

# Vascular Remodeling Protects against Ventilator-induced Lung Injury in the In Vivo Rat

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**Background:** The role of the pulmonary vasculature in the pathogenesis of ventilator-induced lung injury is not well established. In this study, the authors investigated the effect of vascular remodeling due to chronic pulmonary hypertension on susceptibility to ventilator-induced lung injury. The authors hypothesized that the enhanced vascular tensile strength associated with pulmonary vascular remodeling would protect against ventilator-induced lung injury.

**Methods:** Chronic pulmonary arterial hypertension was induced in rats by exposure to hypoxia for 28 days and was confirmed by demonstration of right ventricular hypertrophy. Normotensive and hypertensive groups of rats (as well as a group in which pulmonary hypertension was acutely reversed with a Rho-kinase inhibitor, Y-27632) were exposed to injurious ventilation (respiratory rate 30 min<sup>-1</sup>, 30/0 cm H<sub>2</sub>O) for 90 min. Lung injury was assessed by change in lung mechanics, oxygenation, edema development, and cytokine levels. Electron microscopy was used to examine vascular structure in additional animals.

**Results:** Injurious ventilation caused significant lung injury (lung compliance, oxygenation, pulmonary edema) in the normotensive controls, but not in the presence of pulmonary hypertension; acute reversal of pulmonary hypertension did not alter the lessened susceptibility to ventilator-induced lung injury. Electron microscopy demonstrated capillary endothelial and epithelial breaks in injuriously ventilated normotensive controls that were not seen with pulmonary hypertension,

whether or not the pulmonary hypertension was acutely reversed.

**Conclusions:** Vascular remodeling induced by chronic pulmonary hypertension confers protection against the effects of injurious mechanical ventilation *in vivo* by a mechanism that may involve structural alterations rather than increased pulmonary artery pressure.

CLINICAL data demonstrate that higher tidal volumes are in general associated with greater mortality than lower tidal volumes<sup>1</sup>; this has been attributed to lung injury associated with injurious ventilation. Ventilator-induced lung injury (VILI) has been mainly studied in short-term animal models.<sup>2</sup> Previous examples include surfactant depletion,<sup>3</sup> oleic acid administration,<sup>4</sup> acid aspiration,<sup>5</sup> acute sepsis or lipopolysaccharide exposure,<sup>6</sup> and ischemia-reperfusion.<sup>7</sup>

Most investigations of VILI have focused on the inflammatory<sup>8</sup> or alveolar elements of lung injury,<sup>2</sup> and the pulmonary vasculature—although obviously central to any considerations of pulmonary pathophysiology—has received less attention. Nevertheless, information that is available suggests that the vasculature might be important in the pathogenesis of VILI for the following reasons. First, exposure of the lungs to high stretch during mechanical ventilation is associated with structural failure that has been termed *stress failure*,<sup>9,10</sup> which corresponds to physical fractures in pulmonary endothelial as well as epithelial cell membranes. Second, during positive-pressure ventilation, interactions between intravascular pressure<sup>11-13</sup> or flow<sup>14,15</sup> affect the propensity to lung injury. Third, vasoactive prostanoids that are known to cause or exacerbate pulmonary hypertension are increased in experimental ventilator-induced lung injury,<sup>16</sup> and inhibition of prostanoid synthesis can reduce the pulmonary vascular responses associated with such injury.<sup>17</sup> Fourth, structural elements of the pulmonary vasculature may play a role, e.g., the threshold for mechanical failure of the alveolar-capillary barrier is related to the thickness of the capillary basement membrane.<sup>18</sup> In the current study, we explored the effect of a relatively common clinical condition, secondary chronic pulmonary arterial hypertension, on the development of VILI. We conclude that chronic pulmonary artery hypertension is protective through vascular remodeling which enhances vascular mechanical strength.

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## Materials and Methods

### *Animal Selection and Care*

Male Sprague-Dawley rats were selected (Charles River, Montreal, Quebec, Canada). The study was conducted according to the guidelines of the Canadian Committee for Animal Care and was approved by the Animal Care Committees of the Hospital for Sick Children and Sunnybrook Research Institutes, Toronto, Ontario, Canada.

### *Chronic Hypoxia-induced Pulmonary Hypertension*

Rats were placed for 28–29 days in chambers (80 × 60 × 50 cm) in which the atmosphere was controlled using automated controllers (OxyCycler Model A84XOV; Biospherix, Redfield, NY) as previously described.<sup>19</sup> Gas delivery was automatically adjusted to maintain an oxygen concentration of 10 ± 0.1%, and oxygen sensors were calibrated weekly. Temperature was maintained at 25° ± 1.0°C, humidity at 50%, and carbon dioxide concentration at less than 0.5%. Temperature, humidity, and the ambient oxygen and carbon dioxide concentrations were continuously monitored, recorded, and regulated by personal computer using customized software (AnaWin2 Run-Time, version 2.2.18; Watlow-Anafaze, St. Louis, MO). Food and water were available *ad libitum*. Chambers were opened daily for approximately 5 min to replenish water and food.

