Effects of Positive End-expiratory Pressure Titration and Recruitment Maneuver on Lung Inflammation and Hyperinflation in Experimental Acid Aspiration–induced Lung Injury


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What We Already Know about This Topic

• Positive end-expiratory pressure after recruitment maneuvers are used to improve oxygenation in acute lung injury

What This Article Tells Us That Is New

• The proportion of lung inflammation in experimental acid aspiration lung injury in pigs was increased by positive end-expiratory pressure in the juxta-diaphragmatic lung regions whereas recruitment maneuvers did not cause additional inflammation or hyperinflation

ABSTRACT

Background: In acute lung injury positive end-expiratory pressure (PEEP) and recruitment maneuver are proposed to optimize arterial oxygenation. The aim of the study was to evaluate the impact of such a strategy on lung histological inflammation and hyperinflation in pigs with acid aspiration–induced lung injury.

Methods: Forty-seven pigs were randomly allocated in seven groups: (1) controls spontaneously breathing; (2) without lung injury, PEEP 5 cm H₂O; (3) without lung injury, PEEP titration; (4) without lung injury, PEEP titration + recruitment maneuver; (5) with lung injury, PEEP 5 cm H₂O; (6) with lung injury, PEEP titration; and (7) with lung injury, PEEP titration + recruitment maneuver. Acute lung injury was induced by intratracheal instillation of hydrochloric acid. PEEP titration was performed by incremental and decremental PEEP from 5 to 20 cm H₂O for optimizing arterial oxygenation. Three recruitment maneuvers (pressure of 40 cm H₂O maintained for 20 s) were applied to the assigned groups at each PEEP level. Proportion of lung inflammation, hemorrhage, edema, and alveolar wall disruption were recorded on each histological field. Mean alveolar area was measured in the aerated lung regions. Alveolar mean area was measured in the aerated lung regions.

Results: Acid aspiration increased mean alveolar area and produced alveolar wall disruption, lung edema, alveolar hemorrhage, and lung inflammation. PEEP titration significantly improved arterial oxygenation but simultaneously...
increased lung inflammation in juxta-diaphragmatic lung regions. Recruitment maneuver during PEEP titration did not induce additional increase in lung inflammation and alveolar hyperinflation.

Conclusion: In a porcine model of acid aspiration–induced lung injury, PEEP titration aimed at optimizing arterial oxygenation, substantially increased lung inflammation. Recruitment maneuvers further improved arterial oxygenation without additional effects on inflammation and hyperinflation.

PROTECTIVE-ventilation strategy with tidal volumes of 6 ml/kg or lesser is recommended in patients with acute respiratory distress syndrome for limiting ventilator-induced lung injury. Ventilator-induced lung injury has three identified components: volutrauma-induced lung inflammation and high permeability type pulmonary edema resulting from stress fracture of endothelial cells, air-space enlargement resulting from cyclic hyperinflation of normally aerated lung regions, and biotrauma characterized by increases in pulmonary and systemic inflammatory mediators and resulting either from hyperinflation of previously aerated lung regions or from cyclic lung recruitment/derecruitment. Incremental and decremental positive end-expiratory pressure (PEEP) trials with or without recruitment maneuvers (RM) have been proposed to optimize arterial oxygenation and minimize ventilator-induced lung injury. By recruiting poorly and nonaerated lung regions, RM may theoretically induce a more homogeneous distribution of tidal volume and should reduce lung stretch and the resulting inflammatory reaction. Incremental and/or decremental PEEP titration is per se another type of RM that allows determining the minimum PEEP that sustains the benefits of the RM. The procedure of PEEP titration could, however, overinflate previously aerated lung, which, in turn, could damage the alveolar epithelium and impair alveolar fluid clearance. The high end-inspiratory lung volume resulting from RM may also cause mechanical stress to both alveolar macrophages and epithelial cells, thereby further increasing lung inflammation. A recent randomized trial on high PEEP combined to RM failed to demonstrate a decrease in mortality in patients with acute lung injury (ALI) and acute respiratory distress syndrome.

Despite the numerous studies published on RM and PEEP titration, little is known on how lung histology and morphometry are affected by these maneuvers. We therefore designed the present study to determine the effects of PEEP titration with and without RM aimed at optimizing arterial oxygenation, on lung inflammation, pulmonary hemorrhage, alveolar edema, and air-space enlargement in pigs with acid aspiration–induced ALI.

