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Effect of positive end-expiratory pressure on inflammatory response in oleic acid-induced lung injury and whole-lung lavage-induced lung injury

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Abstract
Purpose. The present study investigated the effects of positive end-expiratory pressure (PEEP) on the inflammatory response in two different lung injury models: edematous lung induced by oleic acid (OA); and atelectatic lung induced by whole-lung lavage (LAV).
Methods. Japanese white rabbits ($n = 28$) were allocated to one of the two lung injury (OA or LAV) groups, and each group was treated with intermittent positive pressure ventilation, using zero end-expiratory pressure (ZEEP) or PEEP (1 cm H_2O above the lower inflection point [LIP]). Thus, the animals were divided into LAV-ZEEP, LAV-PEEP, OA-ZEEP, and OA-PEEP groups. Blood and bronchoalveolar lavage fluid (BALF) were sampled 3 h after ventilatory treatment to analyze interleukin (IL)-8 levels.
Results. Pa_{O_2} was significantly decreased after the induction of lung injury, but was significantly higher in the PEEP groups compared to the ZEEP groups for each lung injury. Serum IL-8 levels were elevated in both experimental models. Serum IL-8 levels were significantly lower in LAV-PEEP than in LAV-ZEEP, whereas no difference was noted between OA-PEEP and OA-ZEEP. BALF IL-8 levels were lower in LAV-PEEP than in LAV-ZEEP. PEEP above LIP attenuated the elevation of IL-8 in BALF and serum in atelectatic lungs, but did not attenuate these increases in the edematous lungs.
Conclusion. These results suggest that the protective effects of PEEP on injured lungs may depend on the underlying lung pathology.

Key words Oleic acid · Lung lavage · Acute respiratory distress syndrome · Ventilator-induced lung injury · Positive end expiratory pressure

Introduction

The pathology of early-phase acute lung injury/acute respiratory distress syndrome (ALI/ARDS) is diverse, involving edema, hemorrhage, and consolidations, in addition to atelectasis [1,2]. Although ventilatory management for ALI/ARDS is primarily aimed at supporting systemic oxygenation, recent studies of the management of ARDS have focused on attempts to improve outcomes by preventing ventilator-induced lung injury [3–8]. High tidal volume, positive end-expiratory pressure (PEEP), or various other kinds of recruiting maneuvers are necessary for recruiting nonaerated lung regions. However, overinflation of lung regions may be induced, particularly in those regions that are normally aerated at end-expiration, increasing the risk of barotrauma. Avoiding overinflation of already aerated normal lung by adopting low tidal volume ventilation is thus recommended [7].

Epithelial shearing stress of the peripheral airways associated with repeated opening and collapse can be another cause of ventilator-induced lung injury [9]. The use of a protective lung strategy for the treatment of ALI/ARDS is based on the hypothesis that PEEP above the lower inflection point (LIP) on the pressure-volume (PV) curve prevents the repeated opening and collapse of airways and some lung units, and protects lung tissue from mechanical injury [3]. Previous clinical trials have demonstrated that ventilator-induced lung injury is associated with increased inflammatory mediators and significantly affects patient morbidity and mortality in ALI/ARDS [6].

Although oleic acid (OA)-induced lung injury and lung injury induced by whole-lung lavage (LAV) are popular laboratory models for studying ALI/ARDS, the pathophysiologies of these injuries may differ. Lung injury induced by LAV represents established lung injury, reflecting surfactant-depleted, collapsed lungs [10–13]. Alveoli in OA-induced lung injury have recently been shown to be completely or partially flooded, rather than collapsed [14–16], and one of these studies suggested that the application of PEEP might induce overinflation of the alveoli [15]. To date, there

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have been few studies investigating the effect of PEEP on OA-induced lung injury, from the inflammatory aspect. One of the key events of lung inflammation is the migration of polymorphonuclear leukocytes (PMNL), which are known to release a variety of enzymes in the lung and to induce damage to type I and II pneumocytes, functional compromise of the surfactant, lung edema, and epithelial necrosis. Among the mediators of chemotactic attraction for PMNL, interleukin (IL)-8 is probably be the most potent chemoattractant factor, and elevation of IL-8 was demonstrated in lung injuries induced by both LAV [17] and oleic acid [18].