### *Anesthesia and Ventilation*

Animals were anesthetized with ketamine (75 mg/kg intraperitoneal) and xylazine (5 mg/kg intraperitoneal), a tracheotomy (14° endotracheal tube) was performed, and mechanical ventilation (Harvard Rodent Ventilator 693; South Natick, MA) was instituted at the following settings: respiratory rate, 45 min<sup>-1</sup>; tidal volume, 7 ml/kg and adjusted to normocapnia; positive end-expiratory pressure, 1 cm H<sub>2</sub>O; and fraction of inspired oxygen (F<sub>IO<sub>2</sub></sub>), 0.21. Anesthesia, instrumentation, and monitoring were as previously described,<sup>20</sup> and the animals were then allowed 10 min for stabilization.

Five groups of animals were studied. Chronic pulmonary arterial hypertension (CPAH) was induced by exposure to chronic hypoxia, and animals were thereafter randomly allocated to one of the following three groups:

- Chronic pulmonary hypertension, no ventilation
- Chronic pulmonary hypertension, ventilated with high tidal volume
- Chronic pulmonary hypertension (acutely reversed), ventilated with high tidal volume

Control rats that had not been exposed to hypoxia (*i.e.*, normotensive controls) were randomly allocated to one of the following two groups:

- Normotensive control, no ventilation
- Normotensive control, ventilation with high tidal volume

### *Study Protocol*

Animals allocated to no ventilation were exsanguinated, and the tissues were collected as outlined below. Animals allocated to high-tidal volume (*i.e.*, injurious) mechanical ventilation were treated as follows. After measurement of baseline respiratory system compliance, ventilation was commenced (respiratory rate, 30 min<sup>-1</sup>; peak inspiratory pressure, 30 cm H<sub>2</sub>O; F<sub>IO<sub>2</sub></sub>, 0.30; fraction of inspired carbon dioxide [F<sub>ICO<sub>2</sub></sub>], 0.05) and continued for 90 min. This ventilation strategy produced lung injury in previously normal control rats in pilot experiments. The peak inspiratory pressure, systemic arterial blood pressure, and body temperature were recorded throughout the experiment. Arterial blood gases at baseline, 30 min, and 90 min were obtained. F<sub>ICO<sub>2</sub></sub> was adjusted when necessary to maintain arterial carbon dioxide tension (P<sub>aCO<sub>2</sub></sub>) at 35–50 mmHg. After 90 min of mechanical ventilation, respiratory system compliance was measured and postmortem investigations were performed.

### *Acute Reversal of Pulmonary Hypertension*

The Rho-kinase inhibitor (ROCK) Y-27632 [(+)-R-trans-4-aminoethyl-N-4-pyridylcyclohexanecarboxamide 2HCl·H<sub>2</sub>O] (Biomol International, Plymouth Meeting, PA) was administered (15 ml/kg intraperitoneal) to reverse pulmonary hypertension, as previously described.<sup>21</sup> Two-dimensional echocardiography was performed at baseline and at 5 and 30 min after administration to assess the change in pulmonary arterial pressure. High-tidal-volume ventilation was started 35 min after injection.

### *Two-dimensional Transthoracic Echocardiography*

Echocardiography was performed using a Hewlett-Packard Sonos 5500 echocardiographic system (M2424A; Andover, MA) with a 7.5 MHz transducer at a sweep speed of 150 cm/s as previously described.<sup>22,23</sup> A high-frequency phased array sector probe (iL10) that allowed image acquisition at a rate of 400 frames per second was used. Pulmonary artery acceleration time and right ventricular ejection time were measured from a pulse wave Doppler trace of pulmonary artery outflow. Specifically, from a short axis view at the level of the aortic valve, the Doppler signal was obtained in the main pulmonary artery at the level of the valve hinge points. The pulmonary artery pressure was then calculated from the pulmonary artery acceleration time as previously reported.<sup>22</sup> Left ventricular contractility was estimated by calculating the shortening fraction. For all echocardiographic data, the average of three readings was used.

### *Lung Sampling and Bronchoalveolar Lavage*

Animals were exsanguinated by transection of the abdominal vena cava and aorta, and the lungs and heart were removed *en bloc*. The left main bronchus was ligated, the left lung was excised, and bronchoalveolar

lavage (BAL) was performed in the right lung. Five milliliters normal saline was slowly instilled (over 30 s), after which the fluid was slowly withdrawn and reinfused another two times; the recovered fluid was snap frozen and stored at  $-80^{\circ}\text{C}$ . The left lung was weighed at this time and again after 1 week of drying time, to calculate lung wet/dry weight ratio.<sup>24</sup>

#### *Respiratory System Compliance*

Quasi-static pressure-volume curves were constructed before and after the injurious ventilation as follows. After standardization of lung volume with a two-breath recruitment maneuver, 1.0-ml aliquots of 100% oxygen were injected, and measurements of pressure were attained 3 s after each injection until a cumulative volume of 5.0 ml had been injected. The pressure was measured and recorded at the external opening of the endotracheal tube with a Validyne MP45 pressure transducer (Validyne Engineering Corp., Northridge, CA).<sup>24</sup>

#### *Cytokine Determination*

The concentration of tumor necrosis factor  $\alpha$ , interleukin  $1\beta$ , and interleukin 6 in the BAL fluid and serum was measured using bead technology (Luminex<sup>®</sup>; Biosource, Camillaro, CA) as previously described.<sup>25,26</sup>

