Positive end-expiratory pressure prevents lung mechanical stress caused by recruitment/derecruitment

Luciana L. Farias, Débora S. Faffe, Débora G. Xisto, Maria Cristina E. Santana, Roberta Lassance, Luiz Felipe M. Prota, Marcelo B. Amato, Marcelo M. Morales, Walter A. Zin, and Patricia R. M. Rocco. Positive end-expiratory pressure prevents lung mechanical stress caused by recruitment/derecruitment. J Appl Physiol 98: 53–61, 2005. First published September 17, 2004; doi:10.1152/japplphysiol.00118.2004.—This study tests the hypotheses that a recruitment maneuver per se yields and/or intensifies lung mechanical stress. Recruitment maneuver was applied to a model of paraquat-induced acute lung injury (ALI) and to healthy rats with (ATEL) or without (CTRL) previous atelectasis. Recruitment was done by using 40-cmH2O continuous positive airway pressure for 40 s. Rats were then, ventilated for 1 h at zero end-expiratory pressure (ZEEP) or positive end-expiratory pressure (PEEP; 5 cmH2O). Atelectasis was generated by infusing a sphygmomanometer around the thorax. Additional groups did not undergo recruitment but were ventilated for 1 h under ZEEP. Lung resistive and viscoelastic pressures and static elastance were computed before and immediately after ventilation at ZEEP alone did not elevate PCIII expression. Ventilation by ZEEP was more deleterious in ALI than in mechanical ATEL, although ZEEP alone did not elevate PCIII expression. Ventilation after recruitment avoided any increment in PCIII expression in all groups. In conclusion, recruitment followed by ZEEP was more deleterious in ALI than in mechanical ATEL, although ZEEP alone did not elevate PCIII expression. Ventilation with 5-cmH2O PEEP prevented derecruitment and aborted the increase in PCIII expression.

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MECHANICAL VENTILATION CAN WORSEN preexisting lung disease because of regional overdistention or shear forces generated by repeated opening and closing of collapsed alveoli (atelectrauma) (20, 49, 50). These processes are associated with increased activation of inflammatory mediators and histological lesions indistinguishable from acute respiratory distress syndrome (ARDS) (41, 50–52). In the last 10 years, avoidance of ventilator-induced lung injury (VILI) has dominated the literature on the management of ARDS (27, 28, 52).

VILI is the result of a complex interplay among various mechanical forces acting on lung structures during mechanical ventilation. Increased mechanical load in parenchyma cells and connective tissues can initiate an adaptive process to high stress, with collagen deposition and vascular cell proliferation (38). The mechanical factors responsible for VILI are thought to be related to recruitment/derecruitment of previously collapsed alveoli and/or pulmonary overdistension, thereby applying tension to lung parenchyma, especially in the context of ARDS (10).

The lung-protective mechanical ventilatory strategy proposed for ARDS emphasizes the need to “open the lung and keep it open” while avoiding alveolar overdistension (32). The reduction of tidal volume (VT) to limit plateau pressure is currently recommended for the ventilatory management of ARDS. However, excessive reduction in VT may result in harmful alveolar derecruitment depending on the level at which positive end-expiratory pressure (PEEP) is set (1, 2, 35, 43, 50). Conversely, high-PEEP strategies can also lead to detrimental consequences, such as the development of air leaks (44).

The use of recruitment maneuvers (RMs) has been proposed as an adjunctive lung-protective strategy to reverse low VT-related derecruitment (2, 21, 29, 43). In experimental studies, RMs have been able to minimize lung injury, allowing the use of lower PEEP and peak airway pressures, thus reducing the potential VILI (44). Because the deleterious effects of high airway pressures over normal or injured lungs have been well established (19, 20, 53), and because the RM itself requires sustained pressures high enough to reach total lung capacity (29), the RM may cause some damage. Given the scant data about the effects of the RM per se on lung remodeling, we envisaged this study to address the following points: 1) whether recruitment per se could yield lung mechanical stress even in normal animals, 2) whether lung recruitment could intensify the lesion in animals with previous lung injury, 3) whether the amount of collapse before the maneuver could modulate this response, and 4) whether the use of PEEP ventilation after recruitment could attenuate the lung injury induced by recruitment and/or derecruitment. For such pur...
poses, respiratory mechanics, pulmonary histology, and type III procollagen (PCHII) mRNA expression were analyzed in control (CTRL) animals and in animals with acute lung injury (ALI) or mechanical atelectasis. Rats were ventilated with a lung-protective strategy with PEEP or zero end-expiratory pressure (ZEEP).