The present study investigated the effects of PEEP on the inflammatory response in two different models of lung injury: edematous lung induced by OA; and atelectatic lung induced by LAV. The goal of the present study was to evaluate the inflammatory aspects involved in the pathogenesis of ventilator-induced lung injury in different lung injury models with or without PEEP. We hypothesized that the protective effects of PEEP on lungs might depend on the underlying lung pathology.

Materials and methods

All experimental protocols were reviewed and approved by the Animal Care and Use Committee of Tokyo Medical and Dental University, and were performed according to National Institutes of Health guidelines. Twenty-eight Japanese white rabbits that weighed between 2.9 and 3.2 kg were randomly separated into four groups of 7 animals each in order to compare the effect of PEEP in two different lung injury models. The animals were anesthetized using intramuscular ketamine hydrochloride (30 mg·kg^{-1}) and xylazine (0.3 mg·kg^{-1}). With the animals supine, a midline cervical incision followed by tracheostomy was performed after subcutaneous infiltration with 0.5% (w/v) lidocaine. The trachea was intubated using a tracheal tube (inner diameter, 4.0 mm). Mechanical ventilation (tidal volume, 10 ml·kg^{-1}; respiratory rate [RR], 30 breaths·min^{-1}; inspiratory:expiratory ratio, 1:2; fraction of inspiratory oxygen [$F_{I_{O_2}}$], 1.0) was commenced, using an SN-480-6 ventilator (Shinano, Tokyo, Japan). The animals were paralyzed using an intramuscular injection of pancuronium (0.5 mg·kg^{-1}). A 4-Fr double-lumen catheter (Arrow International, Reading, PA, USA) was introduced through a jugular vein for fluid and drug infusion. Lactated Ringer's solution was infused intravenously at a rate of 10 ml·kg^{-1}·h^{-1} throughout the study. Anesthesia was maintained using ketamine at 1 mg·kg^{-1}·h^{-1}, propofol at 6 mg·kg^{-1}·h^{-1}, and pancuronium at 0.3 mg·kg^{-1}·h^{-1} through the central venous line. An arterial catheter was placed in the carotid artery for monitoring arterial pressure and sampling arterial blood. Blood sampling and baseline measurements of lung mechanics, hemodynamics, and arterial blood gas were performed. After baseline measurements, animals were allocated to either of the following lung-injury experiments: surfactant-depleted lungs induced by LAV; or OA-induced lung injury.

Lung lavage was performed using warmed (37°C) normal saline (20 ml·kg^{-1}) to produce lung injury. Animals were disconnected from the ventilator, and saline was instilled directly into the lungs via the endotracheal tube. Animals were ventilated using the previous settings for 30 s, and saline was recovered by gentle suctioning. This lavage procedure was repeated every 10 min until $P_{a_{O_2}}/F_{I_{O_2}}$ was less than 200 mmHg. The OA-induced lung injury group received an intravenous injection of 0.1 ml·kg^{-1} OA (Wako, Osaka, Japan) over 30 min.

For the purpose of preventing metabolic acidosis and hypotension, continuous infusion of 0.8 mEq·h^{-1} sodium bicarbonate and 8 μg·kg^{-1}·min^{-1} dopamine was started on induction of lung injury. At 60 min after confirming the establishment of lung injury, control measurements were taken, after which a PV curve was obtained. Static PV curves were constructed using a supersyringe method. Briefly, the animal was disconnected from the ventilator and connected to a specifically designed syringe at the end of a 3-s expiration. A 100-ml syringe was used to inflate the lungs with pure oxygen in 5-ml steps until a volume corresponding to a plateau pressure of 35 cmH_2O was reached.