#### *Confirmation of Chronic Pulmonary Hypertension*

The extent of right ventricular hypertrophy was used to confirm development of chronic pulmonary hypertension as previously described.<sup>27</sup> At the end of each experiment, the heart was excised and the atria removed. The right ventricular free wall was separated from the left ventricle and the septum, and the weights of the right ventricle and of the left ventricle plus septum were recorded. Each specimen was weighed, and chronic pulmonary hypertension was inferred where the ratio of the weights (*i.e.*, right ventricle/[left ventricle + septum]) was greater than 35%.<sup>28</sup>

#### *Exclusion Criteria*

After stabilization and before institution of injurious ventilation, the following values were required for continuing with the experiment: arterial oxygen tension ( $\text{PaO}_2$ ) greater than 65 mmHg,  $\text{PaCO}_2$  35–45 mmHg, and pH 7.35–7.45. Where such criteria were not fulfilled, parameters were reassessed after an additional 10 min of baseline ventilation and stabilization, and in the absence of the above criteria, the animals were not included. Finally, animals that were exposed to chronic hypoxia and did not develop pulmonary hypertension were excluded.

#### *Electron Microscopy*

Electron microscopy was used to examine the ultrastructure in three additional animals in each group as follows:

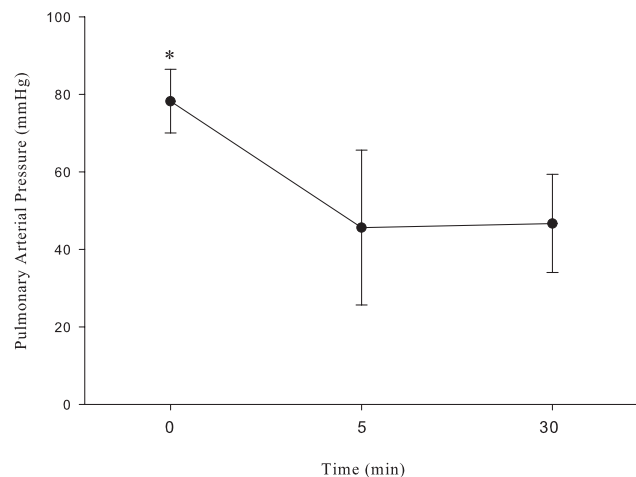
- Pulmonary hypertension, injurious ventilation
- Pulmonary hypertension, no ventilation
- Normotensive controls, injurious ventilation
- Normotensive controls, no ventilation

Induction of CPAH and initiation of injurious ventilation was as described in the Study Protocol section, but in the current series, animals were killed after 40 min (as opposed to 90 min) of ventilation because pulmonary edema develops within 50–60 min of injurious ventilation in this model (pilot data). After 40 min, each animal was killed, and the lungs were prepared for electron microscopy using a fixative (1% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer) instilled through the endotracheal tube at a pressure of 25 cm  $\text{H}_2\text{O}$  until the lungs were fully inflated. The trachea was then ligated, and the lungs were removed and submerged into the fixative. Median segments of lung were then excised from the right caudal lobe of each lung and minced into  $\text{mm}^3$  pieces, fixed for an additional 4 h and stored in 0.1 M phosphate buffer, pH 7.4. Samples were then postfixed in osmium tetroxide, dehydrated in an ascending series of acetone, infiltrated with Embed 812 Araldite (Electron Microscopy Sciences, Hatfield, PA), and polymerized in Embed 812 Araldite using a microwave tissue processor (Pelco, Redding, CA). Light microscopy sections 1  $\mu\text{m}$  thick were cut with an ultramicrotome (Reichert Ultracut; Toronto, Canada) and stained with toluidine blue from five blocks. Sections for electron microscopy were then cut from two blocks from each animal chosen from the sections that contained the most alveoli and were mounted on grids. They were then stained with uranyl acetate and lead citrate before examination in a transmission electron microscope (JEOL JEM 1230; JEOL, Peabody, MA). Samples were then examined qualitatively for the presence of inflammation and endothelial alterations including thinning and separation of the type I cells from the endothelial cells.

#### *Morphometric Analysis*

Eighty random images from the two samples from each animal were captured using the transmission electron microscope and a digital charge-coupled device camera (Advanced Microscopy Technics Corp., Danvers, MA) at a screen magnification of  $\times 30,000$ . The images were then exported to an image analysis program (Scion Image, Frederick, MD), and a computer-generated grid was imposed on each of the images. Measurements were only taken at grid intersects and the following parameters measured: type I cell thickness, alveolar wall thickness, interstitial thickness, endothelial cell thickness, and basement membrane thickness. A minimum of 150 measurements were performed on each animal's defined parameter.





**Fig. 1.** The mean pulmonary arterial pressure at baseline and at 5 and 30 min after intraperitoneal injection of Rho-kinase inhibitor ROCK inhibitor (Y-27632). The pressure was significantly lower compared with baseline (\*  $P < 0.05$ ).

#### Control Normotensive Animals Pretreated with Rho-kinase Inhibitor

An additional study was performed to exclude potential protective effect of ROCK inhibition on lung injury. A group of six normotensive control rats, pretreated with ROCK inhibitor (Y-27632, 15 ml/kg intraperitoneal), were ventilated with high tidal ventilation as described in the Study Protocol section.

