Effects of PEEP levels following repeated recruitment maneuvers on ventilator-induced lung injury

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Background: Different levels of positive end-expiratory pressure (PEEP) with and without a recruitment maneuver (RM) may have a significant impact on ventilator-induced lung injury but this issue has not been well addressed. Methods: Anesthetized rats received hydrochloric acid (HCl, pH 1.5) aspiration, followed by mechanical ventilation with a tidal volume of 6 ml/kg. The animals were randomized into four groups of 10 each: (1) high PEEP at 6 cm H2O with an RM by applying peak airway pressure at 30 cm H2O for 10 s every 15 min; (2) low PEEP at 2 cm H2O with RM; (3) high PEEP alone; and (4) low PEEP alone. Results: The mean arterial pressure and the amounts of fluid infused were similar in the four groups. Application of the higher PEEP improved oxygenation compared with the lower PEEP groups (P < 0.05). The lung compliance was better reserved, and the systemic cytokine responses and lung wet to dry ratio were lower in the high PEEP than in the low PEEP group for a given RM (P < 0.05). Conclusions: The use of a combination of periodic RM and the higher PEEP had an additive effect in improving oxygenation and pulmonary mechanics and attenuation of inflammation.

Accepted for publication 7 November 2007

Key words: Biotrauma; cytokine; compliance.

ACTA ANAESTHESIOLOGICA SCANDINAVICA


ACUTE respiratory distress syndrome (ARDS) is a diffuse pulmonary inflammatory response characterized by severe hypoxemia and impaired pulmonary mechanics. Although mechanical ventilation represents the mainstay in the supportive care of patients with ARDS, a large body of evidence suggests that mechanical ventilation per se may worsen a pre-existing lung injury and produce ventilator-induced lung injury (VILI) (1). Two mechanical theories are speculated to explain the VILI including alveolar overdistention at peak-inspiration (volutrauma) and repetitive alveolar opening-closing at end-expiration (atelectrauma) (2).

Mechanical ventilation with a large tidal volume (VT) may overstretch the aerated alveoli and promote pulmonary inflammation. Protective ventilatory strategies aimed at minimizing the possible iatrogenic effects of mechanical ventilation include a low VT and high positive end-expiratory pressure (PEEP). The ARDS Network study showed that VT of 6 ml/kg could reduce mortality rate by 22% compared with a VT of 12 ml/kg in ARDS patients (3).

However, low VT per se may also induce adverse effects including progressive alveolar collapse and thus an impaired oxygenation in anesthetized surgical patients (4), especially when insufficient levels of PEEP are applied (5).

Patients with ARDS become more prone to developing severe atelectasis than healthy individuals in response to low VT ventilation (4), due to surfactant dysfunction and inflammatory cell infiltration (6). Especially the use of a high fraction of inspired oxygen (FIO2) (7) and repeated tracheal suctioning in ARDS (8) can lead to further alveolar collapse during low VT ventilation.

Recruitment maneuvers (RMs) are designed to open previously collapsed lung units by increasing transpulmonary pressure. Several animal studies...
and clinical trials have confirmed the effectiveness and safety of RMs in ARDS (9–13), although the impacts in long-term care and mortality are unclear (14).

RMs are less likely to induce VILI because high inflation pressures are applied only transiently and intermittently as compared with the use of persistently high PEEP levels. Opened alveoli using RM vary considerably in size and are prone to collapse again if PEEP levels are smaller than their threshold closing pressures (15). An adequate PEEP is thus necessary to maintain the effects of repeated RMs to keep the lung open (12, 16).

However, it is yet to be elucidated as to whether repeated RM with such low PEEP levels can worsen cyclic alveolar opening–closing injury leading to VILI. Therefore, the present study evaluated the effects of repeated RM with either low or high PEEP in the context of VILI by investigating multiple variables including oxygenation, hemodynamics, pulmonary mechanics, and inflammation in an acid aspiration-induced ARDS model in rats.