After the establishment of lung injury, the animals were divided into zero end-expiratory pressure (ZEEP) and PEEP groups. Animals in the ZEEP group were ventilated with a tidal volume (TV) of 15 ml·kg^{-1}, an RR of 30 breaths·min^{-1}, and an end-expiratory pressure of 0 cmH_2O. Animals in the PEEP group were ventilated with a TV of 10 ml·kg^{-1}, an RR of 30 breaths·min^{-1} to achieve target $PaCO_2$, and a PEEP of 1 cmH_2O above LIP, based on the inflation limb of the PV curve. RR was allowed to increase up to 40 breaths·min^{-1} when $PaCO_2$ exceeded 50 mmHg in each group.

A total of four experimental groups were thus formed: the first group received LAV followed by intermittent positive pressure ventilation (IPPV; LAV-ZEEP); the second group received LAV followed by IPPV+PEEP (LAV-PEEP); the third group received intravenous OA followed by IPPV (OA-ZEEP); and the fourth group received intravenous OA followed by IPPV+PEEP (OA-PEEP). The objective of this experiment was to compare the effects of PEEP on gas exchange and inflammatory responses in each type of lung injury.

Arterial blood samples were obtained before and after the lung injury, 60 and 180 min after randomization of the ventilation strategy to determine blood gas

and plasma levels of protein and IL-8. Arterial blood specimens were analyzed for Pa_{O_2}, Pa_{CO_2}, and pH. At 3 h after ventilatory treatment, the animals were killed by injection of a pentobarbital overdose. The lungs and heart were then excised en bloc. Bronchoalveolar lavage fluid (BALF) was harvested from the right lung, which was lavaged with 15 ml·kg^{-1} of 0.9% normal saline. The solution was flushed in and out of the lung five times. Fluid was then centrifuged at 250 G at 4°C for 10 min. Cell-free supernatant and serum were divided into several aliquots and stored at –80°C for the measurement of IL-8 and protein levels. Serum and BALF IL-8 levels were measured using an enzyme-linked immunosorbent assay (OptEIA Set; Pharmingen, San Diego, CA, USA). Protein concentrations in BALF were determined using a protein assay reagent. The left lobe of the lung was fixed by the instillation of formaldehyde solution through the left main bronchus at 20 cmH_2O. At least 48 h after fixation, the left lower lobe was embedded in paraffin, then sections were stained, using hematoxylin and eosin, and examined by light microscopy. Dorsal sections of the left lower lobe (four sections for each animal) were processed for histological analysis. Two observers, blinded to the nature of the experiment, scored lung injury from 0 (no damage) to 3+ (maximal damage), according to five categories of assessment: alveolar congestion; edema; infiltration/aggregation of neutrophils in the airspace or vessel walls; alveolar wall thickening; and alveolar distension or destruction.

Data values are expressed as means ± SD or medians and interquartile ranges, as appropriate. All statistical analyses of recorded data were performed using the StatView statistical software package (J 4.5; Abacus Concepts, Berkeley, CA, USA). Data were compared within the same lung-injury model groups. Intragroup comparisons of blood gas and blood pressure data and intergroup comparisons at each time interval were performed using repeated measures analysis of variance (ANOVA) to determine the effects of treatment. If significant differences were identified, post-hoc tests using Bonferroni's method were performed within and between groups. Nonparametric statistical analysis was applied for IL-8 and protein concentration data. Statistical significance was determined by ANOVA using the Kruskal-Wallis test, followed by the Mann-Whitney *U*-test. Values of $P < 0.05$ were considered statistically significant.

Results

Each lung-injury model displayed deteriorated oxygenation, and values for $Pa_{O_2}/F_{I_{O_2}}$ were less than 200 mmHg, fulfilling the criteria of ARDS. Application of the PEEP improved Pa_{O_2} in both lung-injury models (Table 1). $PaCO_2$ values increased after lung injury, and were maintained in the LAV-ZEEP, OA-ZEEP, and OA-PEEP subgroups, but levels in LAV-PEEP decreased following the use of PEEP. $PaCO_2$ was lower in LAV-PEEP than in LAV-ZEEP. After initiating treatment, pH was higher in LAV-PEEP than in LAV-ZEEP. Peak airway pressure was significantly increased after lung injury, but the values did not exceed the upper inflection point in any animal, and differences in peak airway pressure between the PEEP group and

