nor the alveolar instillate. However, all other aspects of the experimental protocol were the same as in the radioactive studies. Animals were processed after 8 h. After exsanguination, bronchoalveolar lavage was done in these rabbits for protein measurement of the fluid from the acid-instilled and from the contralateral lungs. Lavage was done three times using 5 ml of saline per side. Protein concentration was measured by the Lowry method.[6,7]

Specific Protocols

Control Experiments. After an infusion of saline was begun, six rabbits received intraalveolar PBS followed by the isosmolar rabbit albumin; acid was not instilled. The experiments were completed as described above. An additional five rabbits were begun on a saline infusion and had PBS alone (not followed by the isosmolar protein instillate) instilled into their right lungs for the 8-h experimental interval.

Control and Pentoxifylline. Pentoxifylline infusion was begun in four rabbits that then received the isosmolar rabbit albumin instillation after the PBS instillation. These experiments were done to investigate whether the pentoxifylline infusion affected hemodynamics or other measurements. The experiments were completed as described in group I.

HCL. Six rabbits received saline infusions and then, 1 h later, had HCl acid-instilled before the instillation of the isosmolar rabbit albumin instillation. The experiments were then completed as in the above two groups. An additional five rabbits received the saline

infusion and then had HCl acid-instilled but did not receive a subsequent protein instillate. These rabbits were followed for the usual 8-h experimental interval and then underwent bronchoalveolar lavage. Lavage was done as described above. These studies were done to assess the alveolar epithelial permeability changes of both lungs in acid-instilled animals.

HCl and Pentoxifylline. Six rabbits had pentoxifylline infusions started 1 h before acid was instilled and then received the protein instillate. The pentoxifylline infusion was continued throughout the experiments, which were completed as described above. An additional five rabbits were given the pentoxifylline infusions and had acid instillations, but they did not receive the isosmolar protein instillates. After 8 h, these rabbits were exsanguinated and bronchoalveolar lavage was done for protein measurement. These experiments were done to measure the alveolar epithelial permeability of the acid-instilled and contralateral lungs in the animals receiving pentoxifylline. Details of the assessments of lung barrier permeability are listed below.

Calculations of Alveolar Epithelial Barrier Protein Permeability

Several different methods were used to measure the response of the alveolar epithelium and lung endothelium to injury. First, the bidirectional flux of albumin across the alveolar epithelial barrier[6,7,20] was assessed as a measurement of alveolar epithelial permeability, as we have done before.[6,7,20,22] This method requires the measurement of the residual ^{125}I-albumin (the alveolar protein tracer) in the lung as well as the accumulation of ^{125}I-albumin in the plasma. A second method requires the measurement of ^{131}I-albumin (the vascular protein tracer) in the air space compartment of the lung.

The total quantity of ^{125}I-albumin instilled into the lung was determined by measuring duplicate samples of the alveolar instillate for ^{125}I-albumin total counts (cpm/g) and then multiplying this value by the total volume instilled into the lung. To calculate the residual ^{125}I-albumin remaining in the lung after 8 h, the average of two 0.5-g samples obtained from the lung homogenate was multiplied by the total volume of the lung homogenate. The lung homogenate data was then added to the counts recovered in the aspirated alveolar fluid to assess the quantity of the instilled ^{125}I-albumin remaining in the lung after 8 h. The ^{125}I-albumin in the circulating plasma was measured from a sample of plasma obtained at the end of the experiment. The

plasma fraction was accounted for by multiplying the counts per milligram times the estimated plasma volume [body weight in grams $\times$ 0.07 (1-Hct/100)].

The second method required measurement of the vascular protein tracer, ^{131}I-albumin, in the final alveolar liquid sample. Then, the ^{131}I-albumin counts in the plasma over the course of the experiment were averaged, and the ^{131}I-albumin counts in the air spaces were expressed as a ratio over the mean plasma counts. This ratio provides a good index of equilibration of the vascular protein tracer into the alveolar compartment, as other investigators have shown in prior experimental studies of epithelial permeability.[23]

The accumulation of the vascular protein tracer in the extravascular spaces of the lung was used as an index of endothelial permeability. The accumulation of the vascular protein tracer, ^{131}I-albumin, was expressed as plasma equivalents in milliliters and was calculated by measuring the total extravascular counts of ^{131}I-albumin in the lung divided by the average counts in the plasma after 8 h, as we have done before.[6,7,20,22]

Determination of alveolar epithelial permeability alterations in the nonacid-instilled, contralateral lung was done by measuring protein influx into the alveolar spaces of these lungs. This was done by bronchoalveolar lavage (see *Nonradioactive Studies*).

