Experimental Ventilator-induced Lung Injury

Exacerbation by Positive End-Expiratory Pressure

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Background: Previous experimental studies of ventilator-induced lung injury have shown that positive end-expiratory pressure (PEEP) is protective. The authors hypothesized that the application of PEEP during volume-controlled ventilation with a moderately high tidal volume (VT) in previously healthy in vivo rats does not attenuate ventilator-induced lung injury if the peak airway pressure markedly increases during the application of PEEP.

Methods: Sixty healthy, male Sprague-Dawley rats were anesthetized and randomized to be mechanically ventilated for 4 h at (1) VT of 6 ml/kg, (2) VT of 20 ml/kg, or (3) VT of 20 ml/kg plus 10 cm H2O of PEEP. Peak airway pressures, gas exchange, histologic evaluation, mortality, total lung tissue cytokine gene expression, and serum cytokine concentrations were analyzed.

Results: Peak airway pressures exceeded 30 cm H2O with high VT plus PEEP. All lungs ventilated with high VT had perivascular edema and inflammatory infiltrates. In addition, those ventilated with PEEP had small hemorrhages foci. Five animals from the high VT plus PEEP group died (P = 0.020). Animals ventilated with high VT (with or without PEEP) had a substantial increase in serum interleukin-6 (P = 0.0002), and those ventilated with high VT (without PEEP) had a 5.5-fold increase in systemic levels of tumor necrosis factor-alpha (P = 0.007).

Conclusions: In contrast to previous reports, PEEP exacerbated lung damage and contributed to fatal outcome in an in vivo, mild overdistension model of ventilator-induced lung injury in previously healthy rats. That is, the addition of high PEEP to a constant large VT causes injury in previously healthy animals.

THE use of positive end-expiratory pressure (PEEP) was first reported by Barach et al.1 in 1938, and its use has been widespread since Ashbaugh et al.2 reported in their classic description of the acute respiratory distress syndrome that PEEP improved oxygenation and allowed ventilation with lower inspired oxygen concentration. Extensive experimental and clinical research on the effects of PEEP in healthy and diseased lungs has produced a plethora of information during the past three decades.3–11 However, the beneficial effects of PEEP on morbidity and mortality have not been conclusively demonstrated. It is not well established whether the application of PEEP contributes to lung damage or helps to ameliorate lung injury. In 1974, Webb and Tierney3 first demonstrated that PEEP is protective. They ventilated rats with healthy lungs for 1 h at peak inspiratory pressures of 14, 30, or 45 cm H2O without PEEP. They altered the ventilator frequency and the tidal volume (VT) in each animal to achieve the desired airway pressure. The animals ventilated at a peak pressure of 45 cm H2O and no PEEP developed severe hypoxemia and died with extensive alveolar edema. Those ventilated at a peak pressure of 30 cm H2O had perivascular edema in varying degrees, and those ventilated at a peak pressure of 14 cm H2O showed no abnormalities. They also ventilated other rats with the same high-inspiratory pressures but with 10 cm H2O of PEEP. The most interesting observation was that the application of 10 cm H2O of PEEP, even when 45 cm H2O of peak pressure was used, dramatically reduced edema formation, and no animals died during 1-h ventilation. Similar to Webb and Tierney, Dreyfuss et al.4 reported in 1988 that the application of 10 cm H2O of PEEP produced a marked reduction of alveolar edema in rats with ventilator-induced lung injury (VILI) after being ventilated at peak pressures of 45 cm H2O and no PEEP for 20 min.

To our knowledge, there are no experimental reports showing an increase of morbidity and mortality in healthy animals with VILI when they are ventilated with PEEP as compared to those ventilated without PEEP. In

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this report, we explored the effects of 10 cm H₂O of PEEP on morbidity and mortality in an experimental rat model of VILI induced by mild overdistension. We hypothesized that during volume-controlled mechanical ventilation (MV) with a constant moderately high VT in in vivo healthy rats, the application of PEEP would not attenuate lung injury if the peak airway pressure markedly increases with the application of PEEP.