MATERIALS AND METHODS

Animal preparation. A total of 57 Wistar rats [250 ± 10 g (SD)] were used. They were randomly assigned to three main groups. In the CTRL group (n = 19), saline [0.9% NaCl, 5 ml/kg body wt (BW)] was injected intraperitoneally. In the atelectasis group (ATEL) (n = 19), a model of reproducible atelectasis previously developed was used (16). Briefly, atelectasis was generated by a pediatric sphygmo-manometer wrapped around the thorax of the animal from the axilla to the subcostal plane. The cuff was inflated to a transpulmonary pressure of −8 cmH₂O, which was maintained for 5 s. Then the sphygmo-manometer was taken off to allow further measurements (16). In the ALI group (n = 19), paraquat was injected intraperitoneally (10 mg/kg BW) 24 h before the measurements (45). The animals were sedated (5 mg of diazepam, intraperitoneally), anesthetized (20 mg/kg BW pentobarbital sodium, intraperitoneally), paralyzed (2 mg/kg BW gallamine triethyliodide, intravenously), and mechanically ventilated (Samay VR15, Universidad de la Republica, Montevideo, Uruguay).

All animals received human care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guiding Principles in the Care and Use of Animals” approved by the Council of the American Physiological Society.

An adequate pneumotachograph (1.5-mm inner diameter, length = 4.2 cm, distance between side ports = 2.1 cm) (34) was connected to the tracheal cannula for the measurements of airflow. VT was calculated by digital integration of flow signal. The tracheal pressure (Ptr) was determined (Validyne MP45–2 differential pressure transducer, Engineering, Northridge, CA). The flow resistance of the equipment amounted to 0.12 cmH₂O·s·ml⁻¹ and was subtracted from respiratory system and pulmonary viscous resistances (12). Changes in esophageal pressure (Pes), which reflect chest wall pressure (Pw), were measured with a 30-cm-long water-filled catheter (PE205) with side holes at the tip connected to a PR23-2D-300 Statham differential pressure transducer (Hato Rey, Puerto Rico). The catheter was passed into the stomach and then slowly returned into the esophagus; its proper positioning was assessed using the occlusion test (5a). This test consisted of comparing changes in Pes and Ptr during spontaneous inspiratory efforts subsequent to airway occlusion at end expiration. In all instances, the change in Pes was close to the change in Ptr, with the difference not exceeding 3%. The frequency responses of Ptr and Pes measurement systems were flat up to 20 Hz, without appreciable phase shift between the signals. All signals were conditioned and amplified (Beckman type R Dynograph, Beckman Instruments, Schiller Park, IL). Flow and pressure signals were also passed through eight-pole Bessel filters (902LPF, Frequency Devices, Haverhill, MA), with the corner frequency set at 100 Hz, sampled at 200 Hz with a 12-bit analog-to-digital converter (DT2801A, Data Translation, Marlboro, MA), and stored on a personal computer. All data were collected using LABDAT software (RHT-InfoData, Montreal, Quebec, Canada).

An arterial cannula was inserted into one of the femoral arteries to sample blood for the determination of oxygen saturation (AVL Bio-medical Instruments, Roswell, GA).

Experimental protocol. The experimental protocol is depicted in Fig. 1. Volume control ventilation with a VT of 5 ml/kg BW, a frequency of 80 breaths/min, and inspiratory-to-expiratory ratio of 1:2 was defined as baseline ventilation. The animals were ventilated with an inspired oxygen fraction of 0.21.

A RM consisting of a single continuous positive airway pressure of 40 cmH₂O for 40 s was then performed. After recruitment, baseline VT, respiratory rate, and inspiratory-to-expiratory ratio were resumed. Just after the RM, five animals from each group were ventilated for 1 h under ZEEP, whereas another five rats were ventilated with 5 cmH₂O of PEEP, to avoid derecruitment. To analyze the effects of 1-h ZEEP ventilation by itself without RM, CTRL, ALI, and ATEL animals were ventilated for 1 h at ZEEP, without a previous RM, constituting the CTRL-ZEEP, ALI-ZEEP, and ATEL-ZEEP groups, respectively (n = 4 rats each). To understand the aspects related to each lung preparation per se, animals from the three major groups (CTRL, ALI, and ATEL) were not submitted to RM or mechanical ventilation, i.e., rats were killed and the lungs were removed at end-expiratory volume (nonventilatory CTRL, nonventilatory ALI, and nonventilatory ATEL groups, n = 5 rats each). In ATEL, lungs were removed immediately after the induction of atelectasis.