Determination of Unbound Radioactivity

To evaluate the quantity of ^{125}I and ^{131}I that remained bound to albumin in the instillate, plasma, aspirate, and urine, we used the trichloroacetic acid (TCA) precipitation technique. As the instilled HCl could have broken the bond of ^{125}I to albumin in the instillate, the percentage of unbound ^{125}I at 8 h was determined. The calculations were ^{125}I (cpm/g) at 8 h $\times$ body weight (g) $\times$ 0.07 (1-Ht/100) + total free ^{125}I (cpm) in urine + total ^{125}I in instillate (cpm) $\times$ 100.

Measurements of Hemodynamics and Protein Concentration

Systemic arterial and airway pressures were measured at 60-min intervals.

Statistics

All data are presented as mean $\pm$ SD. The data between the groups were analyzed by ANOVA. Fisher's test was used for comparisons. The within group data were analyzed by Student's paired *t* test. We accepted a $P <$ 0.05 as statistically significant.

Table 1. Influx and Efflux of Protein Tracers between Lung and Vascular Circulation over 8 Hours in Rabbits

Condition	No. of Rabbits	Alveolar Tracer Protein (% of instilled ^{125}I-albumin)		Alveolar fluid/ Plasma ^{131}I-Albumin
		Lung	Blood	
Control	6	85.5 ± 1.9	4.4 ± 2.2	0.26 ± 0.10
Control + PTX	4	86.2 ± 1.8	4.2 ± 1.6	0.24 ± 0.10
HCl	6	69.2 ± 4.1*	27.2 ± 3.6*	0.95 ± 0.05*
HCl + PTX	6	71.0 ± 6.3*	25.5 ± 7.2*	0.83 ± 0.07*

Values are mean ± SD.

* $P < 0.05$ versus control group.

Results

Neutralization of Acid in Lung

Methyl orange xylene cyanol changes color from brown at pH 1 to blue-green at pH 5. After instillation of the HCl (pH 1.25) along with the pH indicator, the pleural surface of the lung demonstrated brown coloration. The lung color then changed to a blue-green within 30 s, suggesting neutralization within this interval. Acid was never detected in the pleural space nor were pH color changes even detected in the noninstilled lung.

Control Experiments (Radioactive and Nonradioactive Studies)

In the control group, the movement of the instilled protein from the alveolar space into the circulation was minimal (table 1). The alveolar tracer (^{125}I-albumin) entered the circulation slowly over 8 h (fig. 2), reflecting an intact epithelium. The movement of the vascular tracer (^{131}I-albumin) into the airspaces of the lung was small (table 1); this also suggests the alveolar epithelium was intact. In the nonradioactive studies, the protein concentration of the lavage fluids obtained from the control animals was very low (fig. 3), suggesting that there was no increase in the protein permeability of the alveolar epithelium in these control animals. Finally, in the radioactive studies, the alveolar epithelium was able to actively transport, in a normal fashion, the instilled fluid from the airspaces into the lung interstitium, as shown by the threefold increase in the protein concentration of the instilled solution in the airspaces after the 8 h interval (ratio of alveolar total protein over final plasma total protein concentration = 3.0 ± 0.7; table 2).

The efflux of ^{131}I-albumin from the vascular space into the lung interstitium (measured in the left non-
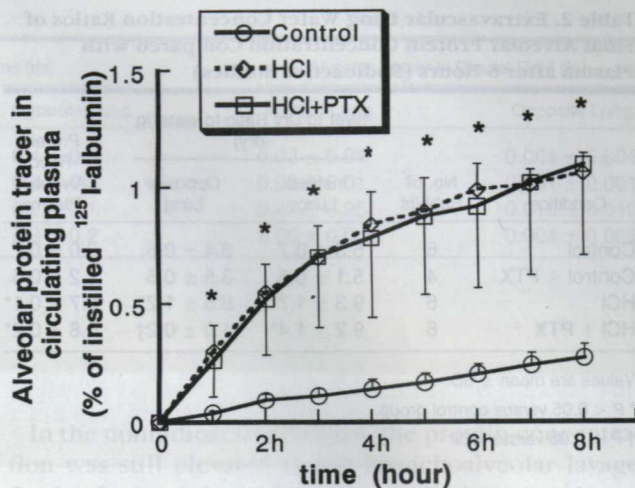