Materials and Methods

Animal preparation

Animal experiments were performed in the Laboratory of Medical Investigation/Anesthesiology—Faculdade de Medicina da Universidade de São Paulo, Brazil. After approval of the Comitê de Ética para Análise de Projetos de Pesquisa (CAPPESQ) do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Brazil, 47 Landrace X large, white, crossbred female pigs were studied. The animals (weighing 38.6 ± 4.2 kg) were restricted from food overnight but had free access to water. Pigs were premedicated with an association of intramuscular ketamine (10 mg/kg), fentanyl (5 µg/kg), and midazolam (0.5 mg/kg). Anesthesia was induced with propofol (5 mg/kg) and animals were intubated orally (7-mm ID cuffed endotracheal tube, Hi-Lo, National Catheter, Argyle, NY). Mechanical ventilation was delivered using volume-controlled ventilation, tidal volume of 6–8 ml/kg, PEEP of 5 cm H₂O and oxygen inspired fraction (FIO₂) of 40% (Galileo Gold—Hamilton Medical AG, Rhazüns, Switzerland). Respiratory rate was adjusted to maintain end-tidal carbon dioxide between 35 and 40 mmHg (Poet IQ, Criticare Systems, Waukesha, WI). Continuous intravenous anesthesia consisting of ketamine (5 mg·kg⁻¹·h⁻¹), fentanyl (5 µg·kg⁻¹·h⁻¹) and pancuronium bromide (0.3 mg·kg⁻¹·h⁻¹) was standardized. The temperature of the pigs was kept at approximately 38°C by using warm blankets (Medi-therm II, Gaymar Industries, Orchard Park, NY). An arterial catheter, a central venous line, and a pulmonary artery catheter with continuous cardiac output measurement (7.5 F Edwards CCO (continuous cardiac output) catheter connected to Edwards Vigilance CCO Monitor; Edwards Lifesciences Corp., Irvine, CA) were inserted in the right femoral artery and vein. The electrocardiogram and arterial pressure were monitored continuously (IntelliVue MP40, Phillips, Boeblingen, Germany). After instrumentation was completed, pigs were placed in the supine position.

Experimental Protocol

Experimental protocol was designed in collaboration between the Laboratory of Medical Investigation/Anesthesiology in São Paulo, Brazil and the Laboratory of Clinical and Experimental Research of the Multidisciplinary Intensive Care Unit in Paris, France. The primary endpoint was histological changes in lung inflammation, pulmonary hemorrhage, alveolar edema, and air-space enlargement induced by PEEP titration with and without RM in pigs with acid aspiration–induced ALI. The secondary endpoints were changes in arterial oxygenation and respiratory mechanics after PEEP titration with and without RM. In animals with ALI, the rationale for PEEP titration and RM was to optimize arterial oxygenation.
Experimental Groups

Seven groups of pigs were randomly allocated. The randomization was performed by means of computer software (Microsoft Office Excel 2003, Microsoft Corporation, Redmond, WA).

Group 1: Controls (n = 5): animals with normal lung without mechanical ventilation.
Group 2: PEEP 5 cm H₂O (n = 6): animals without ALI and mechanically ventilated with PEEP 5 cm H₂O for 1 h.
Group 3: PEEP titration (n = 7): animals without ALI, mechanically ventilated with PEEP 5 cm H₂O for 1 h and treated with incremental and decremental PEEP.
Group 4: PEEP titration + RM (n = 6): animals without ALI, mechanically ventilated with PEEP 5 cm H₂O for 1 h and treated with PEEP titration and three consecutive RM at each PEEP level.
Group 5: ALI–PEEP 5 cm H₂O (n = 7): animals with ALI and mechanically ventilated for 1 h with PEEP 5 cm H₂O.
Group 6: ALI–PEEP titration (n = 8): animals with ALI, mechanically ventilated for 1 h and treated with incremental and decremental PEEP.
Group 7: ALI–PEEP titration + RM (n = 8): animals with ALI mechanically ventilated for 1 h and treated with PEEP titration and three consecutive RM at each PEEP level.

Acid Aspiration–Induced Acute Lung Injury

ALI was induced by intratracheal instillation of hydrochloric acid 0.05 N, pH 1.41, prepared by the Pharmacy Department and instilled (4 ml/kg body weight), at the level of the carina over 3 min by means of a bronchoscope (PentaxFB-15H, Montvale, NJ). Lung injury was established when PaO₂/FIO₂ ratio decreased to 25% from the baseline (blood samples collected at 5 cm H₂O of PEEP; FIO₂ of 40 %), approximately 1 h after airway hydrochloric acid instillation.²²