Respiratory mechanics. Respiratory mechanical data were obtained in CTRL and ALI groups at three occasions: before the RM (Pre), immediately after the RM (RM), and 1 h after RM (Post). Lung histology and molecular biology (type III procollagen mRNA) were analyzed only after 1 h of ventilation. Rats were ventilated for 1 h after recruitment in zero end-expiratory pressure (ZEEP) or positive end-expiratory pressure (PEEP).
immediately after RM, and at the end of the 1-h ventilation period ensuing recruitment (Post).

Respiratory mechanics were measured by end-inflation occlusion method (4, 5, 31). Briefly, constant Vt (5 ml/kg) and airflow (6 ml/s) were applied to all animals (31, 48). After end-inspiratory occlusion, there is an initial fast drop in Ptp from the preocclusion value down to an inflection point, followed by a slow pressure decay until a plateau is reached. This plateau pressure corresponds to the elastic recoil pressure of the respiratory system (Pplat,rs). The initial fast drop in Ptp selectively reflects the pressure spent to overcome the combination of airways, pulmonary, and chest wall Newtonian resistances (5, 5a), and the following slow pressure decay reflects the pressure dissipated by stress relaxation, or viscoelastic properties, of the lung and chest wall tissues, together with a small contribution of pendullum in normal situations (48). The same procedures apply to the Pw, yielding Pw values at the same times as Ptp mentioned above. Transpulmonary pressures were calculated by subtracting the different Pw values from the corresponding values pertaining to the respiratory system (initial drop in Ptp, the inflection point, and the following slow pressure decay). Total pressure drop is equal to the sum of the initial drop value and slow pressure decay value. Respiratory system, lung, and chest wall static elastances were calculated by dividing the elastic recoil pressures of the respiratory system, lung, and chest wall by Vt. All data were analyzed using ANADAT data analysis software (RHT-InfoData, Montreal, Quebec, Canada).

Histological study. At the end of the experiments, the trachea was clamped at end-expiratory volume, and the abdominal aorta and vena cava were sectioned, quickly killing the animals. The chest wall was opened, and the lungs were removed en bloc.

A 3 × 3 × 10 mm strip of subpleural parenchyma was cut from the periphery of the right lung. Pleural tissue was removed, and the strip was stored in liquid nitrogen for analysis of PCIII mRNA expression. To perform the morphological study, the left lung was quick-frozen by rapid immersion in liquid nitrogen (36). Fixation was made with Carnoy’s solution (ethanol-chloroform-acetic acid, 70:20:10) at −70°C. After 24 h, ethanol concentration was progressively increased (70, 80, 90, and 100%, respectively, 1 h each solution, at −20°C). The lungs were then kept in 100% ethanol for 24 h at 4°C. After fixation, tissue blocks were embedded in paraffin and cut 4 μm thick. Slides were stained with hematoxylin-eosin. Morphological analysis was performed with an integrating eyepiece with a coherent system made at a magnification of ×40 across 10 random, noncoincident microscopic fields.

A semiquantitative system was used to account for the severity of alveolar collapse. A five-point semiquantitative severity-based scoring system was used. The pathological findings were graded as negative = 0, slight = 1, moderate = 2, high = 3, and severe = 4 in 10 noncoincident microscopic fields (×100 magnification). A median score for each of the variables (0 = normal lung parenchyma; 1 = 0–25%; 2 = 25–50%; 3 = 50–75%; 4 = 75–100% of areas with alveolar collapse) was then calculated.

PCIII mRNA expression. The relative expression of PCIII mRNA was obtained by semiquantitative RT-PCR of rat lung tissue in all groups.

Total RNA was isolated from the frozen lung tissue by the method of Chomczynski and Sacchi (14). RNA samples were quantitated by absorbance at 260/280 nm.

