Pulmonary lesion induced by low and high positive end-expiratory pressure levels during protective ventilation in experimental acute lung injury

Caroline P. Pássaro, PhD; Pedro L. Silva, MSc; Andréia F. Rzezinski, MSc; Simone Abrantes, RRT;
Viviane R. Santiago; Liliane Nardelli, RRT; Raquel S. Santos, RRT; Carolina M. L. Barbosa, BSc;
Marcelo M. Morales, MD, PhD; Walter A. Zin, MD, PhD; Marcelo B. P. Amato, MD, PhD;
Vera L. Capelozzi, MD, PhD; Paolo Pelosi, MD; Patricia R. M. Rocco, MD, PhD

Objective: To investigate the effects of low and high levels of positive end-expiratory pressure (PEEP), without recruitment maneuvers, during lung protective ventilation in an experimental model of acute lung injury (ALI).

Design: Prospective, randomized, and controlled experimental study.

Setting: University research laboratory.

Subjects: Wistar rats were randomly assigned to control (C) [saline (0.1 mL), intraperitoneally] and ALI [paraquat (15 mg/kg), intraperitoneally] groups.

Measurements and Main Results: After 24 hours, each group was further randomized into four groups (six rats each) at different PEEP levels = 1.5, 3, 4.5, or 6 cm H_2O and ventilated with a constant tidal volume (6 mL/kg) and open thorax. Lung mechanics [static elastance (Est, L) and viscoelastic pressure (ΔP2, L)] and arterial blood gases were measured before (Pre) and at the end of 1-hour mechanical ventilation (Post). Pulmonary histology (light and electron microscopy) and type III procollagen (PCIII) messenger RNA (mRNA) expression were measured after 1 hour of mechanical ventilation. In ALI group, low and high PEEP levels induced a greater percentage of increase in Est, L (44% and 50%) and ΔP2, L (56% and 36%) in Post values related to Pre. Low PEEP yielded alveolar collapse whereas high PEEP caused overdistension and atelectasis, with both levels worsening oxygenation and increasing PCIII mRNA expression.

Conclusions: In the present nonrecruited ALI model, protective mechanical ventilation with lower and higher PEEP levels than required for better oxygenation increased Est, L and ΔP2, L, the amount of atelectasis, and PCIII mRNA expression. PEEP selection titrated for a minimum elastance and maximum oxygenation may prevent lung injury while deviation from these settings may be harmful. (Crit Care Med 2009; 37:1011–1017)

KEY WORDS: oxygenation; elastance; alveolar collapse; cellular stress; electron microscopy

The reduction in tidal volume to limit mechanical stress on the lung parenchyma, associated with adequate gas-exchange, has become the cornerstone of protective mechanical ventilation in patients with acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) (1–5).

Excessive reduction in tidal volume may result in harmful alveolar derecruitment and low end-expiratory lung volume, depending on the level of positive end-expiratory pressure (PEEP) (1–9). Ventilation at low lung volume can exacerbate lung injury because of regional cyclic mechanical stretch of atelectatic parenchymal regions (5, 8–11). This process is associated with increased activation of inflammatory mediators released by the lung and results in adverse systemic effects, contributing to the development of multisystem organ failure (5, 11–15). Therefore, the application of PEEP has been suggested to prevent and/or limit lung injury by avoiding continuous collapse and reopening of alveoli. Conversely, high-PEEP strategies can lead to detrimental consequences, such as the development of alveolar overdistension (8–11, 14–16). Thus, it is important to select the appropriate level of PEEP in order to minimize cyclic forces of alveolar collapse and reopening, as well as lung hyperinflation (2, 3, 6, 7, 17–23). However, there is no general consensus on which physiologic parameters PEEP should be selected at the bedside, thus maximizing the beneficial effects on lung mechanics and gas-exchange, reducing inflammatory process, and minimizing ventilator-induced lung injury. In addition, information on how high PEEP levels may lead to lung injury is scanty.

Our hypothesis was that the application of both lower and higher PEEP levels

Table 1. Lung mechanical parameters

| Groups | Est, L (cm H_2O/mL) | | | | $\Delta P2$, L (cm H_2O) | | | |
| | C | | ALI[a] | | C | | ALI[a] | |
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
PEEP 1.5	2.08 (0.71)	2.44 (0.34)[b]	3.42 (0.27)	4.94 (0.29)[b]	0.80 (0.37)	1.09 (0.17)	1.16 (0.32)	1.81 (0.32)[b]
PEEP 3	2.42 (0.37)	2.95 (0.76)	4.04 (0.29)	5.04 (0.61)[b]	0.75 (0.12)	0.97 (0.17)[b]	0.96 (0.15)	1.29 (0.12)[b,c]
PEEP 4.5	2.51 (1.10)	2.66 (1.18)	3.19 (0.81)	3.95 (1.20)[b,c]	0.89 (0.39)	0.90 (0.41)	1.16 (0.32)	1.39 (0.07)[b,c]
PEEP 6	2.67 (0.44)	3.23 (0.42)[b]	3.16 (0.91)	4.75 (0.32)[b]	0.96 (0.27)	1.03 (0.32)	1.50 (0.37)	2.04 (0.66)[b]

Est, L, lung static elastance; $\Delta P2$, L, lung viscoelastic/inhomogeneous pressure; C, control group; ALI, acute lung injury group; PEEP, positive end-expiratory pressure; ALI, acute lung injury.