Table 1. Arterial blood gas data of animals at baseline following injury (LAV or OA) and during treatment (ZEEP or PEEP)

	Baseline	After injury	60 Min after treatment	180 Min after treatment
pH				
LAV-ZEEP	7.4 ± 0.03	7.32 ± 0.03	7.33 ± 0.04	7.18 ± 0.14
LAV-PEEP	7.41 ± 0.02	7.32 ± 0.02	7.42 ± 0.03*	7.41 ± 0.04*
OA-ZEEP	7.41 ± 0.04	7.38 ± 0.1	7.34 ± 0.07	7.23 ± 0.12
OA-PEEP	7.41 ± 0.02	7.32 ± 0.03	7.33 ± 0.04	7.29 ± 0.13
$PaCO_2$ (mmHg)				
LAV-ZEEP	36.2 ± 2.9	47 ± 2.6	48.4 ± 3.9	60.9 ± 10.8
LAV-PEEP	35.2 ± 2.1	46.6 ± 3.6	37.3 ± 2.8	39.8 ± 6.2*
OA-ZEEP	37.2 ± 1.7	44 ± 5.2	47.8 ± 6.2	49.8 ± 8.1
OA-PEEP	37.2 ± 3	47.2 ± 7	47.2 ± 10.4	49.8 ± 8.8
PaO_2 (mmHg)				
LAV-ZEEP	482 ± 15	97 ± 36	56 ± 19	42 ± 7
LAV-PEEP	511 ± 29	81 ± 15	286 ± 121*	267 ± 169*
OA-ZEEP	525 ± 55	90 ± 17	54 ± 9	50 ± 13
OA-PEEP	501 ± 13	74 ± 19	292 ± 159*	258 ± 121*

*$P < 0.05$ vs ZEEP
Intragroup comparisons of control data and data obtained after treatment, and intergroup comparisons within the same lung-injury models were performed; values are means ± SD
LAV, lung lavage; OA, oleic acid; ZEEP, zero end-expiratory pressure; PEEP, positive end-expiratory pressure

Table 2. Mean arterial pressure and peak airway pressure data of animals at baseline following injury (LAV or OA) and during treatment (ZEEP or PEEP)

	Baseline	After injury	60 Min after treatment	180 Min after treatment
Mean arterial pressure (mmHg)				
LAV-ZEEP	82 ± 15	83 ± 19	92 ± 19	79 ± 22
LAV-PEEP	90 ± 13	89 ± 22	87 ± 20	92 ± 16
OA-ZEEP	82 ± 10	90 ± 5	80 ± 6	76 ± 17*
OA-PEEP	86 ± 17	98 ± 17	85 ± 12	70 ± 15*
Peak airway pressure (cmH_2O)				
LAV-ZEEP	8.2 ± 1.4	21.3 ± 2.1	22 ± 3.5	26.4 ± 3.3*
LAV-PEEP	8.6 ± 1.4	21.3 ± 2.7	23.5 ± 1.7	25.2 ± 1.7*
OA-ZEEP	7.9 ± 1.2	21.2 ± 1.4	26.8 ± 2*	28.5 ± 3.1*
OA-PEEP	7.9 ± 1.6	20.4 ± 2.9	25.9 ± 3.5*	28.1 ± 3.6*
LIP (cmH_2O)				
LAV-ZEEP	—	7 ± 1.8	—	7.8 ± 1.3
LAV-PEEP	—	7.3 ± 1	—	7.7 ± 1.4
OA-ZEEP	—	6.9 ± 0.7	—	8.2 ± 1.4
OA-PEEP	—	7 ± 1	—	7.4 ± 1
UIP (cmH_2O)				
LAV-ZEEP	—	30.3 ± 2.6	—	31.7 ± 3.6
LAV-PEEP	—	28.7 ± 1.6	—	30.6 ± 1.7
OA-ZEEP	—	28.5 ± 2.6	—	29.9 ± 1.7
OA-PEEP	—	28.7 ± 1.4	—	29.4 ± 1