Fig. 2. Time course of the appearance of the alveolar protein tracer in the circulating plasma expressed as percentage of instilled ^{125}I-albumin over 8 h (mean ± SD). Values from 1 to 8 h in HCl and HCl + PTX groups are significantly higher compared to control. HCl + PTX = pentoxifylline pretreatment group. There was no statistical difference between HCl + PTX and HCl groups. *$P < 0.05$ compared to control.

instilled lung) was very low (table 3). This efflux is used as an indicator of lung endothelial permeability to protein. Also, the extravascular lung water content in the noninstilled lung (left lung) is used as another indicator of lung endothelial permeability; this measurement was in the normal range (table 2) and similar to the values that we previously reported.[6,7]

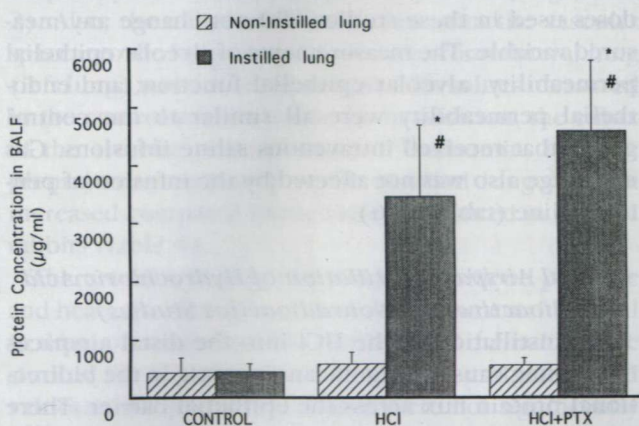**Fig. 3. Protein concentration (μg/ml) in the bronchoalveolar lavage fluids (BALF) obtained from the HCl-instilled lungs and the noninstilled lungs (mean ± SD). *$P < 0.05$ compared to control. #$P < 0.05$ compared with noninstilled lung. HCl + PTX = pentoxifylline pretreatment group. There was no statistical difference between the HCl + PTX and HCl groups.**

Table 2. Extravascular Lung Water Concentration Ratios of Final Alveolar Protein Concentration Compared with Plasma after 8 Hours (Radioactive Studies)

Condition	No. of Rabbits	Wet to Dry Ratio (g water/g dry) Instilled Lung	Opposite Lung	Protein Ratio Alveolar/ Plasma
Control	6	5.3 ± 0.7	3.4 ± 0.5	3.0 ± 0.7
Control + PTX	4	5.1 ± 0.5	3.5 ± 0.5	3.2 ± 0.5
HCl	6	9.3 ± 1.7*	5.5 ± 1.2*	0.7 ± 0.1*
HCl + PTX	6	9.2 ± 1.4*	4.0 ± 0.2†	0.8 ± 0.2*

Values are mean ± SD.

* $P < 0.05$ *versus* control group.

† $P < 0.05$ *versus* HCl.

The efflux of the alveolar protein tracer, ^{125}I-albumin, into the pleural space adjacent to the instilled lung was low (table 4), the expected result in the absence of injury to the alveolar epithelium.[6] Mean arterial blood pressure and heart rate were stable throughout the 8-h experimental interval (table 5). The instillation of the 5% rabbit albumin solution did not affect gas exchange in normal uninjured rabbits (table 6). The rabbits who had received a protein instillate had arterial blood gases after 8 h that were similar to the blood gases measured in control rabbits that had not received the 5% rabbit albumin instillate (fig. 4).

Intravenous Infusion of Pentoxifylline (Radioactive and Nonradioactive Studies)

Pentoxifylline administered intravenously, at the doses used in these studies, did not change any measured variable. The measurements of alveolar epithelial permeability, alveolar epithelial function, and endothelial permeability were all similar to the control group that received intravenous saline infusions. Gas exchange also was not affected by the infusion of pentoxifylline (tables 1–6).