Materials and methods

Animals and instrumentation

The protocol was approved by the Animal Care Committee at the St Michael’s Hospital. Sixty Sprague–Dawley rats, weighing 300–400 g, were anesthetized with ketamine (100 mg/kg) and xylazine (20 mg/kg) intraperitoneally. A tracheostomy was performed and a 14 GA catheter was placed in the trachea and secured with 3-0 silk. A tail vein was cannulated with a 22 GA angiocatheter for continuous anesthetic infusion with ketamine (20 mg/kg/h) and xylazine (4 mg/kg/h). A 24 GA angiocatheter was inserted into the carotid artery for blood sampling and arterial pressure monitoring (90603A; SpaceLabs, Issaquah, WA). Muscle paralysis was achieved by intravenous administration of pancuronium bromide (0.3 mg/kg/h). Core temperature was monitored continuously by a rectal probe, and the rats were maintained in normothermia by a temperature-controlled heating pad.

Experimental protocol

The animals were initially ventilated for 15 min to reach stability after surgery and instrumentation with an $F_{1/2}$O$_2$ of 1.0, a $V_T$ of 8 ml/kg, PEEP of 3 cm H$_2$O, and a respiratory rate of 35 breaths/min under volume control mode at a constant flow delivered by the SAR-830/P animal ventilator (CWE, Ardmore, PA).

Lung injury was induced by endotracheal instillation with 1.5 ml/kg of hydrochloric acid (HCl, pH 1.5) warmed to 37 °C. Briefly, half the amount of HCl solution was instilled into the lung via the tracheostomy catheter. The animal was then turned to lateral recumbency for 5 s. An RM was applied manually by increasing the peak inspiratory pressure at 30 cm H$_2$O for 10 s. The remaining half of HCl was instilled after turning the animal to the other side, followed by an RM maneuver.

Arterial blood gas was measured (CIBA-Corning 248; Bayer, Leverkusen, Germany) after HCl instillation. To insure a similar severity of lung injury, only if the arterial oxygen tension ($P_{aO_2}$) was between 100 and 200 mmHg were the animals included. If $P_{aO_2}$ was >200 mmHg, additional 0.2 ml HCl was given followed by blood gas analysis to confirm the injury. If $P_{aO_2}$ was <100 mmHg, the animal was euthanatized and excluded from the protocol.

Another 15-min stabilization period was allowed following HCl instillation; the animals were then randomized into one of four ventilation strategies: (a) high PEEP (6 cm H$_2$O)+RM; (b) low PEEP (2 cm H$_2$O)+RM; (c) high PEEP only; and (d) low PEEP only. We wanted to include 10 complete animals in each group for an equal statistic power analysis. Because we observed different mortality rates using the ventilatory strategies in a pilot study, we decided to produce a ballot to randomize every animal into one of the designated groups. When the number of complete animals reached ten in a group, the ballot of the group was removed.

RM was defined as an inspiratory pressure of 30 cm H$_2$O that was held for 10 s. RM was conducted every 15 min during the experiments. The ventilatory strategy was set at a $V_T$ of 6 ml/kg, an inspiratory time ($T_I$) of 33%, and an $F_{1/2}$O$_2$ of 1.0 in all four groups. The respiratory rate ranging 55–65 breaths/min was adjusted to achieve normocarbia ($P_{aCO_2}$ 35–45 mmHg). When the mean arterial blood pressure was below 60 mmHg, slow intravenous injections of up to three times of 0.5 ml aliquots of lactated Ringer’s solution (Baxter, Toronto, ON, Canada) were administered to maintain arterial pressure during 4 h mechanical ventilation.