To make the first DNA strand, total RNA isolated from rat lung was reverse-transcribed with SuperScript (GIBCO, Grand Island, NY) at 37°C for 60 min. First-strand cDNA synthesis was performed in a 20-μl reaction containing 1 μg of total RNA, 50 U uridine leukemia virus RT, 20 U RNase inhibitor, 2.5 μM oligo(dT), 2 μl of 5× first strand buffer (250 mM Tris-HCl, 375 mM KCl, 15 mM MgCl2), 1 mM dNTP and diethyl pyrocarbonate (DEPC)-treated water. The reaction was performed in a water bath at 37°C for 80 min and 99°C for 5 min. The negative control (RT replaced with DEPC-treated water) was included with all reverse transcription reactions. The resultant cDNA was diluted in 10 μl of DEPC-treated water and stored at −20°C. PCR technique was used to amplify the synthesized cDNA. The following solution was employed in PCR reaction: 0.2 μM of each dNTP, 50 mM of KCl, 10 mM of Tris·Cl (pH 8.3), 1.5 mM of MgCl2 plus 2.5 U of thermostable DNA polimerase (Taq polimerase, GIBCO), and 0.2 μM of each sense and antisense primers.

For rat PCIII mRNA, one pair of oligonucleotides (5′-CTGCCAT-TGCTGGAGTTG-3′ and 5′-GCAGCATCCTCTAGAAC-3′), corresponding to nucleotides 903–920 and 1,529–1,546, respectively, was synthesized (GeneBank accession no. AJ003595). PCR was performed with 36 cycles of denaturation (94°C, 1 min), annealing (54°C, 1 min), and extension (72°C, 1 min). The final elongation was at 72°C for 10 min. In the PCIII mRNA detection by RT-PCR, the rat glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) primers were added into the same RT-PCR reaction tube, and GAPDH products were used as internal positive control. The rat GAPDH primers predicted to amplify the 211-bp PCR product used were sense: 5′-GTCTTCACCACCATGGAG-3′; antisense: 5′-CGATGC-CAAAGTTGTCATG-3′ (corresponding, respectively, to nucleotides 325–342 and 517–535 of rat GAPDH gene, GeneBank accession no. M17701). The semiquantitative method of RT-PCR, used to quantify the PCIII mRNA expression in the experimental rat lung, was validated in preliminary experiments as previously described (37). All reactions included a negative control of RT replaced with DEPC-treated water. The identity of the amplification was confirmed by determination of the molecular size on agarose gel electrophoresis with a 100-bp DNA molecular marker (GIBCO).

Statistical analysis. The normality of the data (Kolmogorov-Smirnov test with Lilliefors’ correction) and the homogeneity of variances (Levene median test) were tested. Both conditions were satisfied in all instances, and thus one-way ANOVA for repeated measures was used to determine the effect of RM on respiratory mechanics along time in each group. One-way ANOVA was used to compare morphological and mRNA data among all groups. In both cases, if multiple comparisons were required, Tukey’s test was applied. The significance level was set at 5%.

RESULTS

The respiratory system parameters followed the lung mechanical behavior, because chest wall mechanics did not change among groups. Lung mechanics improved immediately after RM both in CTRL and ALI groups (Figs. 2 and 3). After recruitment, 1-h ventilation under ZEEP increased lung static elastance and total and viscoelastic pressure variations. These values were even higher than those observed before the maneuver (Figs. 2 and 3, top). Ventilation with 5-cmH2O PEEP avoided the worsening of respiratory mechanics observed after 1-h ventilation ensuing recruitment (Figs. 2 and 3, bottom).

Table 1 shows the respiratory system plateau pressures achieved in CTRL and ALI groups in all situations. Plateau pressure in the respiratory system was higher in the ALI group than in the CTRL group, fell in both groups after RM, and returned to values similar to or higher than those obtained before pre-RM in ZEEP. One-hour ventilation with 5-cmH2O PEEP post-RM led to a respiratory system plateau pressure lower than the values for pre-RM in the CTRL group but similar to pre-RM in the ALI group.
Histological changes in the nonventilated ALI group included interstitial edema, atelectasis, inflammation with increased amount of polymorphonuclear cells, and hyaline membrane. The ATEL group depicted only atelectasis without cellular infiltration (Fig. 4). RM reduced alveolar collapse in ALI and ATEL groups to the same extent, but 1-h ventilation in ZEEP led to atelectasis even in CTRL animals (Table 2). Interestingly, RM reexpanded collapsed alveoli more homogeneously in the ATEL group than in the ALI group, which still presented areas of patchy atelectasis after recruitment (Fig. 4 and Table 3). Ventilation with PEEP reduced alveolar collapse in the ALI and ATEL groups (Fig. 4 and Table 2).