Distal Airspace Instillation of Hydrochloric Acid (Radioactive and Nonradioactive Studies)

The instillation of the HCl into the distal airspaces of the lung caused a significant increase in the bidirectional protein flux across the epithelial barrier. There was an increased movement of the alveolar tracer (^{125}I-albumin) from the airspaces into the circulation (table 1). The alveolar tracer was already detectable in the systemic circulation within 1 h after instillation, and the alveolar tracer entered the bloodstream exponen-

tially for the first 4 h (fig. 1). Also, the influx of the vascular tracer (^{131}I-albumin) into the airspaces was increased after the HCl had been instilled; the values seen in the animals receiving the HCl were fourfold higher than those measured in the control rabbits (table 1). In the nonradioactive studies, high protein concentrations were measured in the bronchoalveolar lavage fluids obtained from rabbits that had received HCl into their right lower lobes (fig. 3). Interestingly, the protein concentration of the bronchoalveolar lavage fluids obtained from the contralateral, uninjured lungs of these rabbits was low and similar to values measured in control experiments (fig. 3). These results indicate that the protein permeability of the alveolar epithelium of the contralateral, noninstilled lung (left lung) was not increased, despite the administration of hydrochloric acid into the adjacent lung (right lung).

To measure the ability of the alveolar epithelium to actively transport fluid from the airspaces, the final total protein concentration of the alveolar instillate was measured in the radioactive studies. After HCl instillation, the alveolar epithelium was not able to actively transport the instilled fluid from the airspaces into the lung interstitium, as indicated by the decrease in the protein concentration of the solution instilled into the airspaces after 8 h (mean ratio of alveolar over final plasma total protein concentration of 0.7 ± 0.1; table 2).

Measurements of endothelial permeability were done in the radioactive studies. After acid instillation, there was an increase in the extravascular lung water content of the instilled lung (right lung) as well as of the contralateral lung (table 2). These results were confirmed by the significant increase in the efflux of ^{131}I-albumin from the vascular space into the lung interstitium of the contralateral lung (left lung; table 3). The efflux

Table 3. Efflux of ^{131}I-Albumin from Vascular Circulation into the Interstitial Space in the Contralateral Lungs over 8 Hours

Condition	No. of Rabbits	Interstitial ^{131}I-Albumin/ Plasma ^{131}I-Albumin
Control	6	0.70 ± 0.17
Control + PTX	4	0.73 ± 0.15
HCl	6	2.19 ± 1.03*
HCl + PTX	6	0.91 ± 0.21†

Values are mean ± SD.

* $P < 0.05$ *versus* control group.

† $P < 0.05$ *versus* HCl.

Table 4. Effects of Instilled HCl on Pleural Fluid

Condition	No. of Rabbits	Pleural Fluid Volume (ml)		Alveolar Tracer in Pleural Fluid (%)	
		Instilled Lung	Opposite Lung	Instilled Lung	Opposite Lung
Control	6	0.5 ± 0.3	0.4 ± 0.2	0.02 ± 0.01	0.001 ± 0.001
Control + PTX	4	0.5 ± 0.4	0.4 ± 0.3	0.02 ± 0.01	0.001 ± 0.001
HCl	6	0.8 ± 0.5	0.2 ± 0.1	0.47 ± 0.25*	0.010 ± 0.010
HCl + PTX	6	0.4 ± 0.3	0.3 ± 0.2	0.06 ± 0.04†	0.004 ± 0.003

Values are mean ± SD

* $P < 0.05$ versus control group.

† $P < 0.05$ versus HCl.

of the alveolar protein tracer, ^{125}I-albumin, into the pleural space adjacent to the instilled lung was increased in acid-instilled rabbits compared to the measured values in control rabbits (table 4). However, the total volume of pleural fluid measured at the end of the experiment was comparable to that measured in control rabbits (table 4).

Mean arterial blood pressure and heart rate were stable throughout the 8-h experimental interval in both the radioactive (table 5) and nonradioactive studies (data not shown). However, the final arterial oxygen and carbon dioxide values were significantly different in the radioactive animals that had been acid-instilled compared with controls (table 6). The arterial pH decreased significantly over the 8-h experimental interval from 7.40 ± 0.04 to 7.26 ± 0.07 and was associated with a significant change of the mean base excess values from -2.0 ± 2.3 at baseline to -6.7 ± 2.9 at the end of the experiment. A significant decrease in the $Pa_{O_2}/F_{I_{O_2}}$ after 8 h was observed in the rabbits in the nonradioactive studies that had received acid but had not received the 5% rabbit albumin instillate (fig. 4).