Measurements

Plateau airway pressure ($P_{plat}$) was estimated during inspiratory hold over 2 s every 30 min by a data
acquisition system (LabVIEW 5.1; National Instruments, Austin, TX). Static respiratory system compliance (Crs) was calculated by $\text{Crs} = \frac{V_T}{(P_{\text{plat}} - \text{PEEP})}$. The mean arterial blood pressure, heart rate, respiratory rate, and core temperature were recorded every 30 min. Arterial blood gas analysis was performed at baseline, after HCl lung injury, and hourly thereafter.

All animals were killed at the end of the experiment by exsanguination from arterial line. The whole blood was withdrawn and centrifuged (Centrifuge 5415C; Eppendorf, Mississauga, ON, Canada) at 14,000 r.p.m. for 10 min, and the supernatant was maintained at $-80^\circ$C for further analysis.

The lungs were removed en bloc through a midline sternotomy. The whole left lung was used for the measurement of wet vs. dry (W/D) lung weight ratio, which was assessed after placing the lung in an oven (Hotbox Oven; Gallenkamp, Loughborough, UK) at 50°C for 48 h. The right lung was dissected and divided into two portions.

The right caudal lobe was homogenized (UltraTurrax; IKA, Wilmington, NC) in 5 ml of 0.2% Triton X in an ice bath. The homogenate was centrifuged at 5000 r.p.m., 4°C, for 10 min. The supernatants of lung homogenates were stored at $-70^\circ$C for subsequent analysis.

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The upper lobe of the right lung was instilled with 10% formalin solution (Accustain; Sigma-Aldrich, St Louis, MO) at an inflation pressure of 20 cm H2O and embedded in the same solution for histological examination. The hematoxylin and eosin-stained sections of lung tissue from all 40 specimens were examined by a pathologist and scored in a blinded fashion based on the following histological description as for a total lung injury score:

1. **Alveolar distention**: distended alveolar spaces were located with a low-power scanning lens, and the average diameters of these spaces (at least three adjacent ones) were measured with a micrometer eyepiece under a ×10 objective lens and expressed in millimeters.

2. **Alveolar collapse**: The specimens were scanned with a ×2.5 scanning lens for areas of collapse. Specimens with no collapse were scored as ‘0’; those with <5% of cross-sectional area showing collapse were scored as ‘1’; those specimens that showed collapse involving from 5% to 15% of area were scored as ‘2’, and those specimens estimated to have more than 15% of their area collapsed were scored as ‘3’.

3. **Perivascular hemorrhage**: Specimens with no evidence of perivascular or peribronchial hemorrhage were scored as ‘0’; specimens that showed only one or two foci of slight hemorrhage were scored as ‘1’; specimens with more than two areas showing slight to moderate degrees of hemorrhage were scored as ‘2’; and specimens showing severe hemorrhage were scored as ‘3’.

4. **Alveolar hemorrhage**: Ten random fields were examined under a ×40 objective lens. If no evidence of erythrocytes was seen in the alveolar spaces, the specimen was scored as ‘0’; if less than five intraalveolar erythrocytes were seen in one or two alveolar spaces, the specimen was scored as ‘1’; and if five or fewer intraalveolar erythrocytes were seen in more than two alveolar spaces per high-power field, the specimen was scored as ‘2’. If more than five intraalveolar erythrocytes were seen in more than two alveolar spaces per high-power field, the specimen was scored as ‘3’.

5. **Perivascular edema**: The specimens were scanned with a ×2.5 scanning lens for perivascular and peribronchial edema. If this feature was absent, the specimen was scored as ‘0’; if only a mild degree of edema was seen to involve one or two structures, the specimen was scored as ‘1’; if a moderate degree of edema involved more than two structures, the specimen was scored as ‘2’; and any specimen showing a severe degree of edema was scored as ‘3’.

6. **Infiltration by polymorphonuclear leukocytes**: Ten random fields were examined using a ×40 objective lens. The number of polymorphonuclear leukocytes within the alveolar walls and within the alveolar spaces were averaged for three most severely affected alveoli within each of the 10 high-power fields. The average rounded number was entered as the score for each specimen.