Materials and Methods

Animal Preparation
Animal care was supervised and the experimental protocol was approved by the Hospital Universitario N.S. de Candelaria Research Committee and the Committee for the Use and Care of Animals, University La Laguna, Tenerife, Spain. We studied healthy male Sprague-Dawley rats weighing 300–350 g (CRIFFA, Barcelona, Spain). During experiments, animals were anesthetized with an intraperitoneal injection of ketamine hydrochloride (50 mg/kg) and xylazine (2 mg/kg), with additional anesthesia administered as necessary. Proper anesthesia was assessed by paw and tail pinching and maintained throughout all experiments with additional doses as needed. Anesthetized animals were randomly allocated into four groups: nonventilated, ventilated with low VT and no PEEP, ventilated with high VT and no PEEP, and ventilated with high VT and 10 cm H₂O of PEEP. After induction of anesthesia, a catheter (ED 0.96 mm; Intramedic, Clay Adams, Parsippany, NJ) was inserted into the right carotid artery for arterial blood sampling and arterial blood pressure monitoring. While anesthetized and breathing spontaneously, a cervical tracheotomy using a thin-walled 14-gauge Teflon catheter was performed. After the catheter was secured by a ligature around the trachea, those animals allocated to MV were paralyzed with pancuronium bromide (1 mg/kg) and breathing spontaneously, a cervical tracheotomy was performed. Anesthetized animals were randomly allocated into four groups: nonventilated, ventilated with low VT and no PEEP, ventilated with high VT and no PEEP, and ventilated with high VT and 10 cm H₂O of PEEP. After induction of anesthesia, a catheter (ED 0.96 mm; Intramedic, Clay Adams, Parsippany, NJ) was inserted into the right carotid artery for arterial blood sampling and arterial blood pressure monitoring. While anesthetized and breathing spontaneously, a cervical tracheotomy using a thin-walled 14-gauge Teflon catheter was performed. After the catheter was secured by a ligature around the trachea, those animals allocated to MV were paralyzed with pancuronium bromide (1 mg/kg) and connected to a time-cycled, volume-limited rodent ventilator (Ugo Basile, Varese, Italy).

Experimental Protocol
Three groups of healthy animals were ventilated for 4 h: one group (n = 20) at a VT of 6 ml/kg, another group (n = 20) at a VT of 20 ml/kg with no PEEP, and a third group at a VT of 20 ml/kg with 10 cm H₂O of PEEP. Respiratory rate was set to maintain constant minute ventilation and a normal PaCO₂. These settings were maintained constant during the 4 h of MV with room air. All animals were continuously anesthetized with ketamine/xylazine, paralyzed with pancuronium bromide, and maintained supine on a restraining board inclined 20 degrees from the horizontal. Peak airway pressures were continuously monitored. Inspiratory flows were not recorded, but inspiratory time was kept constant in the large VT without PEEP and large VT with PEEP groups. As a result, the peak flow was constant in these two groups. In the low VT group, because of an increased respiratory rate, inspiratory time was shorter than in the large VT groups and thus peak flow was greater. Boluses of isotonic saline were given as needed to maintain a mean arterial blood pressure greater than 60 mmHg. For pH and PaO₂ measurements, arterial blood samples were withdrawn at the end of the 4-h period of MV from six randomly selected surviving rats in each group. Rectal temperature was monitored and maintained at 36–36.5°C with a heating pad.

Outcomes and Pathologic Examination
Overall mortality during the 4-h experimental period was recorded in each group. At the end of the experiment, after sampling arterial blood for gas and serum analysis, six rats randomly selected from each group were sacrificed by a midline thoracotomy/laparotomy and sectioning of abdominal vessels. The heart and lung were removed from the thorax en bloc. Then, the lungs were isolated from the heart, the trachea was cannulated, and the right lung was fixed by intratracheal instillation of 3 ml of 10% neutral buffered formalin. After fixation, the lungs were floated in 10% formalin for a week. Lungs were serially sliced from apex to base, and specimens were processed in the usual manner, embedded in paraffin, and then cut at 10-µm thickness, stained with hematoxylin-eosin and with the Masson-Goldner trichrome technique, and examined under light microscopy. A pathologist (Dr. Valladares), blinded to the experimental history of the lungs, performed the histopathologic evaluation on coded samples. Three random sections of the right lung from each animal were examined with particular reference to alveolar and interstitial damage defined as cellular inflammatory infiltrates, pulmonary edema, disorganization of lung parenchyma, alveolar rupture, and hemorrhage. A semiquantitative morphometric analysis of lung injury was performed by scoring from 0 to 4 (none, light, moderate, severe, very severe) for each parameter. A total histologic lung injury score was obtained by adding the individual scores in every animal and averaging the total values in each group.