*$P < 0.05$ vs "after injury" data
Intragroup comparisons of "after injury" data and data obtained after treatment, and intergroup comparisons within the same lung-injury models were performed; values are means ± SD
LAV, lung lavage; OA, oleic acid; ZEEP, zero end-expiratory pressure; PEEP, positive end-expiratory pressure

the ZEEP group were not significant (Table 2). Serum IL-8 levels were significantly elevated following lung injury in all groups (Figs. 1 and 2). IL-8 values were significantly lower in LAV-PEEP than in LAV-ZEEP at both 60 (150 $pg \cdot dl^{-1}$ [range, 85–220] vs 670 $pg \cdot dl^{-1}$ [range, 413–735], $P < 0.05$) and 180 min (125 $pg \cdot dl^{-1}$ [range, 99–214] vs 614 $pg \cdot dl^{-1}$ [range, 310–825], $P < 0.05$; Fig. 1). Conversely, no significant differences in serum IL-8 levels were noted between OA-PEEP and OA-ZEEP (280 $pg \cdot dl^{-1}$ [range, 160–657] and 154 $pg \cdot dl^{-1}$ [range, 30–350], respectively, at 180 min). IL-8 levels in BALF were also lower in LAV-PEEP compared with LAV-ZEEP (Table 3). No differences in IL-8 levels were noted between OA-ZEEP and OA-PEEP.

The BALF/serum protein ratio tended to be slightly lower in LAV-PEEP than in LAV-ZEEP, but again, no significant differences were detected, nor were any differences found between OA-PEEP and OA-ZEEP.

Representative microscopic images are shown in Fig. 3. Alveolar wall thickening and alveolar collapse were more prominent in LAV-ZEEP in comparison with LAV-PEEP. Conversely, lungs in the OA-ZEEP group were distended rather than collapsed, and lungs in the OA-PEEP group were overdistended and partially destroyed. The difference in the histological score for PMNL infiltration between LAV-PEEP and LAV-ZEEP did not reach statistical significance (Table 4).

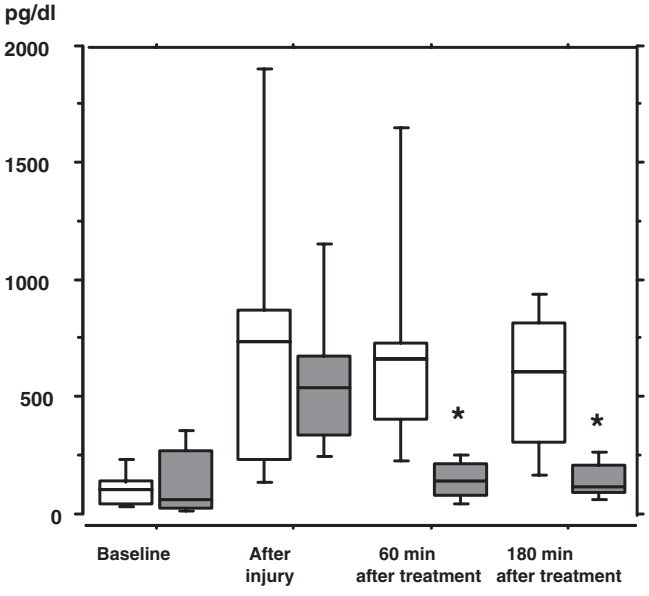

Fig. 1. Serum interleukin (IL)-8 levels in animals at baseline following injury induced by lung lavage (LAV), and during treatment with either zero end-expiratory pressure (ZEEP; *white boxes*) or positive end-expiratory pressure (PEEP; *cross-hatched boxes*). The *ends of the boxes* indicate the 25th and 75th percentiles and the *lines in the boxes* indicate the median values. The 10th and 90th percentiles are indicated with *whiskers*. *$P < 0.05$ vs LAV-ZEEP