#### Statistical Analysis

Data were analyzed using SigmaStat (version 2.0; Jandel Corporation, San Rafael, CA). One-way analysis of variance was performed for multiple group comparisons. First, all data were tested for normality, and where that failed, the data were transformed ( $\log_{10}$  transformation). If the transformation did not result in normalization, the untransformed data were analyzed using analysis of variance on ranks followed by *post hoc* Dunn test. Where transformed data were normally distributed, the transformed data were analyzed using analysis of variance followed by Student-Neumann-Keuls testing. A paired *t* test was performed to evaluate the effect of ventilation within each group. To

protect against type 1 error in the pulmonary hypertension data (fig. 1), a repeated-measures analysis of variance was performed, and a protected *post hoc* comparison was made between each measurement and baseline. Results were expressed either as mean  $\pm$  SD or as median and interquartile range. Statistical significance was set at  $P < 0.05$ .

## Results

#### Baseline Characteristics

All baseline variables are reported in table 1. Animal weight and baseline  $P_{aO_2}$  were comparable among the five groups. Right ventricular hypertrophy was present in the three pulmonary hypertensive groups and not in the two normotensive control groups (table 1).

#### Acute Reversal of Pulmonary Hypertension

Injection of the ROCK inhibitor (Y-27632) resulted in a significant decrease in pulmonary arterial systolic pressure (by approximately 40%) after 5 min, which was maintained at 30 min (fig. 1) and did not alter right ventricular size, heart rate, or left ventricle shortening fraction (data not shown).

#### Effects of Ventilation on Lung Injury

After 90 min of mechanical ventilation with high tidal volume, lung injury was significantly greater in the normotensive *versus* the hypertensive groups, in terms of worsening of oxygenation (fig. 2A), impairment of compliance (fig. 2B), and development of edema (fig. 2C). Reduction in pulmonary hypertension with the ROCK inhibitor (Y-27632) before exposure to high-tidal-volume ventilation had no impact on the development of lung injury (figs. 2A-C). Finally, inhibition of ROCK did not alter the development of VILI in normotensive (control) animals (figs. 2B and C).

#### Effects of Ventilation on Cytokine Release

Cytokine levels in the BAL and serum were similar among all groups in the absence of mechanical ventilation (table 2). After 90 min of high-tidal volume ventilation, the BAL

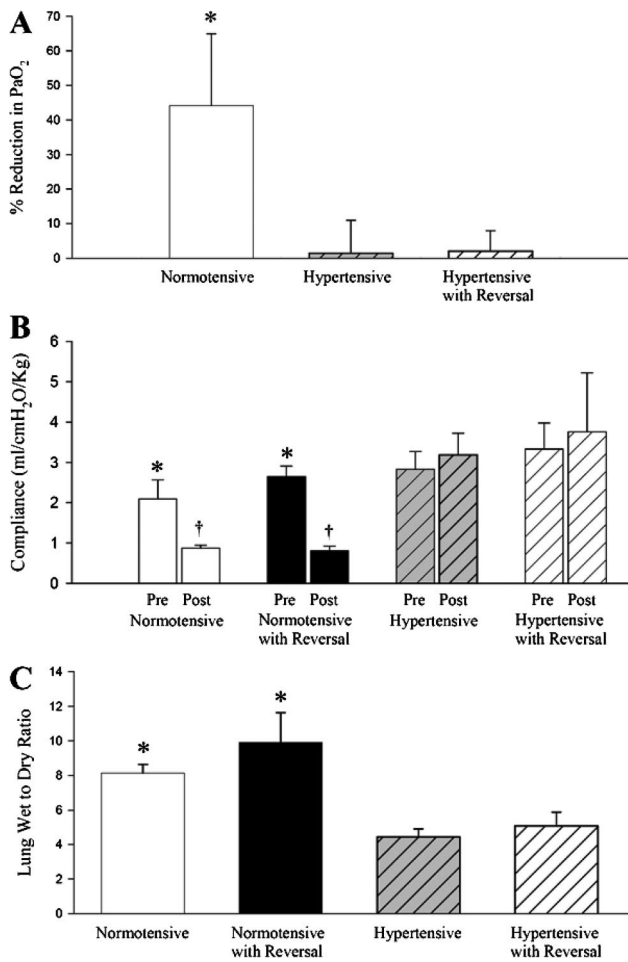
**Table 1. Baseline Characteristics**

	Weight	RV%	Tidal Volume	Compliance
Pulmonary hypertension (18)				
Ventilated (7)	348 $\pm$ 18	44.0 $\pm$ 5.0*	34.4 $\pm$ 6.3	2.7 $\pm$ 0.4*
Vasodilated and ventilated (6)	348 $\pm$ 39	42.3 $\pm$ 6.0*	31.2 $\pm$ 3.7	3.3 $\pm$ 0.5*
Nonventilated (5)	346 $\pm$ 17	41.2 $\pm$ 4.3*	—	—
Normotensive control (12)				
Ventilated (7)	346 $\pm$ 23	20.7 $\pm$ 2.3	28.5 $\pm$ 2.4	1.9 $\pm$ 0.3
Nonventilated (5)	360 $\pm$ 33	22.8 $\pm$ 4.3	—	—

Data are expressed as mean  $\pm$  SD. Animal weight is expressed in grams, tidal volume in ml/kg body weight, and respiratory system compliance in ml  $\cdot$  cm  $H_2O^{-1} \cdot$  kg $^{-1}$ .

\* Significantly greater than normotensive groups ( $P < 0.05$ ).

RV% = (RV  $\times$  100)/(LV + S), where RV = right ventricle, LV = left ventricle, and S = septum.