RNA Extraction and Cytokine Gene Expression in the Lungs
In the same rats used for blood gases and histologic evaluation, the left lung was excised, washed with saline, frozen in liquid nitrogen, and stored at −80°C for subsequent RNA extraction. The entire left lung was homogenized, and total lung tissue RNA was extracted using a commercially available kit (Boehringer Mannheim, Germany), and residual genomic DNA was removed by incubating the RNA samples with RNase-free, FPLC pure DNase I, and RNase inhibitor (Amersham Biosciences, Essex, United Kingdom). Expression lev-
els of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were measured for all samples using published primer pairs. Real-time polymerase chain reaction was used to quantify gene expression using the iCycler iQ Real-Time detection system (Bio-Rad Laboratories, Hercules, CA) and SYBR green I (Molecular Probes, Leiden, The Netherlands). Values for TNF-α and IL-6 gene expression levels were standardized by comparing each group with the one with the lowest levels of gene expression (control group).

Cytokine Serum Levels
At the end of every experiment, blood collected from the carotid artery was centrifuged for 15 min at 3,000 revolutions per minute. Sera were divided into aliquot portions and frozen at −80°C. TNF-α and IL-6 protein concentrations in serum were measured by enzyme-linked immunosorbent assay in dilutions that allowed interpolation from a simultaneously run standard curve. Levels of TNF-α and IL-6 were measured with a commercially available enzyme-linked immunosorbent assay specific for rats (Cytoscreen, Biosource International, Camarillo, CA). Results were analyzed using an enzyme-linked immunosorbent assay microplate reader (ELX800 NB Universal Microplate Reader, Bio-Tek Instruments, Winooski, VT). TNF-α and IL-6 concentrations are expressed as pg/ml. The threshold sensitivity was less than 8 pg/ml for IL-6 and less than 4 pg/ml for TNF-α.

Statistical Analysis
Data are expressed as means ± standard deviations (SD). Statistical analysis was performed with the Fisher exact probability test and paired and unpaired t tests, as appropriate. Comparisons that involved all groups were performed with one-way analysis of variance. If a difference was found, post hoc analysis using a t test were performed. Values derived from cytokine gene expression are expressed as means ± SD for the fold increase levels of gene expression in six surviving rats from each experimental group. Data from enzyme-linked immunosorbent assays were tested for normality (Kolmogorov-Smirnov test with Lilliefors correction) and analyzed by one-way analysis of variance, followed by the Student-Newman-Keuls all pairwise multiple range test. The Scheffé method was used to control for multiple comparisons. Data analyses were performed using Statistical Package for the Social Sciences version 15.0 (SPSS Inc, Chicago, IL). Differences were regarded as significant at P < 0.05.

Results

Outcome and Pathophysiologic Evaluations
All animals from the control and low VT groups survived the 4-h period of spontaneous or mechanical ventilation. Irrespective of PEEP, MV using high VT increased the risk of death within the 4 h of ventilation when compared to animals ventilated with low Vt (P = 0.020) (table 1). At the end of the experiments, mean PaO2 was within normal range in controls and in ventilated animals with low or high VT. Rats ventilated with 6 ml/kg had a small, but significant increase of the PaO2 (P = 0.032) (table 1). PaO2 in the VT 20 ml/kg plus 10 cm H2O PEEP group deteriorated significantly compared to the other studied groups (P < 0.00001). This drop in PaO2 was associated with a significant histologic evidence of increasing lung damage.

In general, the evolution of lung injury at progressively increasing Vt and PEEP was evident from lung histology. Animals ventilated with high Vt showed acute inflammatory infiltrates and perivascular edema, independent of PEEP. Animals ventilated with high VT plus PEEP had small hemorrhagic foci (fig. 1). The histologic score was significantly different among all groups of animals (fig. 2). Rats with VT 20 ml/kg and no PEEP had much greater lung damage than rats with low VT (1.0 ± 0.2 vs. 6.0 ± 1.1, P < 0.00001). The application of PEEP during high VT ventilation was associated with increasing lung damage compared to the group with VT 20 ml/kg and zero PEEP (6.0 ± 1.1 vs. 9.05 ± 1.6, P = 0.001) (fig. 2). Mean peak airway pressures at the end of the experiments never exceeded 16 cm H2O in the low VT group. However, peak airway pressures exceeded 30 cm H2O in those animals ventilated with high VT plus PEEP (table 1).