[a]Significantly different from C group independent of PEEP level both in Pre and Post ($p < 0.05$); [b]significantly different from Pre of the corresponding group($p < 0.05$); [c]Significantly different from PEEP 1.5 in ALI group ($p < 0.05$). Values are mean (SD) of six animals per group. Pre, mechanical ventilation with tidal volume of 6 mL/kg body weight, inspiratory flow of 7 mL/sec, frequency of 100 breaths/min, inspiratory-to-expiratory ratio of 1:2, inspired oxygen fraction (FIO_2) of 21%, and PEEP of 1.5 (PEEP 1.5), 3 (PEEP 3), 4.5 (PEEP 4.5), or 6 (PEEP 6) cm H_2O, POST, after 1-hr mechanical ventilation.

Table 2. Arterial blood gases parameters

| Groups | PaO_2/FIO_2 | | | |
| | C | | ALI[a] | |
	Pre	Post	Pre	Post
PEEP 1.5	340.0 (12.3)	283.0[b] (23.5)	230.2 (69.3)	137.2[b] (45.5)
PEEP 3	300.5 (11.2)	314.0 (11.1)	255.0 (147.0)	218.0[b] (126.5)[c]
PEEP 4.5	343.2 (97.0)	491.9[b] (110.2)[c]	269.2 (59.0)	566.9[b] (27.1)[c]
PEEP 6	343.5 (50.5)	300.8 (38.1)	220.1 (79.1)	95.6[b] (34.0)

PaO_2/FIO_2, arterial oxygen pressure and inspired oxygen fraction ratio; C, control group; PEEP, positive end-expiratory pressure; ALI, acute lung injury.

[a]Significantly different from C group ($p < 0.05$); [b]significantly different from Pre of the corresponding group ($p < 0.05$); [c]Significantly different from PEEP 1.5 in ALI group ($p < 0.05$). Values are mean (SD) of five animals per group; Pre, mechanical ventilation with tidal volume of 6 mL/kg body weight, inspiratory flow of 7 mL/sec, frequency of 100 breaths/min, inspiratory-to-expiratory ratio of 1:2, inspired oxygen fraction (FIO_2) of 1, and PEEP of 1.5 (PEEP 1.5), 3 (PEEP 3), 4.5 (PEEP 4.5), or 6 (PEEP 6) cm H_2O, Post, after 1-hr mechanical ventilation.

than those required for optimal lung mechanics and gas-exchange during protective mechanical ventilation in an experimental model of ALI may deteriorate lung mechanics, promote atelectasis and/or overdistension, and increase the alveolar stress. For this purpose, lung mechanics (static elastance and viscoelastic pressure), arterial blood gases, lung histology (light and electron microscopy), and type III procollagen (PCIII) messenger RNA (mRNA) expression were measured in animals with ALI ventilated with lung-protective strategy at different PEEP levels.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of the Carlos Chagas Filho Institute of Biophysics, Health Sciences Center, Federal University of Rio de Janeiro. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences, USA.

Animal Preparation and Experimental Protocol

Forty-eight Wistar rats (220–260 g) were used. In the control (C) group (n = 24 animals), sterile saline (0.9% NaCl, 5 mL/kg body weight [BW]) was injected intraperitoneally and ALI rats (n = 24) received paraquat (15 mg/kg BW intraperitoneally). After 24 hours, the animals were sedated (diazepam 5 mg intraperitoneally), anesthetized (thiopental sodium 20 mg/kg BW intraperitoneally), and a snugly fitting cannula (1.7 mm inside diameter) was introduced into the trachea. The animals were then paralyzed with pancuronium bromide (2 mg/kg intravenous), and a constant-flow ventilator provided artificial ventilation (Samay VR15, Universidad de la Republica, MTevideo, Uruguay) with the following parameters: tidal volume (V_T) of 6 mL/kg BW, inspiratory flow of 7 mL/s, frequency of 100 breaths/min, inspiratory-to-expiratory ratio of 1:2, and fraction of inspired oxygen (FIO_2) of 1.0. Subsequently, the chest wall was surgically removed and transpulmonary pressure was carefully measured in paralyzed rats when the chest was opened (by occluding the tracheal cannula and measuring the tracheal pressure). The mean transpulmonary pressures in C and ALI groups presented values equal to 2.8 ± 0.3 and 3.3 ± 0.2 (Mean $\pm$ SEM) cm H_2O, respectively, with no significant differences between them. Therefore, we applied a PEEP equal to 3 cm H_2O, which represented the mean transpulmonary pressure considering C and ALI groups together. We were then able to assure that the same transpulmonary pressure was applied and identical conditions used in both groups. Arterial oxygen partial pressure (PaO_2) was measured. FIO_2 was then adjusted to 0.21 to avoid absorption atelectasis and, after 5 minutes, lung mechanics were measured (Pre). Afterward, each animal was randomly assigned to one of the four PEEP groups: 1.5 cm H_2O (PEEP 1.5), 3 cm H_2O (PEEP 3), 4.5 cm H_2O (PEEP 4.5), or 6 cm H_2O (PEEP 6). After 1-hour ventilation period, FIO_2 was set at 1.0 and, after 5 minutes, PaO_2 was measured. Following this step, FIO_2 was reduced to 0.21 and, after 5 minutes, lung mechanics were computed (Post). At the end of the experiments the lungs were prepared for histology and PCIII mRNA expression in lung tissue was analyzed.