Pentoxifylline Pretreatment in Hydrochloric Acid-instilled Rabbits (Radioactive and Nonradioactive Studies)

Pentoxifylline pretreatment did not prevent the deleterious effect of acid instillation on the alveolar epithelial permeability to protein; the significant increase in the bidirectional protein flux across the epithelial barrier persisted in the pretreated animals that subsequently received acid (fig. 1 and table 1). Although there was less influx of ^{131}I-albumin into the alveolar space, the decrease in this influx was not significantly different from the values measured in the rabbits that had not been pretreated before acid instillation (table 1).

In the nonradioactive studies, the protein concentration was still elevated in the bronchoalveolar lavage fluids obtained from the acid-instilled lungs of pretreated rabbits and not different from the values measured in untreated acid-instilled rabbits (fig. 3).

In the radioactive studies, the ability of the alveolar epithelium to actively transport the instilled fluid from the airspaces into the lung interstitium was measured. The pretreated animals that received acid instillations could not concentrate the final alveolar protein (mean ratio of alveolar over final plasma total protein concentration of 0.8 ± 0.2; table 2). Therefore, the pentoxifylline pretreatment did not improve any measured parameter of epithelial permeability or function affected by the acid instillation.

In contrast, the pretreatment with pentoxifylline improved the lung endothelial injury caused by the acid instillation. There was no increase of the extravascular lung water content of the contralateral left lung (table 2). Also, the efflux of ^{131}I-albumin from the vascular space into the lung interstitium of the contralateral lung (left lung) was not increased over the values measured in the control rabbits (table 3). In addition, the efflux of the alveolar protein tracer, ^{125}I-albumin, into the pleural space adjacent to the instilled lung was not increased compared to the values measured in control rabbits (table 4).

There was no change in mean arterial blood pressure and heart rate throughout the 8-h experimental interval in these pretreated rabbits (table 5). In the radioactive studies, the initial and final Pa_{CO_2}, Pa_{O_2}, and arterial pH values in the pretreated rabbits were not significantly different from the nontreated animals that received acid instillations (table 6). In contrast, in the nonradioactive studies, significant hypoxia did not develop in the pentoxifylline-pretreated rabbits that received acid (but did not have a 5% albumin instillate; fig. 4). The per-

Table 5. Hemodynamic Data (Radioactive Studies)

Condition	No. of Rabbits	Mean Systemic Pressure (mmHg)		Pulse Rate (rate/min)	
		Baseline	8 h	Baseline	8 h
Control	6	102 ± 11	92 ± 13	300 ± 39	283 ± 14
Control + PTX	4	100 ± 10	102 ± 11	306 ± 18	298 ± 36
HCl	6	106 ± 9	88 ± 13	313 ± 16	295 ± 40
HCl ± PTX	6	100 ± 10	100 ± 11	302 ± 16	283 ± 40

Values are mean ± SD

sistent decrease in oxygenation seen in the pretreated group of rabbits in the radioactive studies may have been due to the instillation of the 5% rabbit albumin solution. The only difference in experimental protocols between the pretreated radioactive and nonradioactive rabbits was the instillation of the 5% albumin solution into the radioactive rabbits. Therefore, pentoxifylline pretreatment, as shown above, improved endothelial permeability after acid instillation, and this decrease in endothelial permeability probably lead to restoration of normal gas exchange in the rabbits in the nonradioactive studies (fig. 4).

Tracer Binding Studies

More than 98% of the total radioactivity of ^{125}I added to the instilled protein solution was bound to albumin. The percentages of the unbound ^{125}I measured in different biologic fluids at the end of the experiments were less than 1.5% in all groups. TCA precipitation also was done on the fluid obtained at the end of the experiment from the distal airspaces of each rabbits that were instilled with HCl. In these fluids, there was never more than 0.9% of free iodine present.