**Fig. 2.** After 90 min of mechanical ventilation with high tidal volume, lung injury was significantly greater in the normotensive versus the hypertensive groups, in terms of worsening of oxygenation (A), impairment of compliance (B), and development of edema (C). Reduction in pulmonary hypertension with the ROCK inhibitor (Y-27632) before exposure to high-tidal-volume ventilation had no impact on the development of lung injury (A–C). Inhibition of ROCK did not alter the development of ventilator-induced lung injury in normotensive (control) animals (B and C). \* Significantly different ( $P < 0.05$ ) from hypertensive animals. † Significantly different from before ventilation (A).  $P_{aO_2}$  = arterial oxygen tension.

levels of interleukin 6 were significantly increased in the hypertensive groups. The tumor necrosis factor- $\alpha$  levels in plasma were significantly increased only in the normotensive group (table 2). The levels of interleukin 1 $\beta$  were unchanged in all groups except in the group in which pulmonary hypertension was acutely reversed; here, the plasma level increased significantly compared with nonventilated hypertensive animals (table 2).

*Ultrastructural Changes*

The mean thickness of basement membrane in the hypertensive rats was significantly greater compared with the normotensive rats ( $0.047 \pm 0.01$  vs.  $0.025 \pm 0.09 \mu\text{m}$ , respectively;  $P < 0.001$ ), and the thickness of various other layers was not different among groups (hypertensive vs. normotensive) at baseline. However, after 40 min of mechanical ventilation, there was significant thinning of the walls of the alveoli, interstitium, and endothelium in the normotensive animals; this thinning was not observed in the animals with pulmonary hypertension (data not presented). Finally, epithelial and endothelial breaks induced by high tidal volume were observed in the normotensive but not the hypertensive animals (fig. 3).

**Discussion**

The occurrence of ventilator-associated lung injury in humans is extrapolated from the observations that changes in ventilation strategies affect outcome. Most of our knowledge regarding VILI is from animal studies conducted in normal or previously injured lungs.<sup>7,24</sup> In the current study, we chose to investigate the impact of mechanical ventilation in a well-characterized, clinically relevant condition, secondary pulmonary hypertension. The current results suggest that chronic structural changes in the lung vasculature may offer protection against injury from mechanical stretch.

*Lung Injury and the Pulmonary Vasculature*

The exact role of the pulmonary vasculature in the pathogenesis of VILI is not well established. However,

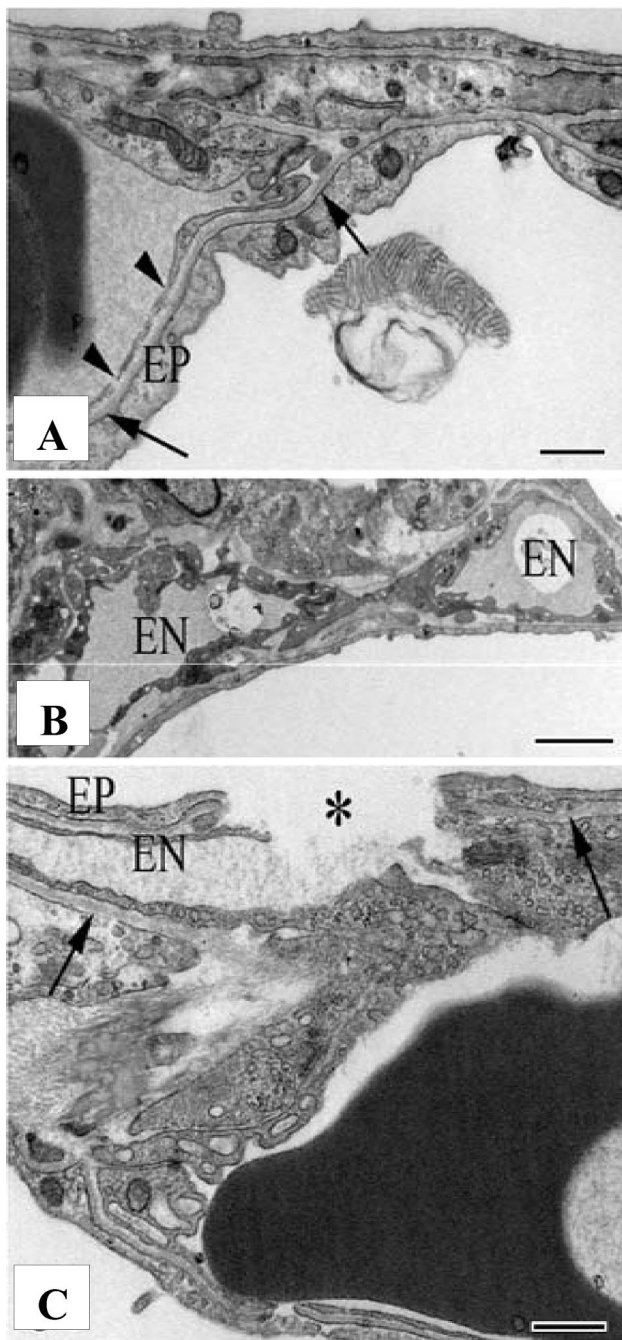
**Table 2. Cytokine Levels in Bronchoalveolar Lavage and Plasma**