Data Acquisition

Air flow, volume and tracheal pressure were registered. Lung static elastance (Est, L) and viscoelastic/inhomogeneous pressure ($\Delta P2$, L) were computed by the end-inflation occlusion method (24). Briefly, after end-inspiratory occlusion there is an initial fast
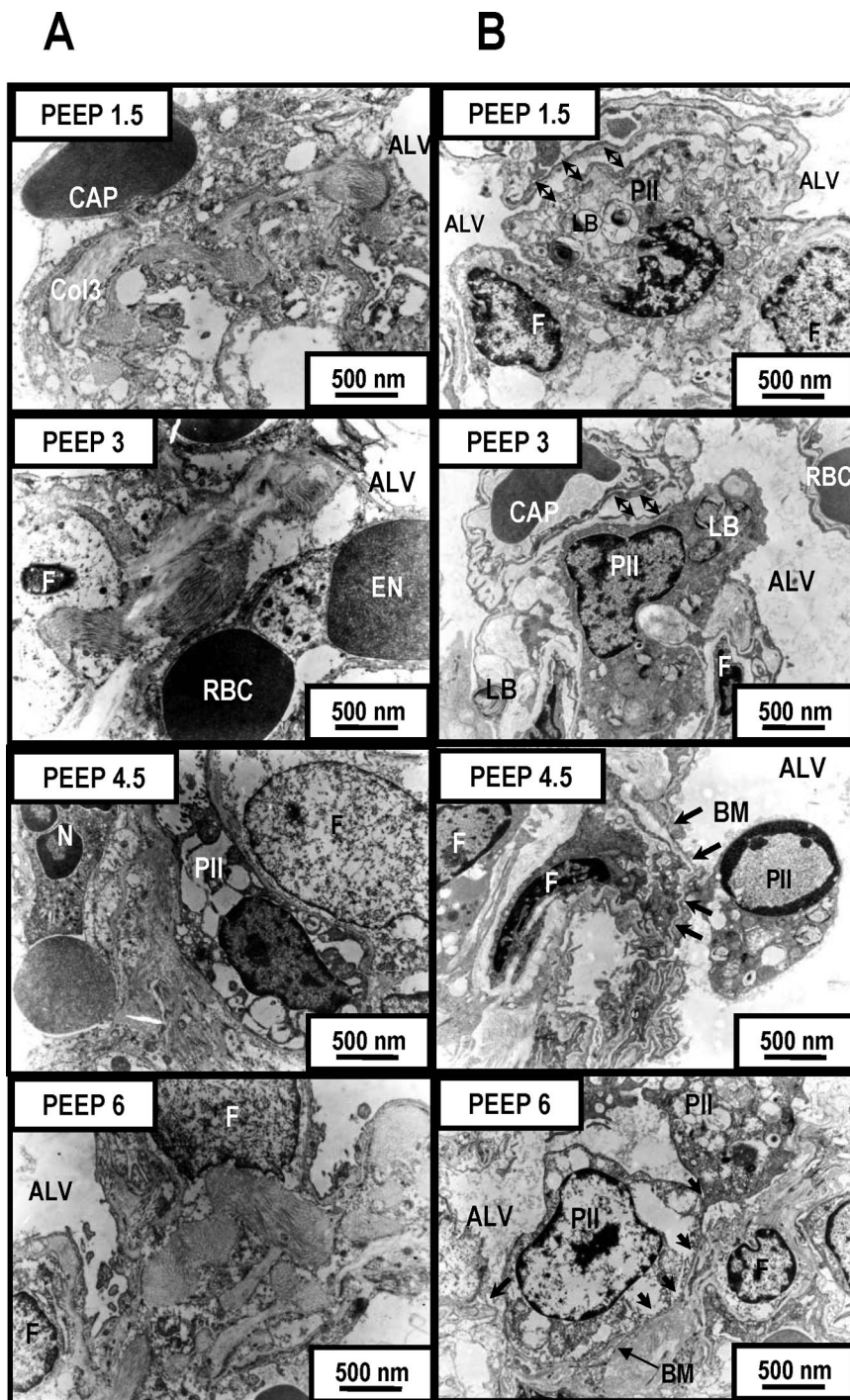
Figure 1. Electron photomicrographs of lung parenchyma in control (*A*) and acute lung injury groups (*B*). *PII*, type II pneumocyte; *ALV*, alveolar space; *CAP*, capillary; *BM*, basement membrane; *LB*, lamellar bodies; *F*, fibroblast; *N*, neutrophil; *EN*, endothelial nucleus; *RBC*, red blood cell; *Col3*, type 3 collagen fiber. Positive end-expiratory pressure (*PEEP*) 1.5, PEEP 3, PEEP 4.5, and PEEP 6 = animals ventilated during 1 hour with 1.5, 3, 4.5, and 6 cm H_2O PEEP levels, respectively.

A polyethylene catheter (PE-10) was introduced into the femoral artery for blood sampling. Blood (300 µL) was drawn into a heparinized syringe for Pao_2 measurement (i-STAT, Abbott Laboratories, IL).

Lung Histology

Light Microscopy. A laparotomy was done immediately after the determination of lung mechanics (Post), and heparin (1000 international units) was intravenously injected into the vena cava. The trachea was clamped at end-expiration, and the abdominal aorta and vena cava were sectioned, yielding a massive hemorrhage that quickly killed the animals. Then, the lungs were removed *en bloc*. The right lungs were quick-frozen by immersion in liquid nitrogen and fixed with Carnoy's solution (25). Slices 4-µm-thick were cut and underwent hematoxylin-eosin. Morphometric analysis was performed using an integrated eyepiece with a coherent system consisting of 100 point and 50 line grid (known length) coupled to a conventional light microscope (Axioplan, Zeiss, Oberkochen, Germany). The volume fraction of the lung occupied by hyperinflated structures (alveolar ducts and sacs or alveoli wider than 120 µm), collapsed alveoli (defined as those that presented rough or plicate walls), or normal pulmonary areas were determined by the point-counting technique (26), magnified at ×200 across ten random, noncoincident microscopic fields.