7. **Alveolar membranes**: Ten random fields were examined using a ×40 objective lens. If no membranes were found, the specimen was scored as ‘0’; if one membrane was found, the specimen was scored as ‘1’; if two membranes were found the specimen was scored as ‘2’; and if more than two membranes were found, the specimen was scored as ‘3’.

8. **Alveolar edema**: Ten random fields were examined using a ×40 objective lens; if no evidence of edema was found, as revealed by amorphous proteinaceous coagulum, or eosinophilic staining fluid with the alveolar spaces, the specimen
was scored as ‘0’; if only two alveolar spaces contained this coagulum, within all ten fields, the specimen was scored as ‘1’; if more than two but less than six alveolar spaces contained evidence of edema, the specimen was scored as ‘2’; and if six or more alveolar spaces contained evidence of edema, the specimen was scored as ‘3.’

Concentrations of tumor necrosis factor-α (TNF-α) and macrophage inflammatory protein-2 (MIP-2) in sera and lung homogenates were measured using rat-specific enzyme-linked immunosorbent assay kits (BioSource, Camarillo, CA). The values of the cytokines measured from lung homogenates were indexed to lung tissue total protein content (Protein Assay, Bio-Rad, Hercules, CA).

**Statistical analysis**
All data are expressed as mean ± SD. Data were analyzed using SPSS (version 12.0; SPSS, Chicago, IL). One-way analysis of variance (ANOVA) was used to compare continuous variables between groups. A value of $P<0.05$ was considered to be significant. When statistical significance was noted, *post hoc* tests with the Bonferroni method were performed for further analysis.

**Results**
Four animals were excluded before randomization due to severe hypoxemia ($P_{a}O_{2}<100\text{mmHg}$ in $F_{I}O_{2}$ 1.0) following HCl installation. Sixteen animals (three in PEEP6+RM, five in PEEP2+RM, two in PEEP6 only, and six in PEEP2 only) died before completion of the experimental protocol and were excluded from the data analysis. Ten animals in each group completed the experimental course. The total amounts of fluid administered were not significantly different and $P_{a}O_{2}$ and Crs at baseline and after acid aspiration were similar in all animals (Table 1).

**Table 1**
Total amounts of fluid, $P_{a}O_{2}$ (mmHg) and static compliance of the respiratory system (Crs, ml/cm H$_{2}$O) at baseline and after acid aspiration.

<table>
<thead>
<tr>
<th></th>
<th>PEEP6+RM</th>
<th>PEEP2+RM</th>
<th>PEEP6 only</th>
<th>PEEP2 only</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid (ml)</td>
<td>10.4 ± 0.5</td>
<td>11.2 ± 0.4</td>
<td>9.7 ± 0.4</td>
<td>10.7 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>$P_{a}O_{2}$ at baseline</td>
<td>387 ± 77</td>
<td>377 ± 51</td>
<td>395 ± 47</td>
<td>362 ± 40</td>
<td>NS</td>
</tr>
<tr>
<td>$P_{a}O_{2}$ post-acid</td>
<td>128 ± 30</td>
<td>122 ± 15</td>
<td>116 ± 14</td>
<td>140 ± 30</td>
<td>NS</td>
</tr>
<tr>
<td>Crs at baseline</td>
<td>0.53 ± 0.06</td>
<td>0.52 ± 0.09</td>
<td>0.51 ± 0.05</td>
<td>0.52 ± 0.08</td>
<td>NS</td>
</tr>
<tr>
<td>Crs post-acid</td>
<td>0.28 ± 0.04</td>
<td>0.29 ± 0.07</td>
<td>0.26 ± 0.04</td>
<td>0.27 ± 0.04</td>
<td>NS</td>
</tr>
</tbody>
</table>

PEEP, positive end-expiratory pressure; RM, recruitment maneuver.