Discussion

A major objective of this study was to quantitate the effects of unilateral lung acid instillation on the permeabilities of the alveolar epithelium and lung endothelium and to compare these effects to the permeabilities caused by other injuries. The administration of 1.2 ml/kg of *p*H 1.25 HCl into one lung caused a comparable increase in the permeability of the alveolar epithelium as the instillation of 10^9 cfu of a virulent *P. aeruginosa*.[24] Both instillations led to an efflux of approximately 20–30% of the instilled alveolar protein tracer over an 8-h interval (table 1). Both the acid and the bacteria instillations led to an influx of the vascular

protein tracer into the airspaces of the instilled lung. However, the instillation of the bacteria lead to an alveolar ^{131}I-albumin to plasma ratio for about 0.6, whereas the instillation of acid lead to a ratio of 0.9 (table 1). The instillation of acid lead to an increase in extravascular lung water content up to of 9.3 g/g of dry lung (table 2), whereas the instillation of bacteria caused less of an increase in the extravascular lung water content; levels of 6–8 g/g of dry lung were measured in the bacterial experiments.[24] The instillation of bacteria led to an inability of the alveolar epithelium to concentrate the instilled protein.[24] The instillation of acid similarly was associated with an inability of the alveolar epithelium to concentrate the instilled protein (table 2). Therefore, both bacteria and acid increased the alveolar epithelial permeability to protein to about the same degree and both appeared to affect the alveolar epithelium's ability to clear liquid and concentrate liquid. However, the instillation of acid had a more profound effect on the permeability of the lungs' endothelium, as demonstrated by the significant increase in the influx of the vascular tracer into the instilled lung's alveolar space (table 1), the increase in the extravascular lung water of the instilled lung (table 2), and the increase in the extravascular (interstitial) ^{131}I albumin counts in the contralateral lung (table 3).

Although the contralateral lung clearly demonstrated evidence of increased endothelial permeability (table 3), there was no evidence of increased epithelial permeability of the contralateral lung. The bronchoalveolar lavage data suggest that the alveolar epithelial permeability to protein of the contralateral lung is similar to that of the control lungs that had PBS instilled or had no instillations (fig. 2).

Other investigators have attempted to quantitate the permeability of the pulmonary epithelial barrier after acid instillation. They demonstrated that large solutes entered the airspaces of an acid-instilled lung more rapidly than in a control lung.[8,25,26] These studies were

done for only 60 min and cannot be compared to other injuries in a quantitative fashion. Our experiments are unique in that the acid injury used in the current experiments can be compared to other injurious agents in a quantitative fashion and that both the permeabilities of the alveolar epithelium and lung endothelium are measured.

Pentoxifylline was chosen in these experiments for several reasons. Pentoxifylline is a drug that is now used clinically to treat claudication and therefore is a drug that could be used in patients with lung acid aspiration injury. Pentoxifylline has multiple effects on leukocyte function, including reducing leukocyte adhesion,[27,28] reducing degranulation,[15,29] reducing superoxide production by leukocytes,[15-28] and reducing TNF-α production by macrophages.[30,31] Furthermore, pentoxifylline has been investigated in other studies in which endothelial injury was produced. Pentoxifylline was found to attenuate endotoxin induced increased endothelial permeability and to prevent TNF-α lung injury.[11,12,32] However, there have not been previous investigations as to whether pentoxifylline modifies alveolar epithelial injury.

The administration of pentoxifylline to animals who only had received PBS instillations did not affect any of the variables measured except for the peripheral neutrophil counts (data not shown). The increase in the peripheral neutrophils may be due to pentoxifylline's reduction of neutrophil adhesion to the endothelium.[27,28]

The administration of pentoxifylline to animals that had received acid instillations did not decrease the alveolar epithelial permeability changes; the efflux of ^{125}I-albumin into the circulation and the influx of ^{131}I-albumin into the alveolar space remained increased compared to control animals (tables 1 and 2). However, pentoxifylline administered before the instillation of acid had a significant effect on the extravascular lung
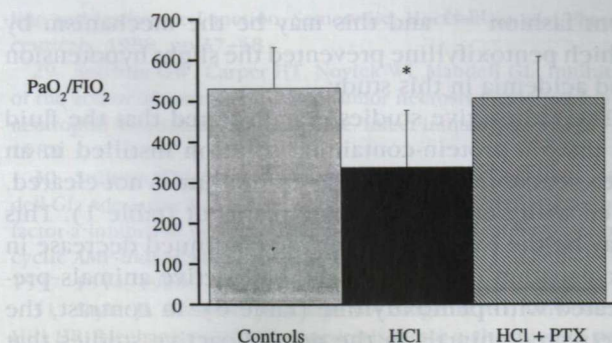