Transmission Electron Microscopy

Three slices ($2 \times 2 \times 2$ mm^3) were cut from three different segments of the left lung to obtain a stratified random sample. A specimen was then fixed in 2.5% glutaraldehyde and phosphate buffer. Ultrathin sections were observed using a transmission electron microscope (JEOL 1010 Transmission Electron Microscope, Tokyo, Japan). The following parameters were analyzed: a) alveolar barrier; b) types I and II pneumocytes; and c) endothelial cells, according to a 5-point semiquantitative severity-based scoring system. The pathologic findings were graded as 0, normal lung parenchyma; 1, changes in 1% to 25%; 2, changes in 26% to 50%; 3, changes in 51% to 75%; and 4, changes in 765 to 100% of the examined tissue.

Semiquantitative Reverse-Transcription and Polymerase Chain Reaction

In C and ALI groups, additional nonventilated animals were used (n = 4 each). These rats received the same sedation and anesthesia protocol as the other groups, but were not submitted to mechanical ventilation. Their lungs were removed *en bloc* immediately after anesthesia was completed, and parenchyma

drop in pressure from the preocclusion value [lung peak inspiratory pressure] down to an inflection point followed by slow pressure decay ($\Delta P2$, L) until a plateau is reached. This plateau corresponds to the lung elastic recoil pressure [transpulmonary plateau pressure (Pplat, L)]. $\Delta P2$ reflects lung viscoelastic properties together with a small contribution of time-constant inhomogeneities (*pendelluft*). Est, L was calculated by dividing Pplat, L by the V_T. Pulmonary mechanics measurements were performed ten times in each animal, and analyzed using ANADAT data analysis software (RHT-InfoData).

Table 3. Morphometric data

| Groups | Normal Area (%) | | Alveolar Collapse (%) | | Alveolar Hyperinflation (%) | |
	C	ALI	C	ALI	C	ALI
PEEP 1.5	95.6 (1.8)	77.0 (16.1)[a]	4.5 (1.8)	23.0 (16.1)[a]	0.0 (0.0)	0.0 (0.0)
PEEP 3	99.3 (0.7)	92.9 (2.6)[a,b]	0.7 (0.7)	6.8 (2.1)[a,b]	0.0 (0.0)	0.3 (0.7)[a]
PEEP 4.5	96.9 (3.0)	94.4 (2.5)[b]	3.1 (3.0)	4.5 (2.5)[b]	0.0 (0.0)	1.1 (1.6)[a]
PEEP 6	96.9 (2.7)	71.5 (21.9)[a]	2.3 (1.6)	22.8 (21.6)[a]	0.8 (1.8)	5.8 (0.9)[a,b]

C, control group; ALI, acute lung injury group; PEEP, positive end-expiratory pressure.

[a]Significantly different from C group ($p < 0.05$); [b]significantly different from PEEP 1.5 in ALI group ($p < 0.05$). Values are mean (SD) of six animals per group. Ten random, noncoincident microscopic fields were analyzed after the determination of lung mechanics (1-hr period) in each subgroup; PEEP 1.5, PEEP 3, PEEP 4.5, and PEEP 6, animals ventilated during 1 hr with 1.5, 3, 4.5, and 6 cm H_2O PEEP level, respectively.

Table 4. Semi-quantitative analysis of electron microscopy

| Groups | Alveolar Capillary Membrane | | Type II Epithelial Cell | | Endothelial Cell | |
	C	ALI[a]	C	ALI[a]	C	ALI[a]
PEEP 1.5	0 (0–1)	3 (3–4)	0 (0–0)	3 (3–3.25)	0 (0–0)	3 (3–4)
PEEP 3	0 (0–0.25)	2 (1.75–2.25)	0 (0–0)	3 (2.75–3.25)	0 (0–0)	3 (2.75–3)
PEEP 4.5	0 (0–0)	1 (1–2)[b]	0 (0–0)	2 (1.75–3)[b]	0 (0–0)	2 (2–2.25)[b]
PEEP 6	0 (0–1)	4 (3–4)	0 (0–0.25)	4 (3–4)	0 (0–0)	4 (3.75–4)

C, control group; ALI, acute lung injury group; PEEP, positive end-expiratory pressure.

[a]Significantly different from C group ($p < 0.05$); [b]significantly different from PEEP1.5 in ALI group ($p < 0.05$). Lung tissue score was done independently by two different investigators. The pathologic findings were graded according to a 5-point semiquantitative severity-based scoring system: 0, normal lung parenchyma; 1, changes in 1% to 25%; 2, changes in 26% to 50%; 3, changes in 51% to 75%; and 4, changes in 76% to 100% of the examined tissue. Electron microscopy of lung parenchyma in C (control) and ALI (acute lung injury) groups. PEEP 3, animals ventilated with PEEP of 3 cm H_2O; PEEP 1.5, animals ventilated with PEEP of 1.5 cm H_2O; PEEP 4.5, animals ventilated with PEEP of 4.5 cm H_2O; PEEP 6, animals ventilated with PEEP of 6 cm H_2O. Values are median (25th percentile–75th percentile) of five rats in each group.

strips ($3 \times 3 \times 10$ mm^3) were longitudinally cut from left lungs. Total RNA was isolated from the frozen lung tissue (27). The relative expression of PCIII mRNA was obtained by semiquantitative reverse-transcription and polymerase chain reaction. In the PCIII mRNA detection by reverse-transcription and polymerase chain reaction, the rat glyceraldehyde-3-phosphate-dehydrogenase primers were used as internal positive control. The semiquantitative method of reverse-transcription and polymerase chain reaction, used to quantify the PCIII mRNA expression in rat lung, was validated in preliminary experiments (28, 29). All reactions included a negative control reverse transcriptase(−). The identity of the amplification was confirmed by determination of the molecular size on agarose gel electrophoresis with 100 bp DNA molecular marker (GIBCO BRL, Grand Island, NY).