Figure 5 shows PCIII mRNA expression among Nonvent, CTRL-RM-ZEEP, CTRL-ZEEP, ATEL-ZEEP, ATEL-RM-ZEEP, ALI-ZEEP, and ALI-RM-ZEEP groups. In this set of experiments, data were related to the values obtained in the Nonvent group. The ALI group showed higher PCIII expression than the ATEL or CTRL groups. RM followed by ZEEP ventilation increased PCIII mRNA expression similarly in all groups (Fig. 5). The results cannot be attributed to a possible effect of mechanical ventilation alone since there were no changes in nonrecruited CTRL animals after 1 h of ventilation in ZEEP (Fig. 5). To clarify the beneficial effects of PEEP in each group (CTRL, ATEL, and ALI), all values were related to ZEEP ventilation without RM. PEEP ventilation after RM prevented the increase in PCIII mRNA expression in all three main conditions (Fig. 6).

DISCUSSION

RM improved lung mechanics, but its beneficial effects disappeared as soon as 1 h of ventilation on ZEEP had elapsed. On the other hand, the RM itself increased PCIII mRNA expression even in healthy lungs (Fig. 5). By contrast, ventilation with 5-cmH₂O PEEP after recruitment avoided the worsening of mechanical and histological parameters, as well as PCIII mRNA production, observed in all lung preparations (Fig. 6). Interestingly, although the plateau pressures achieved during PEEP after recruitment were higher than those observed during ZEEP (Table 2), the PCIII mRNA expression remained much lower under PEEP than under ZEEP. It has been shown that type III collagen increases early in the evolution of lung
fibrotic process (40); thus PCIII mRNA expression was used as a marker of lung parenchyma remodeling. It is noteworthy that derecruitment also has to be considered as an additional possible mechanism of damage in this model, since a relatively low recruitment pressure was used. However, we decided to use similar pressures that have been applied in clinical studies. The increase in PCIII mRNA expression in the CTRL group submitted to RM together with the absence of a rise in PCIII mRNA expression in ATEL without RM suggest that recruitment itself is playing a role in lung damage.

Table 1. Plateau pressures over the course of the experiments in control and acute lung injury groups

<table>
<thead>
<tr>
<th>Pplat, cmH2O</th>
<th>ZEEP</th>
<th>PEEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTRL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>2.75±0.04</td>
<td>8.22±0.28</td>
</tr>
<tr>
<td>RM</td>
<td>1.97±0.02*</td>
<td>7.17±0.23*</td>
</tr>
<tr>
<td>Post</td>
<td>3.85±0.13*†</td>
<td>7.77±0.16*†</td>
</tr>
<tr>
<td>ATEL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>3.79±0.37‡</td>
<td>9.17±0.31</td>
</tr>
<tr>
<td>RM</td>
<td>2.32±0.31*</td>
<td>7.15±0.31*</td>
</tr>
<tr>
<td>Post</td>
<td>4.00±0.13†</td>
<td>8.90±0.38†</td>
</tr>
</tbody>
</table>

Values are means ± SE of 5 animals in each group (15 determinations in each situation/rat). Plateau pressure (Pplat) was determined before recruitment maneuver (Pre), just after recruitment (RM), and after 1-h ventilation (Post) in zero end-expiratory pressure (ZEEP) or positive end-expiratory pressure (PEEP; 5 cmH2O). CTRL, control rats; ALI, rats with paraquat-induced acute lung injury. *Significantly different from Pre (P < 0.05). †Significantly different from group RM (P < 0.05). ‡Significantly different from CTRL-Pre (P < 0.05). §Significantly different from group CTRL-Post (P < 0.05).

The ALI model used in the present study leads to a well reproducible moderate lung injury, characterized by alveolar collapse, interstitial edema, and hyaline membrane, without alveolar edema (45). ALI reproducibility was of great importance since lung injury severity as well as ventilation settings could be standardized among all animals, thus avoiding the usual limitations observed in clinical trials. The ATEL model (16) was used to discriminate whether the deleterious effects of RMs were related to the previous degree of atelectasis present in our ALI model (45) or, alternatively, to parenchymal inflammation.