PEEP and Recruitment Maneuver

Sixty minutes after mechanical ventilation, in the groups 3, 4, 6, and 7, PEEP was increased step by step in 5-cm H₂O increment from initial values of 5 cm H₂O until 20 cm H₂O, while the tidal volume was held constant. The descending PEEP followed the same procedure. At each step, PEEP was kept constant for 20 min. In groups 4 and 7, recruitment maneuvers were added at each PEEP level by inflating the lungs three times, using the continuous positive airway pressure-mode on the ventilator, until an inspiratory pressure of 40 cm H₂O was reached and sustained for 20 s. Between each RM, the pigs were ventilated for 10 s, using regular volume-controlled ventilation. The experimental protocol is detailed in figure 1.
Measurements of Respiratory Parameters
Inspiratory peak pressure, plateau pressure and respiratory compliance, pulmonary shunt, and blood gases were measured at baseline, 60 min after hydrochloride acid instillation and 20 min after PEEP titration, and 5 min after the third RM. Mechanical respiratory parameters were obtained directly from the ventilator monitor and recorded on a personal computer, using Data Logger software, v.3.27.1 (Hamilton Medical AG, Bonaduz, Switzerland).

Lung Samples Collection
In the control group, anesthetized animals were immediately killed by 25 mEq of potassium chloride. In other groups, 60 min after PEEP had been returned to 5 cm H\textsubscript{2}O, animals received additional propofol and fentanyl, and the chest cavity was opened. The lungs were carefully examined and animals were killed by 25 mEq of potassium chloride. After death, the lungs were removed, weighted, and instilled step by step in the vertical position by a solution composed of formalin, ethanol, polyethylene glycol, and water until a pressure of 30 cm H\textsubscript{2}O was reached. After fixation juxta-pleural lung samples were taken from the apical, middle, and diaphragmatic lobes in healthy and sick lung areas, based on macroscopy. The blocks were processed for routine histological preparation and embedded in paraffin. The hematoxylin and eosin-stained 4-μm-thick histological sections of lung tissue from all specimens were examined and scored in a blinded fashion.

Histological Analysis
Histological analysis was performed by the Laboratory of Clinical and Experimental Research of the Multidisciplinary Intensive Care Unit and the Department of Pathology of La Pitié-Salpêtrière hospital in Paris, France, according to techniques previously described.\textsuperscript{23,24} Each histological section was examined at a magnification of ×4 and histological analysis was made for each histological field by two independent physicians who were unaware of study conditions. The presence of inflammatory cells infiltrate, alveolar wall thickening, atelectasis, hemorrhage, alveolar edema, and alveolar disruption was evaluated and recorded according to the following criteria:
- Hemorrhage: presence of red blood cells in the lung tissue and alveolar space.
- Inflammation: presence of inflammatory cells infiltrate (interstitial and alveolar) and/or alveolar wall thickening.
- Alveolar wall disruption: presence of alveolar walls rupture.
- Alveolar edema: presence of intraalveolar pink staining fluid.

The presence or absence of these alterations was semiquantitatively analyzed in each adjacent microscopic field. The presence of each category (hemorrhage/inflammation/disruption-/edema) in one field was defined as the observation of at least 25% areas of the category in this field. The percentage of each category was calculated as the number of fields of the category observed divided by the total field analyzed. The representative image of each category is illustrated in figure 2. These histological measurements were representative of the impact of different ventilatory strategies on selected lung regions of apical, middle, and diaphragmatic lobes.

Histomorphometrical Analysis
Histomorphometrical analysis was performed by the Laboratory of Clinical and Experimental Research of the Multidisciplinary Intensive Care Unit and the Department of Pathology of La Pitié-Salpêtrière hospital in Paris, France. Alveolar dimensions were measured in lungs areas remaining aerated according to a technique previously described.\textsuperscript{7,25}

Fig. 2. Representative images (magnification x4) of histological evidence of alveolar wall disruption (A), hemorrhage (B), edema (C), and inflammation (D).
Each histological section was examined at a magnification of \( \times 4 \). The alveolar area and the linear intercept were measured using an image analyzer computer system (Leica Qwin, Cambridge, United Kingdom) connected through a high-resolution color camera (JVC KYF 3 CCD; JVC, Yokohama, Japan) to an optical microscope. Mean alveolar area was determined as the average area of the aerated alveoli present on all examined fields. Mean linear intercept was defined as the mean distance between alveolar walls on 10 parallel transverse lines drawn in each examined field.

**Statistical Analysis**

The study was designed to demonstrate the effect of incremental and decremental PEEP titration with and without recruitment maneuvers on ventilator-induced lung injury. Lung injury was assessed on histological lung slices and defined according to five criteria: lung inflammation, hemorrhage, edema, alveolar wall disruption, and mean alveolar area. The primary analysis compared each of the five criteria among the three groups of pigs with acid aspiration–induced lung injury (group 5: ALI–PEEP 5 cm H\(_2\)O, group 6: ALI–PEEP titration, and group 7: ALI–PEEP titration + RM). Assumptions for the sample-size for the primary analysis were calculated using XL Stat (Addinsoft SARL, Paris, France). On the basis of an 80% statistical power, a two-sided nominal \( \alpha \) value of 0.01 (Bonferroni correction for five criteria and an actual \( \alpha \) value of 0.05) and an effect size of 2.5, eight pigs in each group would be needed.