Statistical Analysis

Two-way analysis of variance was used to compare pulmonary effects using the lung injury and the different PEEP levels as the two factors for analysis. One-way analysis of variance was used to compare morphologic and mRNA data among all groups. In both cases, if multiple comparisons were required, Tukey test was applied. SigmaStat 3.0 statistical software package (Jandel Corporation, San Rafael, CA) was used. In all instances, the significance level was set at 5% ($\alpha = 5\%$).

RESULTS

There was no significant difference in flow and tidal volume among the groups. Est, L, and ΔP2, L were higher in ALI than in C groups at baseline (Pre) (Table 1). Est, L, and ΔP2, L increased in ALI groups at the end of 1 hour mechanical ventilation with different PEEP levels (Post) (Table 1). The percentage of the increase in Est, L and ΔP2, L in Post values related to Pre was significantly higher in PEEP 1.5 (44% and 56%, re-

spectively) and PEEP 6 (50% and 36%, respectively) groups.

PaO$_2$ was lower in ALI than in C groups at baseline (Table 2). In ALI groups, PEEP 1.5 group showed better oxygenation than the other groups after 1-hour mechanical ventilation (Table 2).

Figure 1 shows the alveolar-capillary barrier in C and ALI groups at different PEEP levels under electron microscopy. In C groups the alveolar-capillary barrier was preserved and supported the maintenance of the alveolar architecture observed by light microscopy (Table 3). In contrast, in ALI groups there was a distortion of the alveolar-capillary barrier, with type II cell apoptosis, denudation of basal lamina, interstitial edema, and an increase in the amount of type III collagen fibers (Fig. 1 and Table 4), concurrent with higher fraction area of alveolar collapse mainly in PEEP 1.5 and PEEP 6 groups (Table 4).

PCIII mRNA expression in C and ALI groups at different PEEP levels and in nonventilated animals is depicted in Figure 2. In this set of experiments, data were related to the values obtained in the nonventilated-C group. PCIII mRNA expression was significantly increased in ALI compared with C, mainly in PEEP 1.5 and PEEP 6 groups (Fig. 2).

DISCUSSION

This study demonstrated that protective mechanical ventilation with inappropriate PEEP levels resulted in impaired lung mechanics, increased atelectasis, hyperinflation, and PCIII mRNA expression compared with a ventilatory strategy where PEEP is titrated in order to obtain minimum elastance and maximum oxygenation.

Different clinical studies demonstrated that mortality rate associated with the iatrogenic effects of mechanical ventilation may be reduced by using lung protective ventilation strategies in ALI/ARDS patients (2, 3). Such strategies share the common goal of diminishing tidal tissue stress by reducing the tidal volume, but the role of PEEP is still under debate. PEEP simply stabilizes lung avoiding derecruitment of collapsed areas, minimizing cyclic alveolar collapse, and re-opening during tidal breathing, which is associated with increased shear stress and progressive lung injury (15, 16, 30). However, excessive PEEP levels may induce overinflation, promoting pulmonary lesion (17–21).
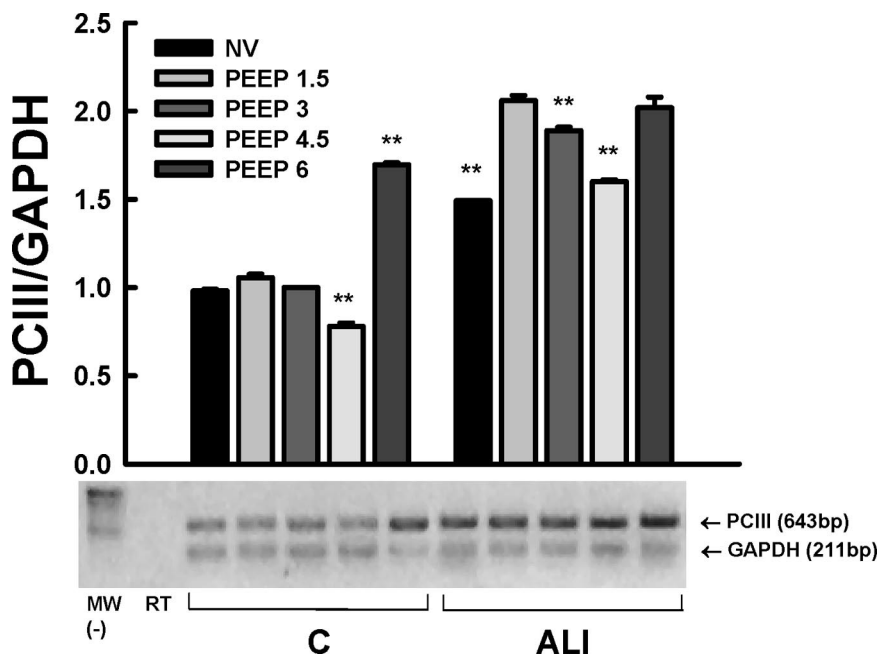
Figure 2. Relative expression of type III procollagen mRNA (*PCIII*) obtained by amplification of PCIII and glyceraldehydes-3-phosphate-dehydrogenase (*GAPDH*) by semiquantitative reverse-transcription and polymerase chain reaction (*RT*-PCR) of rat lung tissue in different situations. *C*, control group; *ALI*, acute lung injury group; *NV*, nonventilated animals; Positive end-expiratory pressure (*PEEP*) 1.5, PEEP 3, PEEP 4.5, and PEEP 6, animals ventilated during 1 hour with 1.5, 3, 4.5, and 6 cm H_2O PEEP level, respectively. *MW*, Molecular weight. Values are mean + SD (n = 4) of the ratio between the densitometric values of PCIII and GAPDH bands obtained in RT-PCR experiments. **Significantly different from PEEP 1.5 of the corresponding group ($p < 0.05$).