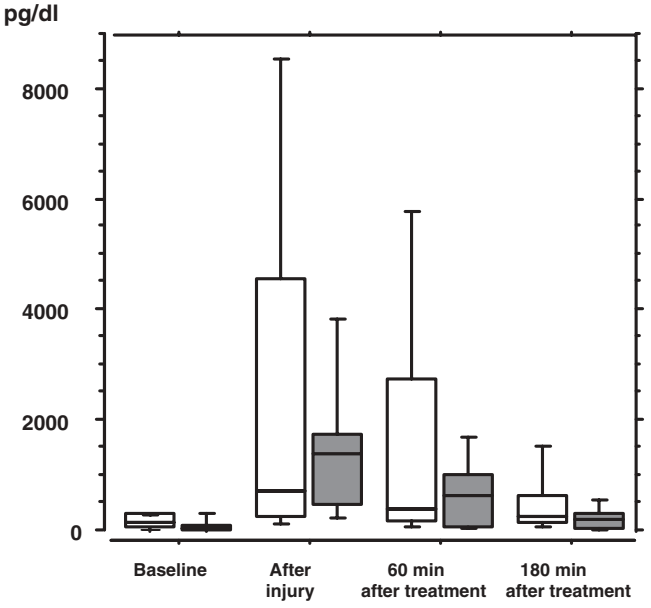

Fig. 2. Serum IL-8 levels in animals at baseline following injury induced by oleic acid (OA), and during treatment with either zero end-expiratory pressure (ZEEP; *white boxes*) or positive end-expiratory pressure (PEEP; *cross-hatched boxes*). The *ends of the boxes* indicate the 25th and 75th percentiles and the *lines in the boxes* indicate the median values. The 10th and 90th percentiles are indicated with *whiskers*

Discussion

The mechanisms and pathogenesis of ventilator-induced lung injury have attracted a great deal of attention, but the details are not yet fully understood. Currently, two important mechanisms are assumed to be involved: overstretch of fully opened alveoli by high tidal volume or high airway pressure [19]; and shearing trauma to the epithelium of terminal airway units during repetitive closure and forceful reopening at high pressure [9,20]. On the basis of this second assumption, the hypothesis has emerged that PEEP above the LIP point would protect lung tissue from mechanical injury [9,21,22] by preventing alveolar collapse and opening during tidal ventilation. The inflation limb of the PV curve has been proposed to identify a safe range of ventilatory pressures during mechanical ventilation in patients with ARDS [23].

In the present study, the PV curve displayed an LIP in both experimental models of ARDS. The presence of LIP suggests that dependent regions of injured lungs collapse at the end of expiration, and that expanding a lung from a degassed state initially meets with a large impedance. However, in edematous lungs, Martynowicz et al. [15] rejected the idea that dependent lungs were atelectatic at lower transpulmonary pressure. They measured regional volume during tidal ventilation in dog lungs, using a parenchymal marker technique after

Table 3. BALF IL-8 and total protein (TP) after treatment with or without PEEP in different lung-injury models

	BALF IL-8 (ng/dl)[a]	BALF TP (g/dl)[b]	BALF TP / serum TP[b]
LAV-ZEEP	21.8 (13–36.6)	0.56 ± 0.17	0.22 ± 0.1
LAV-PEEP	9.2 (4.6–20.4)*	0.45 ± 0.2	0.17 ± 0.09
OA-ZEEP	8.4 (3.6–13.5)	0.77 ± 0.21	0.32 ± 0.09
OA-PEEP	2.5 (1.4–11.3)	0.8 ± 0.23	0.41 ± 0.21

* $P < 0.05$ vs LAV-ZEEP
Intergroup comparisons within the same lung-injury model were performed
[a] Median (interquartile range)
[b] Mean ± SD
LAV, lung lavage; OA, oleic acid

Table 4. Histological injury scores after ventilatory treatments in different lung-injury models

	Infiltration of PMNL	Hemorrhage	Alveolar thickening	Alveolar collapse	Overinflation
LAV-ZEEP	2.13 ± 0.83	1.5 ± 0.54	1.25 ± 0.89	2.13 ± 1.13	0.52 ± 0.76
LAV-PEEP	1.55 ± 0.89	1.5 ± 0.93	0.25 ± 0.46*	0.5 ± 0.54*	0.88 ± 0.64
OA-ZEEP	1.25 ± 0.56	1.75 ± 0.46	0.5 ± 0.5	0.375 ± 0.51	1.5 ± 0.53
OA-PEEP	0.95 ± 0.46	0.75 ± 0.46	0	0	2.5 ± 0.76