	Interleukin 1 $\beta$		Interleukin 6		Tumor Necrosis Factor $\alpha$	
	BAL	Plasma	BAL	Plasma	BAL	Plasma
<b>Pulmonary hypertension (18)</b>						
Ventilated (7)	10 (8, 14)	15 (11, 19)	523 (415, 644)*	230 (168, 358)	19.5 (14, 35)	292 (135, 308)
Vasodilated and ventilated (6)	12 (11, 16)	16 (13, 20)*	425 (197, 658)*	1,348 (816, 2,442)*	26 (13, 29)	175 (108, 388)
Nonventilated (5)	10 (9, 12)	10 (9, 10)	17 (16, 38)	100 (64, 302)	0.5 (0.5, 5)	11 (11, 14)
<b>Normotensive control (12)</b>						
Ventilated (7)	7 (7, 10)	12 (10, 14)	197 (70, 382)	208 (122, 444)	20 (4, 91)	276 (90, 370)†
Nonventilated (5)	9 (7, 10)	11 (9, 12)	28 (23, 39)	58 (39, 90)	0.5 (0.5, 1)	12 (11, 12)

Data are expressed as median (interquartile range). Cytokine concentrations are expressed in pg/ml.

\* Significantly different from nonventilated pulmonary hypertensive animals ( $P < 0.05$ ). † Significantly different from nonventilated groups.

BAL = bronchoalveolar lavage.



**Fig. 3.** Electron micrographs showing the ultrastructure of the blood gas barrier with epithelial (EP) and endothelial (EN) cells, basement membrane (arrows), and interstitial matrix (\*). **A** (normal, normotensive nonventilated control) demonstrates normal EP cells and a normal basement membrane (arrows, EP side; arrowheads, EN side). Bar = 500 nm. **B** (hypertensive, high-tidal-volume ventilation) demonstrates thickened interstitial matrix and normal basement membrane without endothelial or epithelial breaks. Bar = 2  $\mu$ m. **C** (normal, normotensive high-tidal-volume ventilation) demonstrates severe breaks in the EN and EP cell membranes (\*), as well as in the basement membrane (arrow). Bar = 500 nm.

injurious ventilation in small animals can result in alveolar hemorrhage,<sup>29,30</sup> and several studies in isolated perfused lungs indicate that acute changes in pulmonary

artery pressure,<sup>13</sup> left atrial pressure,<sup>11</sup> or increased pulmonary blood flow<sup>14</sup> can worsen VILI. Others described the effects of increased pressure and shear forces in the pulmonary vessels on mediator release and VILI,<sup>31</sup> and that the combination of increase in transmural pressure and lung volume cause a stress failure of the microvascular (*i.e.*, capillary) vessel walls.<sup>9</sup> In addition, activation of the renin-angiotensin system may be pathogenic in VILI, through involvement of the lung vasculature.<sup>32,33</sup>

The current study demonstrated that animals with normal pulmonary vasculature that are exposed to injurious ventilation developed pulmonary edema with significant impairment of compliance and gas exchange whether they were pretreated or not with ROCK inhibitor. The pulmonary edema was preceded by structural deformity of the blood gas barrier, which occurred within the first 40 min of ventilation, but animals with CPAH that were exposed to the same ventilation strategy—indeed with even higher tidal volume because of higher baseline compliance as previously described<sup>34</sup>—were not affected, whether or not pulmonary artery pressure was acutely reduced.

Confirmation of these effects by electron microscopy underscores the site of structural deformity, *i.e.*, the blood gas barrier, and the pivotal role of “mechanical” injury in the early development of pulmonary edema and VILI in such small animal models.

#### Potential Mechanisms

The striking structural failure at the level of the alveolar capillary barrier observed on electron microscopy in the control animals without pulmonary hypertension and the absence of these findings in hypertensive animals suggest that the protection could be attributed to the structural changes associated with CPAH<sup>35,36</sup> and specifically the changes in the basement membrane.<sup>36</sup> Indeed, others have shown that the integrity of the alveolar-capillary barrier is related to the capillary basement membrane strength,<sup>18</sup> which is in turn related to both its thickness<sup>37</sup> and the matrix collagen content.<sup>38</sup> These data do not prove the mechanism but constitute supportive evidence. Capillary stress failure through increase in capillary transmural pressure and transpulmonary pressure plays a major role in lung injury during ventilation with high tidal volume.<sup>9,18,39</sup> Therefore, we suggest that the greater thickness of basement membrane observed in the hypertensive group offers greater mechanical strength to the alveolar capillary barrier and protection against the stress failure during high-tidal-volume ventilation.

Finally, a similar trend in cytokine release was observed among groups (table 2), where interleukin 6 and tumor necrosis factor  $\alpha$  increased in the BAL and plasma after injurious ventilation in normotensive and pulmonary hypertensive animals (table 2). Although the sample size is small and the variability high, the data may suggest that



pulmonary hypertension results in greater resistance to the inflammatory effects of mediators or that such mediators play a lesser role in the pathogenesis of VILI than previously thought.<sup>40</sup>

### Potential Clinical Implications

Secondary pulmonary hypertension is a relative common complication of chronic lung disease and may be associated with increased morbidity and mortality. Although this study shows that chronic pulmonary vascular changes may offer protection against injury from mechanical stretch, the time course and the model design are very different from lung injury observed in the clinical context.