Cytokine Gene Expression in the Lungs
Although it was small, MV had an upregulatory effect on TNF-α gene expression in the lungs of healthy animals. Real-time polymerase chain reaction values, expressed relative to control animals, showed that TNF-α gene expression was doubled in animals ventilated with high VT on no PEEP (3.5 ± 0.6 vs. 1.6 ± 0.3, P = 0.004) (fig. 3). Animals ventilated with high VT and PEEP

| Table 1. Respiratory Parameters, Oxygenation, and Mortality Rate after 4 h of Spontaneous Breathing or Mechanical Ventilation |
|---|---|---|---|---|
| Parameters | Control | VT 6 ml/kg | VT 20 ml/kg | VT 20 ml/kg + 10 cm H2O PEEP |
| RR, breath/min | 101 ± 5 | 90.5 ± 0.2 | 36.3 ± 0.1 | 36.2 ± 0.1 |
| Peak airway pressure, cm H2O | — | 14 ± 1 | 25 ± 1.5 | 35 ± 2.5* |
| Pao2, mmHg | 85.4 ± 3.7 | 96.1 ± 5.7 | 89.1 ± 6.4 | 45.4 ± 7.9† |
| Paco2, mmHg | 36 ± 3.1 | 45.7 ± 3.0 | 34.8 ± 2.5 | 36.9 ± 1.4 |
| pH | 7.41 ± 0.03 | 7.34 ± 0.02 | 7.46 ± 0.01 | 7.29 ± 0.03 |
| Mortality, % (n) | 0 (0/20) | 0 (0/20) | 5% (1/20) § | 25% (5/20) § |

* P = 0.0001, when compared to groups ventilated without PEEP. † P = 0.00001, when compared to all groups. ‡ P = 0.032, when compared to control group. § P = 0.020, when compared to all ventilated groups. PEEP = positive end-expiratory pressure; RR = respiratory rate; Vt = tidal volume.
showed the highest expression of TNF-α (4.9 ± 0.7) \((P = 0.007,\) when compared to low \(V_T\); \(P = 0.024,\) and when compared to high \(V_T\) without PEEP). Although the combination of high \(V_T\) with 10 cm H\(_2\)O of PEEP had a synergistic effect on IL-6 gene expression, significant differences were only found between the low \(V_T\) versus high \(V_T\) plus PEEP groups (1 ± 0 \(vs.\) 1.9 ± 0.25, \(P = 0.016\). Four hours of MV with a \(V_T\) of 6 or 20 ml/kg did not change significantly the IL-6 expression \((P = 0.586)\) (fig. 3).

**Cytokine Serum Levels**

In animals ventilated with high \(V_T\), serum concentrations of TNF-α did not show significant variations after 4 h of MV compared to low \(V_T\) or control animals (9.3 ± 3.1, 6.39 ± 4.28, and 4.13 ± 2.0 pg/ml, respectively). However, animals ventilated with high \(V_T\) and PEEP had a 5.5-fold increase in serum concentrations of TNF-α when compared to animals ventilated with low \(V_T\) (35.4 ± 12.6 \(vs.\) 6.39 ± 4.28 pg/ml, \(P = 0.007\)). In addition, animals ventilated with high \(V_T\) with or without PEEP (69.9 ± 19.2 and 59.13 ± 17.04 pg/ml, respectively) had a substantial increase of systemic levels of IL-6 compared to animals ventilated with low \(V_T\) (15.96 ± 5.9 pg/ml) \((P = 0.0002)\) (fig. 4).

**Discussion**

The findings of this study can be summarized as follows. PEEP applied to healthy rats ventilated with a \(V_T\) of 20 ml/kg was not protective in any of the studied parameters. These findings challenge the assumption held by some that, when PEEP is added, lung damage can be attenuated in settings where VILI develops. Specifically, the addition of high PEEP to a constant large \(V_T\) causes injury in previously healthy animals.

The concept of VILI has been examined experimentally since 1970 by several investigators. How-
ever, most published studies have been performed under experimental situations that differ from the clinical setting; *ex vivo* lung models, lung injury models induced by lung lavage, aspiration, drugs or endotoxin injection, and the use of extremely large *V*Ts or high inspiratory pressures. In addition, in some of these studies the number of animals was insufficient to support any statistically valid conclusion, and some reported contradictory results. It is difficult to compare these published reports with our study because we used a different animal model, different *V*Ts, constant minute ventilation, and only 0 or 10 cm H2O of PEEP. Most studies showing the protective effects of PEEP were performed in experimental models of preexisting lung injury. Our intention was not to propose a new ventilatory strategy during MV, but to examine the morbidity and mortality at the same PEEP level reported in several hallmark studies on VILI in healthy animals. As other investigators, we studied the effects of simultaneous application of a moderately high *VT* plus 10 cm H2O of PEEP, although we did not explore whether this level of PEEP applied a few hours after injuring the lungs with high *VT* was protective.