**Hemodynamic**
The MAP was not significantly different among the groups (Fig. 1A). At the end of 4h ventilation, all animals showed some hypotension at MAP of 60–70 mmHg. It was observed that a transient hypotension with a decrease of MAP by 20–30 mmHg occurred during RM, which recovered spontaneously within 10min.
Arterial oxygenation and respiratory system compliance

Figure 1B shows the mean values of $P_aO_2$ over the entire experiment. An initial identical decrease in $P_aO_2$ and Crs was observed after acid aspiration in all groups, but the mean values of $P_aO_2$ were significantly higher in the PEEP6 groups than in the PEEP2 groups during the 4 h ventilation. At a given PEEP of 6 cm H$_2$O, application of RM resulted in a further increase in $P_aO_2$ during the initial 3 h of mechanical ventilation. On the contrary, the $P_aO_2$ values were identically lower in the PEEP2 groups than in the PEEP6 groups irrespective of RM application.

Figure 1C shows that the mean value of Crs was higher in the PEEP6+RM group than in the PEEP2+RM and the PEEP6 groups, respectively. The PEEP2 group had the lowest Crs, suggesting a worsened lung compliance using low PEEP without applying RM maneuvers.

Lung injury indices

The lung W/D ratio was significantly lower in the PEEP6+RM group than in any other groups (5.7 ± 0.5 in the PEEP6+RM, vs. 6.6 ± 0.4 in the PEEP2+RM, 6.1 ± 0.6 in the PEEP6 only, and 6.2 ± 0.4 in the PEEP2 only groups, respectively, $P < 0.05$ vs. all, Fig. 2A). Similar to the results of the lung W/D ratio, the PEEP6+RM group had a lower lung injury score than the PEEP2+RM group (Fig. 2B).

Inflammatory responses

We further examined biotrauma profiles induced by mechanical ventilation by measuring the pro-inflammatory cytokine TNF-$
\alpha$ and the chemokine MIP-2. The serum levels of TNF-$\alpha$ and MIP-2 were lower in the PEEP6+RM group than in the PEEP2+RM group (Fig. 3A and B). The concentrations of TNF-$\alpha$ and MIP-2 in the lung tended to be lower in the PEEP6+RM than in the other groups, but the differences did not reach statistical significance (Fig. 3C and D).

Discussion

A major finding of the present study is that repeated RM at an inadequate/low PEEP of 2 cm H$_2$O aggravates VILI compared with RM at a high PEEP of 6 cm H$_2$O.

Application of PEEP is mandatory for the management of patients with ARDS (17), but it has been shown that high PEEP alone might be unable to prevent new alveolar collapse while using low $V_T$. Richard et al. (18) demonstrated a significant development of alveolar collapse after reducing $V_T$ from 10 to 6 ml/kg in ARDS lungs at a PEEP level that was set above the lower inflection point of a pressure–volume curve. The use of repeated RM has been proposed as an adjunct to mechanical ventilation to re-expand collapsed lung tissue (19). Animal data showed that the effect of a single RM is transient, lasting <30 s in injured mouse lung (20). Rothen et al. (21) showed that performing an RM for 1.8 s could reduce alveolar collapsed lung by 50% in anesthetized healthy subjects, and on prolonging the RM to 7.8 s a 95% reduction was observed. Fujino et al. (22) reported an accumulative effect of multiple RMs by which a maximal recruitment was achieved after three to four RMs in a sheep model of ARDS. In the present study, we applied RM for 10 s to maximize the effectiveness, and repeated RM at a 15-min interval to reach a
We observed that PEEP6, in combination with RM, had the lowest degree of lung injury as reflected by better oxygenation, greater lung compliance, a lower lung W/D ratio, a lower lung injury score, and attenuated cytokine responses. It is noteworthy that twice as many as the number of animals died in the PEEP2 groups than in the PEEP6 groups ($n = 2–3$ vs. $n = 5–6$) before completion of the experimental protocol and these animals were excluded from the data analysis. We speculated that the variables of lung mechanic and injury could worsen should the experiments be prolonged in the low PEEP groups.