* $P < 0.05$ vs ZEEP
Intergroup comparisons within the same lung-injury model were performed; values are means ± SD
LAV, lung lavage; OA, oleic acid; PMNL, polymorphonuclear leukocytes

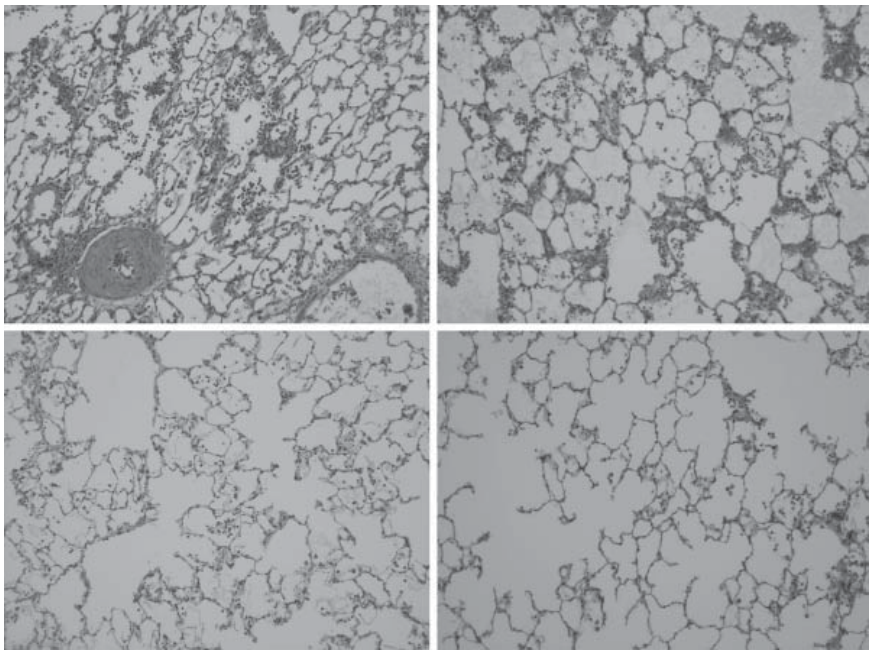

Fig. 3A–D. Lung sections. **A** Lung injury induced by whole-lung lavage (LAV) in animal treated with control mechanical ventilation with zero end-expiratory pressure (ZEEP); **B** LAV animal treated using PEEP (1 cmH_2O above lower inflection point); **C** oleic acid (OA)-induced lung injury in animal treated with ZEEP; **D** OA animal treated with PEEP. Alveolar wall thickening and alveolar collapse were significant in LAV animals treated with ZEEP in comparison with LAV animals treated with PEEP. In the OA-induced injury, alveolar collapse was not recognized in the ZEEP group and the lungs of the PEEP group were overdistended and partially destroyed. **A–D** H&E, ×200

OA-induced lung injury. OA injury did not produce the collapse of dependent lung units, and no evidence of cyclic reopening and collapse of dependent lung regions was found during mechanical ventilation. However, the PV curves of dependent regions of injured lungs display a sigmoidal shape, expressing the transition from a fluid-filled to an air-filled, high surface-tension state [24].

In the present study, PEEP was applied according to a LIP on the PV curve. The failure of PEEP to attenuate IL-8 production in OA-induced lung injury may be partially explained by the full recruitment of dependent lung regions during expiration. The lungs of OA-induced injury were inflated rather than collapsed, as seen in the histopathological findings. The level of PEEP necessary to produce full recruitment of a dependent lung might induce overinflation of fluid-filled alveoli or nondependent aerated lung regions in OA lung injury [16]. On the other hand, PEEP preserved alveolar structures in lavaged lungs and attenuated the elevation of IL-8 levels in serum and BALF. So far, very few in vivo studies have clearly demonstrated that PEEP decreases shear stress-induced lung injury and the subsequent release of cytokines. Steinberg et al. [25] recently showed that PEEP stabilized surfactant-deactivated lungs and significantly reduced modest increases in IL-6, in addition to histological evidence of lung injury.