RMs are characterized by a sustained increase in airway pressure (26). The use of RMs has been suggested as adjunct to lung-protective ventilation strategies in ARDS patients (2). It is also of use during general anesthesia to improve arterial oxygenation, which is mainly thwarted by atelectasis (25, 46, 47, 54). However, controversy exists over the possibility of harm being caused by recruitment because of excessively high intrathoracic pressure and volume (8, 11, 19, 53). The literature has a paucity of data on various methods of performing an RM in animal or human subjects (30). In the present study, we chose to perform a single RM with 40-cmH2O continuous positive airway pressure for 40 s (2), because most investigators use a single RM consisting of a continuous positive airway pressure of ~30–40 cmH2O (9, 18).

ALI increased lung static elastance and resistive and viscoelastic/inhomogeneous pressures. The increase in static elastance could be attributed to an increase in stiffness of lung tissue due to larger surface forces and airway closure and/or alveolar collapse (17). The mechanisms that account for the increase of viscoelastic pressure variations are related to lung...
inhomogeneities due to alveolar collapse/overdistension, edema, and surfactant functional changes. Furthermore, alveolar collapse could pull open alveolar ducts and might distort the parenchyma, thereby affecting local tissue mechanics (45). The variation in lung resistive pressure increased in the ALI group because of the reduction of central airway caliber caused by edema, fluid accumulation, reflex bronchoconstriction, and/or reduced lung volume. The immediate improvement in lung mechanics after RM was transitory (Figs. 2 and 3), as previously described by other authors (23, 39). Interestingly enough, mechanical parameters were even worse after 1 h of ventilation under ZEEP (33). The decrease in static elastance indicates recruitment of previously closed alveolar space, whereas the reduction in viscoelastic pressure variations suggests a decrease in mechanical inhomogeneities and tissue viscoelasticity. Additionally, the variation in lung resistive pressure diminished after recruitment probably because of the parenchymal tethering that distends the airways. Many studies suggest that the high potential for recruitment is related to chest wall mechanical behavior (22, 39). However, in the present study, we observed no change in chest wall mechanical parameters, probably because of the lack of abdominal distension or pleural effusions. Thus our findings suggest that the beneficial effects of recruitment in extrapulmonary ARDS are not fundamentally linked to chest wall behavior.

We demonstrated that RM increases PCIII mRNA expression in pulmonary tissue in three different conditions (Fig. 5). The relative high pressure necessary to reexpand and open collapsed lung units with a single RM may expose the alveoli to shear forces, increasing PCIII mRNA expression similarly in the three groups. Our results are in accordance with previous reports demonstrating increased procollagen mRNA expression in lungs submitted to high airway pressures (38), high inflation (6), or cyclic mechanical strain (10). Propeptides of collagen are released during fibril formation as a result of cleavage by specific extracellular N- and C-terminal proteinases (3). The N-terminal peptide of PCIII has been used as a biological marker of collagen synthesis (13). Many cell types in lung, e.g., fibroblasts and alveolar macrophages, may contribute to the increase in lung parenchyma mRNA for PCIII. However, few studies have focused on cells of peripheral lung parenchyma.

D’Angelo et al. (17) showed that prolonged low-volume ventilation on ZEEP induces peripheral airway injury, even in normal lungs. Although the viscoelastic deterioration observed after 1 h of ZEEP (Fig. 3) supported their findings, it is interesting to note that this was not enough to increase PCIII mRNA expression in lung tissue. PCIII mRNA expression was only triggered when 1-h ZEEP ventilation was preceded by a recruiting maneuver (Fig. 5).

ALI animals, not submitted to RM, showed increased PCIII mRNA expression in the pulmonary tissue (Fig. 5). These results are in accordance to those observed in ARDS, where type III collagen predominates early at the early phase of the disease (15, 40).

Kloot et al. (30) demonstrated that responses to PEEP, Vt, and recruitment differ among models of ALI induced by lavage, oleic acid, and intratracheal instillation of *Escherichia coli*. Our results showed that, although the RM reexpanded collapsed alveoli in both the ATEL and ALI groups (Table 2), this beneficial effect was more significant in atelectatic lungs without inflammation (Table 3). Bilek et al. (7) reported that pulmonary surfactant protects lung epithelium from mechanical stresses associated with airway reopening injury. However, RM is deleterious both in the models of mechanical atelectasis and ALI and in the CTRL group. Thus the tissue stress induced by RM is probably the major component that accounts for the increase in PCIII mRNA.