Several experimentally and clinically relevant conclusions can be drawn from the current study. As in several other reports, our data strongly supports the induction of VILI as a result of prolonged, repetitive overdistension by high *VT*. In our study, 1 of 20 healthy animals died when they were ventilated with high *VT*; this mortality rate increased to 1 of 4 when PEEP was added, contrary to the findings of Webb and Tierney. They found that 10 cm H2O of PEEP dramatically reduced lung damage in rats with healthy lungs. However, there are marked differences between their model and our experiments. First, they ventilated healthy rat lungs for 1 h; second, a peak pressure of 45 cm H2O was maintained regardless of the level of PEEP (0 or 10 cm H2O), and the *VT* required to reach such inspiratory pressures was above 40 ml/kg. Since Webb and Tierney fixed the respiratory rate, the lungs were hyerventilated with such a high minute ventilation that animals rapidly died from severe alkalosis and from severe hypoxemia caused by barotrauma, alveolar edema, and shock. In all our ventilated animals, we maintained minute ventilation by manipulating the ventilator rate, insuring a normal PaCO2. However, the major differences with our study were the peak pressure and *VT* after the application of PEEP. Webb and Tierney kept the peak pressure constant as PEEP was applied, allowing the *VT* to decrease (level not indicated). However, we kept the *VT* constant at 20 ml/kg as PEEP was increased, resulting in a large increase in peak pressure. It is the large increase in peak pressure with the application of PEEP that we believe offset the PEEP benefit observed by Webb and Tierney. There seems to be no need to use *VT* much greater than 20 ml/kg to develop VILI. Moriondo et al.

![Graph A](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931179/)

**Fig. 4.** Effects of 4 h of mechanical ventilation with low tidal volume (*VT*), high *VT*, and high *VT* plus 10 cm H2O of positive end-expiratory pressure (PEEP) on systemic levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). (A) The combination of high *VT* with 10 cm H2O of PEEP had a synergistic effect on serum protein concentrations of TNF-α. ‡ Significantly different from low *VT* and high *VT* (*P* = 0.007). (B) The combination of high *VT* with 10 cm H2O of PEEP had a synergistic effect on serum protein concentrations of IL-6. * Significantly different from control (*P* = 0.01); ** significantly different from low *VT* (*P* = 0.0002); *** significantly different from high *VT* (*P* = 0.03). Bars represent the means ± SD values of six surviving rats per group.
and leads to edema and structural changes.\textsuperscript{29,30} As in the Webb and Tierney report, none of our animals developed interstitial emphysema or pneumothorax. However, increases in microvascular permeability and edema formation can occur without actual rupture of the lung. High levels of PEEP and peak inspiratory airway pressures can increase filtration pressures across pulmonary vessels and enhance edema formation.\textsuperscript{30} When PEEP is applied, the intrathoracic and alveolar vessel transmural pressures increase throughout the respiratory cycle. High levels of PEEP can reduce stroke volume and increase the pulmonary arterial pressure. The effect of MV on cardiac output is a function of mean airway pressure; therefore, animals ventilated with high $V_T$ plus PEEP presumably should have the greatest impact on hemodynamics. We maintained a mean arterial pressure above 60 mmHg in all animals by giving intermittent boluses of normal saline.

Dreyfuss \textit{et al.}\textsuperscript{31} reported in healthy rats the effects of increasing $V_T$ at increasing PEEP and found that rats ventilated with low $V_T$ (7 ml/kg) and 15 cm H$_2$O of PEEP developed pulmonary edema; those rats ventilated with the same $V_T$ plus 10 cm H$_2$O of PEEP did not. These authors found that doubling $V_T$ at 0 PEEP did not produce any significant variation of edema indexes when compared with low $V_T$ at 0 PEEP. However, when 10 cm H$_2$O of PEEP was added, a mild edema was produced. We were unable to reproduce these results, and we propose two major reasons in addition to the use of a different rat breed: (1) duration of MV in all their experiments was only 20 min in most cases and 5 min in some cases, whereas we maintained ventilation for 4 h, and (2) although peak inspiratory pressures in the low $V_T$ plus PEEP or in the medium-high $V_T$ without PEEP were similar to both our groups, low $V_T$ and high $V_T$ without PEEP (12–23 cm H$_2$O), the peak inspiratory pressures in their low $V_T$ plus 15 cm H$_2$O, or medium high $V_T$ plus 10 cm H$_2$O increased at the same level as in their animals ventilated with 40 ml/kg without PEEP (45 cm H$_2$O).