Both OA- and LAV-induced lung injuries represent established models of ARDS and mimic the pathophysiology of ARDS in humans, but do not seem to correspond to clinical ARDS from the perspective of pathogenesis. OA-induced lung injury can be regarded as a prototypical model of primary ARDS in which intravenously administered OA directly injures the pulmonary vascular endothelium [26]. Several authors have reported differences between ARDS originating from pulmonary disease and that originating from extrapulmonary disease [27,28]. Gattinoni et al. [27] reported that lungs in patients with pulmonary-origin ARDS were less compliant and did not respond well to PEEP in comparison with ARDS of extrapulmonary origin. In the present study, OA-induced lung injury, as well as the lung injury induced by LAV, at least during the early experimental phase, may have behaved like extrapulmonary ARDS, because the OA-induced lung injury responded to PEEP. Van der Kloot et al. [28] also found that alveolar recruitment by PEEP was prominent in OA-induced lung injury and LAV-induced lung injury compared with a model of bacterial pneumonia. In evaluating the effects of PEEP or ventilatory treatment in acute lung-injury models, it is important to note that these treatments may produce different results depending on the injury model.

A recent multicenter trial has shown that higher PEEP for ARDS does not improve the survival rate in comparison with lower PEEP, provided that a low tidal volume is applied [8]. Although that trial did not measure individual PV curves, higher PEEP levels might sufficiently exceed a LIP and result in alveolar recruitment. The trial suggests that PEEP is effective for improving gas exchange in ARDS, and the protective effects, such as the prevention of barotrauma or ventilator-induced lung injury, may be limited, due to a non-uniform process and heterogeneous pathology.

High PEEP levels should not be indiscriminately administered, and need to be adjusted according to the individual lung pathology.

One of the main problems of our study design was that a high and "antique" tidal volume ($15\,ml \cdot kg^{-1}$) was chosen for the control group. On the other hand, a tidal volume of $10\,ml \cdot kg^{-1}$ was used for the ventilation setting of the PEEP group, which might appear a little high in light of recent guidelines recommending ventilation with lower tidal volumes [3–8]. However, ventilation with lower tidal volumes of $6–8\,ml \cdot kg^{-1}$ caused severe hypoxia and hypercapnia in our preliminary experiments. We think it would have been better to apply similar tidal volumes in all groups, but static PV curves before and after treatment proved that peak airway pressure was almost identical in the two groups within the same lung-injury model, and peak airway pressure did not exceed the upper inflection point in any animal. Therefore, the lung overinflation seen in our OA-PEEP group must have been due to a combination of underlying alveolar edema and PEEP. The second problem in the present study design was that only a single cytokine was used for the evaluation of inflammatory responses. Although changes in other inflammatory cytokines such as IL-6 or tumor necrosis factor-α should have been examined, IL-8 was the only cytokine that we could measure in rabbits. The role of IL-8 in the pathogenesis of ventilator-induced lung injury remains unclear, but this cytokine plays a major role in the pathogenesis of acute lung injury. IL-8 is increased in patients at risk for and with ALI/ARDS, and a recent multicenter trial strongly suggested that the improved outcome in patients ventilated with low tidal volume ventilation was associated with an attenuation of IL-8 plasma levels [29]. In addition, stretch-induced low molecular weight (LMW) hyaluronan (HA) from fibroblasts plays a key role in augmenting the induction of proinflammatory cytokines in ventilator-induced lung injury [30]. Mascarenhas et al. [30] demonstrated that high tidal volume ventilation of rat lung increased LMW HA production and the synthesis of HA synthase 3 mRNA, and they identified an increase in IL-8 levels following the exposure of human type II-like A549 cells to LMW HA in an in vitro model of lung cell stretch.

In conclusion, PEEP based on the PV curve significantly improved gas exchange in both types of lung injury, but its effects on inflammatory responses in the lungs differed. Ventilatory strategies that are expected to promote alveolar stability may cause overdistension of alveoli, and the protective effects of PEEP for injured lungs may depend on the underlying lung pathology.

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