High PEEP levels may result in a reduction in mortality rate (2, 7). Nevertheless, the beneficial effects of high PEEP in patients with ARDS have been recently questioned (2, 3, 7, 21–23). In agreement with other studies (2, 7, 15, 16, 28, 31–36), we observed that ventilation in ALI rats at low PEEP levels was accompanied by an increase in Est, L (Table 1) and alveolar collapse (Table 3), a reduction in oxygenation (Table 2), elevation of PCIII expression (Fig. 2), and ultrastructural changes in the alveolar-capillary barrier suggesting ventilator-induced lung injury (Fig. 1 and Table 4). We also observed that ΔP2, L increased after ALI induction and, most importantly, varied according to the optimal PEEP levels. ΔP2, L reflects lung viscoelastic properties together with a small contribution of time-constant inhomogeneities and may be an important parameter to evaluate, at bedside, the role of relative overdistension, as well as stress and strain at different levels of PEEP. However, when ALI animals were ventilated with high PEEP levels we found similar deterioration in lung mechanics and oxygenation, development of alveolar hyperinflation with areas of atelectasis (Tables 1–3), and

changes in the electron microscopy characterized by detachment of the epithelial cells from the basement membrane, and apoptotic type II pneumocytes (Fig. 1 and Table 4). The presence of overinflation with areas of alveolar collapse may be essentially explained by two phenomena: a) increased lung injury and edema, resulting in an increase in the weight of the lung and subsequent atelectasis formation, and b) compression of the most dependent part of the lung due to the inhomogeneous distribution of alveolar pressure. We did not measure the wet-to-dry ratio and, hence, we cannot confirm the increased amount of lung water. Furthermore, previous reports in ALI/ARDS patients showed a more important degree of atelectasis in the most dependent part of the lung at higher airway pressure levels, similar to that found in our study (30, 33).

Different methods have been suggested for the appropriate selection of PEEP at bedside in ALI/ARDS patients such as: a) lung mechanics, mainly elastance (2, 32); b) gas-exchange, considering better oxygenation (15, 32, 33); c) oxygen delivery (34); and d) radiology, using CT scan (31, 35, 36). We observed

that the parameters which corresponded best to minimize ventilator-induced lung injury during PEEP selection were minimum lung elastance and maximum oxygenation (Tables 2 and 3).

Some authors described that mechanical forces can modify the gene expression of several molecules of extracellular matrix (28, 29, 37–40). In this context, we evaluated lung tissue expression of PCIII mRNA, since it is the first collagen to be remodeled in the evolution of lung fibrogenesis (40), and has been used as an early marker of lung parenchyma remodeling (11, 30, 31, 37–40). We observed that PCIII mRNA expression was increased mainly in ALI group (Fig. 2). To our knowledge, this is the first study showing the effect of different PEEP levels on PCIII mRNA expression.

In agreement with our results, previous studies reported that PEEP levels, higher than those required for optimal gas-exchange and/or lung mechanics, may increase pulmonary damage and bacterial translocation (41). It was also shown that bacterial growth and translocation can be attenuated by reducing atelectasis in an ALI/ARDS model of experimental pneumonia, which suggests that the use of individualized PEEP titration resulted in less volutrauma and atelectrauma, and therefore, in fewer permeability disturbances and subsequent less bacterial translocation (42, 43). Bacterial translocation was not analyzed, but our data suggest that applying a low tidal volume on a higher PEEP did not prevent atelectrauma, although it still led to alveolar hyperinflation due to high end-inspiratory stretch (Table 3). It is also conceivable that high PEEP levels did not prevent the influx of fluids and proteins into the alveolar space (Fig. 1 and Table 4).

This study has several limitations. First, we used paraquat to create an experimental ALI model, which may not fully reflect all aspects of this disease. Thus, our findings may be particular to this model. In this line, the degree of ALI was established based on the following parameters: 1) oxygenation index (Pa_{O_2}/F_{IO_2} was equal to 137 mm Hg at PEEP 1.5 group and 218 mm Hg at PEEP 3), in agreement with ALI/ARDS definition (44); 2) Est, L was increased in ALI compared with C groups (Table 1); 3) lung histology showed alveolar collapse (21%) in agreement with that reported by Gattinoni et al (31) (20% of collapsed areas); and 4) electron microscopy depicted endothelial lesion, type II cell damage, and interstitial edema, accord-