Ventilation with 5-cmH2O PEEP after recruitment avoided the stimulation of PCIII mRNA expression induced by the maneuver (Fig. 6). Recruiting the lung and preventing derecruitment decreases the potential for lung injury by avoiding the repetitive shear stress associated with opening and closing unstable lung units (24, 28). Therefore, we cannot discard that the increase in PCIII mRNA expression was avoided in PEEP ventilation also by avoiding derecruitment.

It has already been demonstrated that hypoxia leads to increased collagen synthesis in rat pulmonary artery (6). However, the beneficial effect of 5-cmH2O PEEP ventilation after RM cannot be attributed to an improvement in oxygenation, since there was no statistically significant difference in oxygen saturation between groups.

### Table 2. Morphometrical parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal, %</th>
<th>Alveolar Collapse, %</th>
</tr>
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<tbody>
<tr>
<td>CTRL</td>
<td>94.00±0.80</td>
<td>6.00±1.20</td>
</tr>
<tr>
<td>RM-ZEEP</td>
<td>78.28±1.27*</td>
<td>21.72±1.27*</td>
</tr>
<tr>
<td>RM-PEEP</td>
<td>91.11±0.32†</td>
<td>8.89±0.32†</td>
</tr>
<tr>
<td>ALI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonvent</td>
<td>56.67±2.56</td>
<td>43.33±0.99</td>
</tr>
<tr>
<td>RM-ZEEP</td>
<td>65.67±0.81*</td>
<td>34.33±0.81*</td>
</tr>
<tr>
<td>RM-PEEP</td>
<td>85.63±0.26†</td>
<td>14.37±0.26†</td>
</tr>
</tbody>
</table>

Values are means ± SE of 5 animals in each group. All values are percentage of normal, collapsed areas in 10 random, noncoincident fields per rat. ATEL, rats submitted to mechanical atelectasis; Nonvent, nonrecruited and nonventilated rats; RM-ZEEP and RM-PEEP, rats ventilated for 1 h after recruitment in ZEEP or PEEP (5 cmH2O), respectively. *Significantly different from Nonvent group (P < 0.05). †Significantly different from group RM-ZEEP (P < 0.05).

### Table 3. Lung histology score of alveolar collapse

<table>
<thead>
<tr>
<th>Group</th>
<th>Alveolar Collapse (Score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTRL</td>
<td></td>
</tr>
<tr>
<td>Nonvent</td>
<td>0 (0–0.3)</td>
</tr>
<tr>
<td>RM-ZEEP</td>
<td>1 (1–2)*</td>
</tr>
<tr>
<td>RM-PEEP</td>
<td>0 (0–1)†</td>
</tr>
<tr>
<td>ALI</td>
<td></td>
</tr>
<tr>
<td>Nonvent</td>
<td>4 (3–4)</td>
</tr>
<tr>
<td>RM-ZEEP</td>
<td>3 (2–3)*</td>
</tr>
<tr>
<td>RM-PEEP</td>
<td>1 (1–2)‡</td>
</tr>
<tr>
<td>ATEL</td>
<td></td>
</tr>
<tr>
<td>Nonvent</td>
<td>4 (2.8–4)</td>
</tr>
<tr>
<td>RM-ZEEP</td>
<td>2 (1–2)*</td>
</tr>
<tr>
<td>RM-PEEP</td>
<td>1 (0–1)†‡</td>
</tr>
</tbody>
</table>

Values are as medians (25th percentile, 75th percentile) of 5 animals in each group. Lung tissue injury was scored by 2 investigators who were blinded with regard to the group analyzed. *Significantly different from Nonvent group (P < 0.05). †Significantly different from group RM-ZEEP (P < 0.05). ‡Significantly different from group RM-PEEP in ALI group (P < 0.05).
saturation between ZEEP and 5-cmH2O PEEP ventilation groups (ALI-RM-ZEEP: 93.8% ± 3.9%; ALI-RM-PEEP: 92.4% ± 3.8%). Although low VT ventilation has been established as an essential element in lung protective strategy, controversy still exists over the approach used to set PEEP (1). PEEP levels as high as 15–20 cmH2O have been applied to avoid alveolar derecruitment (1). Recently, Halter et al. (24) demonstrated that recruitment followed by inadequate PEEP results in unstable alveoli and may induce VILI despite improved oxygenation. In this line, we observed that optimal compliance was achieved with 5 cmH2O in this model of moderate lung injury in rats. In accordance with our findings, Rimensberger et al. (44) demonstrated that lungs could be ventilated at optimal compliance when using RMs followed by a PEEP below the lower inflection point but above the closing pressure. It is possible, therefore, that a significant portion of the lung was kept open in our animals, even at these low PEEP levels, which was enough to decrease damage. Accordingly, the extent of end-expiratory collapse was much smaller in animals ventilated for 1 h after recruitment under PEEP compared with those under ZEEP (Table 2 and Fig. 4).