Fig. 4. The Pa_{O_2}/F_{IO_2} measured after 8 h in the nonradioactive studies (where no 5% albumin is instilled). HCl + PTX = pentoxifylline pretreatment group. All groups received an F_{IO_2} of 1.0. The pentoxifylline pretreated group had restoration of their oxygenation. *$P < 0.05$ compared to control.

water content of the contralateral lung (table 2) and on the extravascular lung concentration of ^{131}I-albumin in the contralateral lung (table 3). The administration of pentoxifylline also decreased the amount of the alveolar tracer entering the right pleural space adjacent to acid-instilled lungs (table 4). These results suggest that pentoxifylline had an effect on decreasing the interstitial water in the instilled lungs, as the amount of alveolar tracer entering the pleural space depends on the extent of alveolar injury and on the amount of interstitial lung water.[33]

The preservation of the mean arterial blood pressure in the animals receiving acid instillations and pentoxifylline infusions could be due to several known effects of pentoxifylline. Puranapanida et al. has reported that pentoxifylline improves erythrocyte deformity in septic shock[34] and could improve blood flow in the microcirculations of the body. Pentoxifylline prevents the appearance of detectable serum TNF after administration of intravenous LPS to rats.[13] Pentoxifylline suppresses LPS-induced TNF production in a dose-depen-

Table 6. Gas Exchange Data (Radioactive Studies)

Condition	Pa_{O_2}/F_{IO_2}		Pa_{CO_2} (mmHg)		Arterial pH	
	Baseline	8 h	Baseline	8 h	Baseline	8 h
Control	513 ± 35	503 ± 34	39 ± 3	40 ± 3	7.44 ± 0.07	7.41 ± 0.05
Control + PTX	520 ± 35	516 ± 28	36 ± 5	38 ± 6	7.42 ± 0.06	7.41 ± 0.08
HCl	485 ± 26	177 ± 53*	36 ± 3	45 ± 4*	7.40 ± 0.04	7.26 ± 0.07*
HCl + PTX	506 ± 24	160 ± 60*	36 ± 6	37 ± 5	7.41 ± 0.06	7.38 ± 0.08

Values are mean ± SD.

* $P < 0.05$ versus control groups.

dent fashion[17,35] and this may be the mechanism by which pentoxifylline prevented the slight hypotension and acidemia in this study.

The radioactive studies demonstrated that the fluid in the 5% protein-containing solution instilled in an area of acid-injured alveolar epithelium is not cleared, even with pentoxifylline pretreatment (table 1). This may be the explanation for the continued decrease in oxygenation observed in the radioactive animals pretreated with pentoxifylline (table 6). In contrast, the pretreated animals in the nonradioactive studies that had not received the protein instillate had their oxygenation restored to normal levels after acid instillation (fig. 4). The increase in the $Pa_{O_2}/F_{I_{O_2}}$ observed in these pentoxifylline-pretreated animals demonstrates that the presence of alveolar epithelial injury does not preclude improvement in gas exchange if lung endothelial permeability can be decreased. The clinical utility of such therapy is obvious; if oxygenation can be improved sufficiently, mechanical ventilation might be avoided or the duration of such ventilation decreased.

In summary, the instillation of acid causes a large increase in alveolar epithelial permeability; but in contrast to other alveolar epithelial injuries, it also causes a dramatic increase in the endothelial permeability in the instilled lung and in the contralateral lungs of experimental animals. Pentoxifylline administered before and after the instillation of acid decreases the endothelial injury of the contralateral lung and it decreases the interstitial edema of the instilled lung, in that the pleural fluid from the instilled lung contains less alveolar tracer. Restoration of gas exchange to control levels probably was due to this decrease in endothelial permeability. As pentoxifylline has multiple effects, its mechanism for attenuation of endothelial injury can only be speculated on. However, the secondary effects on blood pressure, pH, and carbon dioxide elimination suggest that TNF blockade may be involved. Future studies are needed to explore the potential of therapy using pentoxifylline after the establishment of acid-induced lung injury.

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