ing to a moderate-severe lung injury. Second, we used an open-chest approach. Therefore, the transpulmonary pressure applied to the lung may substantially differ from those reached during ventilation with closed chest. In other words, it is likely that the lung volumes at end-expiration were lower at low PEEP levels and higher at high PEEP than expected during closed-chest ventilation. This, in turn, may also have affected regional pulmonary perfusion and its effects on lung injury (45, 46). However, we aimed to investigate systematically the effects of different transpulmonary pressure levels. Thus, in our experiment, we were able to carefully control the exact transpulmonary pressure from a lower end-expiratory lung volume to higher volumes using different PEEP levels. Third, it is possible that we used excessively high PEEP levels (6 cm H_2O), leading to hyperinflation. Considering that the experiments were done on rats, where the height of the thorax (from sternum to vertebra) is roughly equivalent to 2 cm compared with 8 cm in humans, the application of 3 cm H_2O was roughly equivalent to 10–12 cm H_2O in humans, while 6 cm H_2O was roughly equivalent to 20–24 cm H_2O. These PEEP levels are often applied in rat experiments (47) and in patients with ALI/ARDS. Fourth, the study period was short (1 hour), and thus our results cannot be directly shifted to longer periods of ventilation. Fifth, although we accurately evaluated lung injury from a morphologic point of view by light and electron microscopy, we limited our analysis of stress to the quantification of PCIII, and not other inflammatory mediators. Sixth, we did not perform recruitment maneuver before adding any PEEP as in previous studies (28, 31, 32). It is possible that different results were obtained by using recruitment maneuver before PEEP selection. Nevertheless, even without recruitment maneuver, beneficial effects were observed after adding correct PEEP levels. Seventh, even though precautions were taken by reducing the F_{IO_2}, the use of F_{IO_2} equal to 1 at the final 5 minutes of ventilation could be enough to promote absorption atelectasis. However, since this protocol was performed in both C and ALI groups, it may not have influenced the final results.

CONCLUSIONS

This study indicates that in the present nonrecruited experimental model of ALI, protective lung mechanical ventilation with lower and higher PEEP levels than required for a minimum elastance and maximum oxygenation increased lung static elastance, the amount of atelectasis, and PCIII mRNA expression. PEEP selection according to minimum elastance and maximum oxygenation may prevent lung injury. Our results could partially explain controversial results obtained in clinical trials regarding the efficacy of PEEP at reducing morbidity and mortality in ALI/ARDS patients.

REFERENCES

1. Muscedere JG, Mullen JB, Gan K, et al: Tidal ventilation at low airway pressures can augment lung injury. *Am J Respir Crit Care Med* 1994; 149:1327–1334
2. Amato MB, Barbas CS, Medeiros DM, et al: Effect of a protective-ventilation strategy on mortality in the acute respiratory distress syndrome. *N Engl J Med* 1998; 338:347–354
3. The Acute Respiratory Distress Syndrome Network: Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med* 2000; 342:1301–1308
4. Richard JC, Maggiore SM, Jonson B, et al: Influence of tidal volume on alveolar recruitment. Respective role of PEEP and a recruitment maneuver. *Am J Respir Crit Care Med* 2001; 163:1609–1613
5. Meade MO, Cook DJ, Guyatt GH, et al: Ventilation strategy using low tidal volumes, recruitment maneuvers, and high positive end-expiratory pressure for acute lung injury and acute respiratory distress syndrome: A randomized controlled trial. *JAMA* 2008; 299: 637–645
6. Mercat A, Richard JC, Vielle B, et al: Positive end-expiratory pressure setting in adults with acute lung injury and acute respiratory distress syndrome: A randomized controlled trial. *JAMA* 2008; 299:646–655
7. Villar J, Kacmarek RM, Pérez-Méndez L, et al: A high positive end-expiratory pressure, low tidal volume ventilatory strategy improves outcome in persistent acute respiratory distress syndrome: A randomized, controlled trial. *Crit Care Med* 2006; 34: 1311–1318
8. Verbrugge SJ, Lachmann B, Kesecioglu J: Lung protective ventilatory strategies in acute lung injury and acute respiratory distress syndrome: From experimental findings to clinical application. *Clin Physiol Funct Imaging* 2007; 27:67–90
9. Halter JM, Steinberg JM, Gatto LA, et al: Effect of positive end-expiratory pressure and tidal volume on lung injury induced by alveolar instability. *Crit Care* 2007; 11:R20
10. Slutsky AS: Lung injury caused by mechanical ventilation. *Chest* 1999; 116:9S–15S
11. Dos Santos CC, Slutsky AS: The contribution of biophysical lung injury to the development of biotrauma. *Ann Rev Physiol* 2006; 68:585–618
12. Takata M, Abe J, Tanaka H, et al: Intraalveolar expression of tumor necrosis factor-α gene during conventional and high-frequency ventilation. *Am J Respir Crit Care Med* 1997; 156:272–279
13. Ranieri VM, Suter PM, Tortorella C, et al: Effect of mechanical ventilation on inflammatory mediators in patients with acute respiratory distress syndrome. *JAMA* 1999; 282: 54–61
14. Takeuchi M, Goddon S, Dolhnikoff M, et al: Set positive end-expiratory pressure during protective ventilation affects lung injury. *Anesthesiology* 2002; 97:682–692
15. Gattinoni L, Carlesso E, Cadringher P, et al: Physical and biological triggers of ventilator-induced lung injury and its prevention. *Eur Respir J Suppl* 2003; 47:15s–25s
16. Gattinoni L, Caironi P, Carlesso E: How to ventilate patients with acute lung injury and acute respiratory distress syndrome. *Curr Opin Crit Care* 2005; 11:69–76
17. Rouby JJ, Brochard L: Tidal recruitment and overinflation in acute respiratory distress syndrome: Yin and yang. *Am J Respir Crit Care Med* 2007; 175:104–106
18. Vieira SR, Nieszkowska A, Lu Q, et al: Low spatial resolution computed tomography underestimates lung overinflation resulting from positive pressure ventilation. *Crit Care Med* 2005; 33:741–749
19. Nieszkowska A, Lu Q, Vieira SR, et al: Incidence and regional distribution of lung overinflation during mechanical ventilation with positive end-expiratory pressure. *Crit Care Med* 2004; 32:1496–1503
20. Brochard L, Roudot-Thoraval F, Roupie E, et al: Tidal volume reduction for prevention of ventilator-induced lung injury in acute respiratory distress syndrome. The Multicenter Trail Group on Tidal Volume reduction in ARDS. *Am J Respir Crit Care Med* 1998; 158:1831–1938
21. Brower RG, Shanholtz CB, Fessler HE, et al: Prospective, randomized, controlled trial comparing traditional versus reduced tidal volume ventilation in ARDS. *Crit Care Med* 1999; 27:1492–1498
22. Brower RG, Lanken PN, MacIntyre N, et al: Higher versus lower positive end-expiratory pressures in patients with the acute respira-