### Study Limitations

The current study was design to determine whether changes associated with chronic pulmonary hypertension could affect the development of VILI. A well-established model of CPAH, the chronic hypoxia model, was used.<sup>27</sup> Then, to isolate the confounding effects of pressure,<sup>13,41</sup> an additional group of animals were treated with a ROCK inhibitor to acutely reverse raised pulmonary vascular resistance.<sup>42</sup> However, the current study has several limitations. First, we cannot exclude the possibility that mechanical ventilation may have differentially affected pulmonary artery flow and pressure. Second, the plateau pressure was not measured. Therefore, it is possible that in the different groups, although exposed to identical peak inspiratory pressure and similar tidal volume (based on body weight), were in fact subjected to different degrees of local stress or strain. Third, because wet/dry weight was not measured in nonventilated animals, it is theoretically possible that the wet/dry differences observed between the normotensive and hypertensive ventilated animals reflect a pre-existing (before ventilation) difference in this ratio. However, we believe that the reduction in respiratory system compliance and  $P_{aO_2}$  in combination of capillary breaks and obvious pulmonary edema observed in the normotensive animals at termination of the experiment support the differences observed in wet/dry and the occurrence of pulmonary edema.

### Summary and Conclusions

Chronic pulmonary hypertension reduced the susceptibility to VILI in rats; these effects do not seem to be due to the presence of pulmonary hypertension *per se*, but rather to due to the associated vascular remodeling.

### References

1. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The Acute Respiratory Distress Syndrome Network. *N Engl J Med* 2000; 342:1301-8.

2. Dreyfuss D, Saumon G: Ventilator-induced lung injury: Lessons from experimental studies. *Am J Respir Crit Care Med* 1998; 157:294-323
3. Burger R, Fung D, Bryan AC: Lung injury in a surfactant-deficient lung is modified by indomethacin. *J Appl Physiol* 1990; 69:2067-71
4. Dickey BF, Thrall RS, McCormick JR, Ward PA: Oleic-acid-induced lung injury in the rat: Failure of indomethacin treatment or complement depletion to ablate lung injury. *Am J Pathol* 1981; 103:376-83
5. Folkesson HG, Matthay MA, Hebert CA, Broaddus VC: Acid aspiration-induced lung injury in rabbits is mediated by interleukin-8-dependent mechanisms. *J Clin Invest* 1995; 96:107-16
6. Rinaldo JE, Dauber JH, Christman J, Rogers RM: Neutrophil alveolitis following endotoxemia: Enhancement by previous exposure to hyperoxia. *Am Rev Respir Dis* 1984; 130:1065-71
7. Laffey JG, Tanaka M, Engelberts D, Luo X, Yuan S, Tanswell AK, Post M, Lindsay T, Kavanagh BP: Therapeutic hypercapnia reduces pulmonary and systemic injury following *in vivo* lung reperfusion. *Am J Respir Crit Care Med* 2000; 162:2287-94
8. Tremblay L, Valenza F, Ribeiro SP, Li J, Slutsky AS: Injurious ventilatory strategies increase cytokines and c-fos mRNA expression in an isolated rat lung model. *J Clin Invest* 1997; 99:944-52
9. Fu Z, Costello ML, Tsukimoto K, Prediletto R, Elliott AR, Mathieu-Costello O, West JB: High lung volume increases stress failure in pulmonary capillaries. *J Appl Physiol* 1992; 73:123-33
10. Hotchkiss JR, Simonson DA, Marek DJ, Marini JJ, Dries DJ: Pulmonary microvascular fracture in a patient with acute respiratory distress syndrome. *Crit Care Med* 2002; 30:2368-70
11. Broccard AF, Vannay C, Feihl F, Schaller MD: Impact of low pulmonary vascular pressure on ventilator-induced lung injury. *Crit Care Med* 2002; 30:2183-90
12. Guery BP, DeBoisblanc BP, Fialdes P, Sarphy TG, Nelson S, Chidiac C, Beaucaire G, Summer WR, Mason CM: Pulmonary stress injury within physiological ranges of airway and vascular pressures. *J Crit Care* 1998; 13:58-66
13. Hotchkiss JR Jr, Blanch L, Naveira A, Adams AB, Carter C, Olson DA, Leo PH, Marini JJ: Relative roles of vascular and airspace pressures in ventilator-induced lung injury. *Crit Care Med* 2001; 29:1593-8
14. Broccard AF, Hotchkiss JR, Kuwayama N, Olson DA, Jamal S, Wangenstein DO, Marini JJ: Consequences of vascular flow on lung injury induced by mechanical ventilation. *Am J Respir Crit Care Med* 1998; 157:1935-42
15. Lopez-Aguilar J, Piacentini E, Villagra A, Murias G, Pascotto S, Saenz-Valiente A, Fernandez-Segoviano P, Hotchkiss JR, Blanch L: Contributions of vascular flow and pulmonary capillary pressure to ventilator-induced lung injury. *Crit Care Med* 2006; 34:1106-12
16. Imai Y, Kawano T, Miyasaka K, Takata M, Imai T, Okuyama K: Inflammatory chemical mediators during conventional ventilation and during high frequency oscillatory ventilation. *Am J Respir Crit Care Med* 1994; 150:1550-4
17. Perlman MB, Johnson A, Malik AB: Ibuprofen prevents thrombin-induced lung vascular injury: Mechanism of effect. *Am J Physiol* 1987; 252:H605-14
18. West JB, Tsukimoto K, Mathieu-Costello O, Prediletto R: Stress failure in pulmonary capillaries. *J Appl Physiol* 1991; 70:1731-42
19. Kantores C, McNamara PJ, Teixeira L, Engelberts D, Murthy P, Kavanagh BP, Jankov RP: Therapeutic hypercapnia prevents chronic hypoxia-induced pulmonary hypertension in the newborn rat. *Am J Physiol Lung Cell Mol Physiol* 2006; 291:L912-22
20. Tsuchida S, Engelberts D, Roth M, McKerlie C, Post M, Kavanagh BP: Continuous positive airway pressure causes lung injury in a model of sepsis. *Am J Physiol Lung Cell Mol Physiol* 2005; 289:L554-64
21. Uehata M, Ishizaki T, Satoh H, Ono T, Kawahara T, Morishita T, Tamakawa H, Yamagami K, Inui J, Maekawa M, Narumiya S: Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. *Nature* 1997; 389:990-4
22. Jones JE, Mendes L, Rudd MA, Russo G, Loscalzo J, Zhang YY: Serial noninvasive assessment of progressive pulmonary hypertension in a rat model. *Am J Physiol Heart Circ Physiol* 2002; 283:H364-71
23. Duggan M, McNamara PJ, Engelberts D, Pace-Asciak C, Babyn P, Post M, Kavanagh BP: Oxygen attenuates atelectasis-induced injury in the *in vivo* rat lung. *ANESTHESIOLOGY* 2005; 103:522-31
24. Kornecki A, Tsuchida S, Ondiveeran HK, Engelberts D, Frndova H, Tanswell AK, Post M, McKerlie C, Belik J, Fox-Robichaud A, Kavanagh BP: Lung development and susceptibility to ventilator-induced lung injury. *Am J Respir Crit Care Med* 2005; 171:743-52
25. Vignali DA: Multiplexed particle-based flow cytometric assays. *J Immunol Methods* 2000; 243:243-55
26. Prabhakar U, Eirikis E, Miller BE, Davis HM: Multiplexed cytokine sandwich immunoassays: Clinical applications. *Methods Mol Med* 2005; 114:223-32
27. Rabinovitch M, Gamble W, Nadas AS, Miettinen OS, Reid L: Rat pulmonary circulation after chronic hypoxia: Hemodynamic and structural features. *Am J Physiol* 1979; 236:H818-27
28. Hislop A, Reid L: New findings in pulmonary arteries of rats with hypoxia-induced pulmonary hypertension. *Br J Exp Pathol* 1976; 57:542-54
29. Webb HH, Tierney DF: Experimental pulmonary edema due to intermittent positive pressure ventilation with high inflation pressures: Protection by positive end-expiratory pressure. *Am Rev Respir Dis* 1974; 110:556-65
30. Dreyfuss D, Basset G, Soler P, Saumon G: Intermittent positive-pressure