It is interesting to note that in animals with either ALI or atelectasis, ventilation with 5-cmH2O PEEP avoided the increase in PCIII mRNA expression despite the higher plateau pressure achieved in these groups (Table 1). These findings suggest that the protective effect of an adequate PEEP level overcomes the expected deleterious effect of high tonic tissue stretching. Our results showed a key role of PEEP after recruitment. This finding is supported by the recent demonstration that once plateau pressure is maintained below 30–35 cmH2O by reducing VT, increasing PEEP markedly augments recruitment (42). Richard et al. (42) suggested that, in this situation, PEEP might play a fundamental role in the lung protective strategy aiming to minimize lung collapse.

In a clinical scenario, patients undergo high levels of PEEP to achieve optimal recruitment, and during these interventions they may be exposed to sudden derecruitment of previously recruited lung by a sudden withdrawal of PEEP. Sudden loss of recruitment may accentuate ventilation inhomogeneity and cause further closing of small airways, rendering the lung more vulnerable to injury. Recently, Suh et al. (50) showed that derecruitment of initially recruited lung may increase lung injury associated with mechanical ventilation. In our study, RM followed by ZEEP led to derecruitment, showing an increased expression of PCIII mRNA (Fig. 5). Conversely, a PEEP level as low as 5 cmH2O after RM avoided derecruitment in this model of moderate ALI. Thus not only RM per se but also the derecruitment after previous reopening of collapsed alveoli can be the triggering factor for procollagen expression.

Fig. 5. Relative expression of type III procollagen mRNA (PCIII) obtained by amplification of PCIII and glyceraldehydes-3-phosphate-dehydrogenase (GAPDH) by semi-quantitative RT-PCR of rat lung tissue in different situations. Nonvent, nonrecruited and nonventilated normal rats; CTRL-ZEEP and CTRL-RM-ZEEP, CTRL rats ventilated for 1 h in ZEEP without recruitment maneuvers and after recruitment maneuvers, respectively; ATEL-ZEEP and ATEL-RM-ZEEP, ATEL rats ventilated for 1 h in ZEEP without recruitment maneuvers and after recruitment maneuvers, respectively; ALI-ZEEP and ALI-RM-ZEEP, ALI rats ventilated for 1 h in ZEEP without recruitment maneuvers and after recruitment maneuvers, respectively; MW, molecular weight. Values are means ± SE (n = 4) of the ratio between the densitometric values of PCIII and GAPDH bands obtained in RT-PCR experiments. *Significantly different from Nonvent group (P < 0.05). #Significantly different from all other groups (P < 0.05).

Fig. 6. Relative expression of PCIII mRNA obtained by amplification of PCIII and GAPDH by semiquantitative RT-PCR in CTRL (top), ATEL (middle), and ALI (bottom) rats. Animals from each group were nonrecruited but ventilated for 1 h in ZEEP (ZEEP), or submitted to recruitment maneuvers and ventilated for 1 h in ZEEP (RM-ZEEP) or PEEP (RM-PEEP). All values were related to ZEEP ventilation without recruitment maneuvers. RT(−), reverse transcription in the absence of RT. Values are means ± SE (n = 4) for the ratio between the densitometric values of PCIII and GAPDH bands obtained in RT-PCR experiments. *Significantly different from the ventilated nonrecruited rats in each situation (P < 0.05). Note the different ordinate values.
In conclusion, recruitment/derecruitment triggered PCIII expression in healthy lungs with atelectasis and with previous lung inflammation. In addition, the use of low PEEP levels right after the maneuvers aborted the increase in PCIII expression, being an effective strategy to minimize the potential harm associated with cellular mechanical stress.

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