tory distress syndrome. *N Engl J Med* 2004; 351:327–336

23. Grasso S, Fanelli V, Cafarelli A, et al: Effects of high versus low positive end-expiratory pressures in acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2005; 171:1002–1008

24. Bates JHT, Rossi A, Milic-Emili J: Analysis of the behavior of the respiratory system with constant flow. *J Appl Physiol* 1985; 58: 1840–1848

25. Nagase T, Lei M, Robatto FM, et al: Tissue viscance during induced constriction in rabbit lungs: Morphological-physiological correlations. *J Appl Physiol* 1992; 73:1900–1907

26. Weibel ER: Morphometry: Stereological theory and practical methods. *In:* Models of Lung Disease-Microscopy and Structural Methods. Gil J (Ed). New York, Marcel Dekker, 1990, pp 199–247

27. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidium thiocyanate–phenol–chloroform extraction. *Anal Biochem* 1987; 162:156–159

28. Farias LL, Faffe DS, Xisto DG, et al: Positive end-expiratory pressure prevents lung mechanical stress caused by recruitment/derecruitment. *J Appl Physiol* 2005; 98: 53–61

29. Garcia CS, Rocco PR, Facchinetti LD, et al: What increases type III procollagen mRNA levels in lung tissue: Stress induced by changes in force or amplitude? *Respir Physiol Neurobiol* 2004; 144:59–70

30. Gattinoni L, Pelosi P, Crotti S, et al: Effects of positive end-expiratory pressure on regional distribution of tidal volume and recruitment in adult respiratory distress syndrome. *Am Respir Crit Care Med* 1995; 151:1807–1814

31. Gattinoni L, Caironi P, Cressoni M, et al: Lung recruitment in patients with the acute respiratory distress syndrome. *N Engl J Med* 2006; 354:1775–1786

32. Victorino JA, Borges JB, Okamoto VN, et al: Imbalances in regional lung ventilation: A validation study on electrical impedance tomography. *Am J Respir Crit Care Med* 2004; 169:791–800

33. Kunst PW, Böhm SH, Vazquez de Anda G, et al: Regional pressure volume curves by electrical impedance tomography in a model of acute lung injury. *Crit Care Med* 2000; 28: 178–183

34. Suter PM: Lung Inflammation in ARDS—Friend or foe? *N Engl J Med* 2006; 354: 1739–1742

35. Rouby JJ: Recruitment in pulmonary and extrapulmonary acute respiratory distress syndrome: The end of a myth? *Anesthesiology* 2007; 106:203–204

36. Gattinoni L, Caironi P, Valenza F, et al: The role of CT-scan studies for the diagnosis and therapy of acute respiratory distress syndrome. *Clin Chest Med* 2006; 27:559–570

37. Breen EC: Mechanical strain increases type I collagen expression in pulmonary fibroblasts in vitro. *J Appl Physiol* 2000; 88:203–209

38. de Carvalho ME, Dolhnikoff M, Meireles SI, et al: Effects of overinflation on procollagen type III expression in experimental acute lung injury. *Crit Care* 2007; 11:R23

39. Parker JC, Breen EC, West JB: High vascular and airway pressures increase interstitial protein mRNA expression in isolated rat lungs. *J Appl Physiol* 1997; 83:1697–1705

40. Raghu G, Striker LJ, Hudson LD, et al: Extracellular matrix in normal and fibrotic human lungs. *Am Rev Respir Dis* 1985; 131: 281–289

41. Lachmann RA, van Kaam AH, Haitsma JJ, et al: High positive end-expiratory pressure levels promote bacterial translocation in experimental pneumonia. *Intensive Care Med* 2007; 33:1800–1804

42. van Kaam AH, Lachmann RA, Herting E, et al: Reducing atelectasis attenuates bacterial growth and translocation in experimental pneumonia. *Am J Respir Crit Care Med* 2004; 169:1046–1053

43. Nahum A, Hoyt J, Schmitz L, et al: Effect of mechanical ventilation strategy on dissemination of intratracheally instilled *Escherichia coli* in dogs. *Crit Care Med* 1997; 25: 1733–1743

44. Artigas A, Bernard GR, Carlet J, et al: The American-European Consensus Conference on ARDS, Part 2. Ventilatory, pharmacologic, supportive therapy, study design strategies, and issues related to recovery and remodeling. Acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1998; 157: 1332–1347

45. Hotchkiss JR Jr, Blanch L, Naveira A, et al: Relative roles of vascular and airspace pressures in ventilator-induced lung injury. *Crit Care Med* 2001; 29:1593–1598

46. Constantin JM, Cayot-Constantin S, Roszyk L, et al: Response to recruitment maneuver influences net alveolar fluid clearance in acute respiratory distress syndrome. *Anesthesiology* 2007; 106:944–951

47. Valenza F, Sibilla S, Porro GA, et al: An improved *in vivo* rat model for the study of mechanical ventilatory support effects on organs distal to the lung. *Crit Care Med* 2000; 28:3697–3704