- hyperventilation with high inflation pressures produces pulmonary microvascular injury in rats. *Am Rev Respir Dis* 1985; 132:880-4
31. Uhlig S: Ventilation-induced lung injury and mechanotransduction: Stretching it too far? *Am J Physiol Lung Cell Mol Physiol* 2002; 282:L892-6
32. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, Yang P, Sarao R, Wada T, Leong-Poi H, Crackower MA, Fukamizu A, Hui CC, Hein L, Uhlig S, Slutsky AS, Jiang C, Penninger JM: Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 2005; 436:112-6
33. Marshall RP, Webb S, Bellingan GJ, Montgomery HE, Chaudhari B, McAnulty RJ, Humphries SE, Hill MR, Laurent GJ: Angiotensin converting enzyme insertion/deletion polymorphism is associated with susceptibility and outcome in acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2002; 166:646-50
34. Okubo S, Mortola JP: Respiratory mechanics in adult rats hypoxic in the neonatal period. *J Appl Physiol* 1989; 66:1772-8
35. Howell K, Preston RJ, McLoughlin P: Chronic hypoxia causes angiogenesis in addition to remodelling in the adult rat pulmonary circulation. *J Physiol* 2003; 547:133-45
36. Stenmark KR, Fagan KA, Frid MG: Hypoxia-induced pulmonary vascular remodeling: Cellular and molecular mechanisms. *Circ Res* 2006; 99:675-91
37. Mathieu-Costello O, Willford DC, Fu Z, Garden RM, West JB: Pulmonary capillaries are more resistant to stress failure in dogs than in rabbits. *J Appl Physiol* 1995; 79:908-17
38. West JB, Mathieu-Costello O: Vulnerability of pulmonary capillaries in heart disease. *Circulation* 1995; 92:622-31
39. Hachenberg T, Rettig R: Stress failure of the blood-gas barrier. *Curr Opin Anaesthesiol* 1998; 11:37-44
40. Ricard JD, Dreyfuss D: Cytokines during ventilator-induced lung injury: A word of caution. *Anesth Analg* 2001; 93:251-2
41. Broccard AF, Hotchkiss JR, Suzuki S, Olson D, Marini JJ: Effects of mean airway pressure and tidal excursion on lung injury induced by mechanical ventilation in an isolated perfused rabbit lung model. *Crit Care Med* 1999; 27:1533-41
42. Hyvelin JM, Howell K, Nichol A, Costello CM, Preston RJ, McLoughlin P: Inhibition of Rho-kinase attenuates hypoxia-induced angiogenesis in the pulmonary circulation. *Circ Res* 2005; 97:185-91