![Serum TNF-α](A) and MIP-2](B) in serum and lung tissue homogenates. The concentrations of TNF-α and MIP-2 in lung tissue homogenates were indexed to lung tissue protein content. *$P < 0.05$ vs. PEEP2+RM and PEEP2, respectively. MIP, macrophage inflammatory protein; PEEP, positive end-expiratory pressure; RM, recruitment maneuver; TNF, tumor necrosis factor-α.

The estimated respiratory system compliance was similar between the PEEP6 alone and the PEEP2+RM groups, suggesting that the use of a high PEEP alone could result in comparable lung compliance as seen by using RM at a low PEEP. It is intriguing that at a given respiratory system compliance, the oxygenation was higher in the PEEP6 group than in the PEEP2+RM group. Given the fact that the chest wall compliance was not significantly altered during a short period of time (i.e., 4 h) with high-pressure ventilation (23), the respiratory system compliance could represent the lung compliance in a short experimental condition in rats. Taken together, this phenomenon of an increased lung compliance with little improvement of oxygenation at a low PEEP level could be explained by an enhanced intratidal recruitment rather than an increased number of aerated alveoli.

It is important to point out that the lung W/D ratio and lung injury score were significantly lower in the PEEP6+RM group than in the PEEP2+RM group. It appears that the severity of the lung injury was associated with an increase in cytokine responses as reflected by the increased levels of TNF-α and MIP-2 in the lung. These findings indicate that periodic RM with inadequate PEEP might have exaggerated lung injury.

The respiratory rate used did not produce intrinsic PEEP and we do not believe it played a significant role in preventing lung collapse in the high PEEP group. Given a similar plateau pressure between the PEEP2+RM and the PEEP6+RM groups (data not shown), we observed a transient effect (i.e., 1–2 min) at the lower PEEP while a sustained effect on lung compliance at the higher PEEP level at a given RM.

RM could attenuate repeated derecruitment-associated lung injury by intermittent intentional disconnection of the ventilatory circuit (24). In injured lungs, however, alveolar recruitment and overdistention occur simultaneously at a higher intrathoracic pressure (25). RMs can damage or transiently alter the integrity of the alveolar–
capillary barrier (26). The application of zero PEEP following single RM increased type III procollagen mRNA expression as a marker of lung parenchyma remodeling (27). Our study supported the concept that at an inappropriate PEEP setting, repeated RM might induce more deleterious effects than a single RM.

There were several limitations in the present study. First, we arbitrarily defined a high PEEP at 6 cm H₂O and a low PEEP at 2 cm H₂O because a premature mortality rate was observed in a pilot study. Although it would be best to define optimal PEEP according to a PV curve (28), the construction of a PV curve per se could cause harm in the relatively small animals. Second, the acid-aspiration-induced lung injury model represents some clinical features seen in human ARDS;Gattinoni et al. (29) showed that PEEP and RMs had less effect in ARDS of pulmonary origin than that of extrapulmonary origin.

While recent clinical trials have demonstrated a survival benefit using low Vₜ ventilation in patients with ARDS (3, 30, 31), this ventilatory strategy may induce alveolar atelectasis and alveolar instability if used inadequately (32). Strategies combining a reduction in Vₜ alveolar recruitment and a sufficient level of PEEP may be more important to limit VILI (11, 33).

In conclusion, a combination of periodic RM and adequate PEEP levels has an additive effect of improving oxygenation and pulmonary mechanics, and reduces pulmonary inflammation. On the contrary, we speculate that periodic RM with an insufficient level of PEEP may cause further lung damage. A thorough and accurate monitoring of lung mechanics is important to adjust ventilatory maneuvers.

References


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