There is experimental evidence to indicate that PEEP attenuates VILI, even when high $V_T$ is used.\textsuperscript{6} Tremblay \textit{et al.}\textsuperscript{3} ventilated isolated rat lungs for 2 h with a moderately high $V_T$ and 10 cm H$_2$O of PEEP and found that ventilating isolated healthy lungs with a $V_T$ of 15 ml/kg plus 10 cm H$_2$O of PEEP was associated with lower lung damage and lower pulmonary inflammation than those ventilated with no PEEP. However, a more detailed examination of the data reported by Tremblay \textit{et al.} shows conflicting findings. First, average peak pressures in lungs ventilated with 15 ml/kg increased 2.5-fold in the first 30 min (from 23.3 to 56.2 cm H$_2$O) and twofold by the end of 2 h of ventilation when 10 cm H$_2$O of PEEP was applied (from 27.6 to 50.9 cm H$_2$O), a fold increase that has not been reproduced by us or by other investigators. In contrast, the average peak pressure of lungs ventilated with extremely high $V_T$ (40 ml/kg) was much lower than in the moderate $V_T$ group (41.1 cm H$_2$O in the first 30 min and 46.7 cm H$_2$O at the end of 2h period), a finding that we cannot reproduce in our \textit{in vitro} animal model. Second, according to their TNF-$\alpha$ messenger RNA images, it seems that TNF-$\alpha$ expression is higher in lungs ventilated with 10 cm H$_2$O of PEEP than with no PEEP, although the histogram representation of the densitometric values showed the opposite. On the basis of our reinterpretation, it seems that, although 10 cm H$_2$O of PEEP was protective in rats previously treated with endotoxin, as our group has similarly reported in a sepsis-induced lung injury model,\textsuperscript{5} it was deleterious in lungs from healthy rats.

Factors that produce regional or global alveolar overdistension facilitate local tissue damage. Several experimental and clinical studies\textsuperscript{6,9,10,16,20,21} have demonstrated that MV induces an inflammatory response, suggesting that the upregulation of local cytokines may contribute to VILI. We measured TNF-$\alpha$ and IL-6 as representative proinflammatory cytokines because of their clinical importance, as indicated by their correlation with outcome in critically ill patients.\textsuperscript{32} Studies demonstrating that MV induces a pulmonary inflammatory response suggest that the upregulation of local cytokines by MV may contribute to VILI\textsuperscript{6,9,10,16} and, as a result, translate into ventilator-induced death.\textsuperscript{33} Some reports\textsuperscript{6,9,10} support the hypothesis that, because the pulmonary endothelial barrier is damaged during acute lung injury, lung cytokines released into the circulation can initiate or propagate a systemic inflammatory response and play an active role in the development of multiple organ dysfunction. Therefore, a ventilatory strategy that prevents alveolar overdistension and collapse may attenuate the inflammatory response. However, as shown in our study, the addition of PEEP during high $V_T$ MV of healthy lungs is harmful.

The primary limitation of this study is the fact that only one setting (20 ml/kg $V_T$ with and without PEEP) was evaluated. However, as stated above, when our results are compared to those of others, it seems logical to assume that the increase in airway pressure with the application of PEEP is the cause of the extension of lung injury that we observed. We did not measure blood gasses in rats that died within the 4h experimental period; however, we think that rather than being a limitation of the study, it is plausible that a greater deterioration of the alveolar gas exchange occurred in those animals and it was probably the cause of death. We did not explore the changes in other mediators involved in VILI, although we are aware that components of the extracellular tissue matrix (including proteoglycan, collagen, and elastin) could play a key role.\textsuperscript{22}

In summary, the current study challenges the assumption reported in previous studies that lung damage can be attenuated in all settings of VILI when PEEP is added. Although our study has similarities with few others, we
were unable to reproduce their observations because we did not reduce $V_T$ with the application of high levels of PEEP. The superimposed alveolar overdistension caused by the application of high PEEP to the already high $V_T$ extended the injury induced by the high $V_T$ alone in previously healthy intact animals. That is, the addition of high PEEP to a constant large $V_T$ causes injury in previously healthy animals.

References

\alpha$ gene during conventional and high frequency ventilation. Am J Respir Crit Care Med 1997; 156:272–9