A two-tailed hypothesis was tested in the statistical methods. Percentages of alveolar wall disruption, hemorrhage, edema and inflammation, and the differences of mean alveolar area and mean linear intercept among the groups were tested for each criterion, using linear mixed models, with group and lobes as fixed effects and animal as random effects. Pair-wise comparisons were tested using Tukey tests. A Bonferroni correction was performed, and nominal \( \alpha \) values less than 0.01 were considered as significant. Comparisons of respiratory parameters were made by one-way ANOVA for repeated measures. The regional distribution of lung inflammation and mean alveolar area in apical middle and diaphragmatic lobes was compared by a two-way ANOVA for repeated measures (group and lobe factors). For these analyses, statistical significance level was fixed at \( \alpha \) value less than 0.05. The values are reported as means ± SD or as median and 25–75% interquartile range according to the data distribution. The statistical analysis was performed using SAS (SAS Institute Inc., Cary, NC).

**Results**

**Oxygenation and Respiratory Mechanics**

In pigs without ALI, PEEP titration and PEEP titration + RM did not change Pa\(_{O_2}\), but significantly decreased respiratory compliance at PEEP 20 cm H\(_2\)O compared with baseline: 15 ± 5 (PEEP titration 20 cm H\(_2\)O) versus 26 ± 6 ml/cm H\(_2\)O (baseline), \( P \) value less than 0.05, and 17 ± 4 (PEEP titration 20 cm H\(_2\)O + RM) versus 22 ± 3 ml/cm H\(_2\)O (baseline), \( P \) value less than 0.05. Peak airway pressure and plateau airway pressure increased significantly when PEEP was 15 cm H\(_2\)O or higher, \( P \) value less than 0.05. At PEEP 20 cm H\(_2\)O, plateau airway pressure reached 35.8 ± 4.5 cm H\(_2\)O after PEEP titration and 33.8 ± 4.2 cm H\(_2\)O after PEEP titration + RM, \( P \) value less than 0.05.

In pigs with ALI, acid instillation significantly decreased Pa\(_{O_2}/FIO_2\) ratio (table 1). During PEEP titration, the maximum Pa\(_{O_2}/FIO_2\) ratio of 15 cm H\(_2\)O. However, the initial Pa\(_{O_2}/FIO_2\) ratio before ALI could not be restored. PEEP titration + RM successfully reestablished the Pa\(_{O_2}/FIO_2\) ratio to the initial baseline values for PEEP levels higher than 5 cm H\(_2\)O. After PEEP titration + RM, Pa\(_{O_2}\) decreased significantly to values less than 300 mmHg. PEEP titration and PEEP titration + RM induced significant increase in Peak airway pressure and plateau airway pressure and decrease in respiratory compliance when PEEP was 15 cm H\(_2\)O or higher.

**Lung Weight and Macroscopic Aspects**

In animals of the control group, lungs were macroscopically normal (fig. 3A). Lung lesions caused by HCl aspiration were unevenly distributed, but predominated in dependent lung areas and diaphragmatic lobes. As shown in figure 3E, lung injury was macroscopically more extended after hydrochloric acid instillation and PEEP titration. Lung weight was significantly greater in animals with ALI when compared with those without ALI (\( P < 0.01 \)); group 1 (control without ventilation) = 300 ± 44 g; group 2 (PEEP 5 cm H\(_2\)O for 1h) = 354 ± 62 g; group 3 (without ALI, PEEP titration) = 285 ± 53 g; group 4 (without ALI, PEEP titration + RM) = 360 ± 49 g; group 5 (with ALI, PEEP 5 cm H\(_2\)O) = 430 ± 77 g; group 6 (with ALI, PEEP titration) = 590 ± 51; and group 7 (with ALI, PEEP titration + RM) = 505 ± 25. In pigs with ALI, lungs submitted to PEEP titration were heavier than those undergoing ALI without PEEP titration (\( P < 0.01 \)).

**Histological and Histomorphometrical Characteristics of Lungs with and without Acid Aspiration–Induced Acute Lung Injury**

The presence of inflammatory cells infiltrate, hemorrhage, alveolar edema, and alveolar wall disruption were analyzed in 4,978 fields and compared among group 1 (control), group 2 (PEEP 5 cm H\(_2\)O), and group 5 (ALI–PEEP 5 cm H\(_2\)O). As shown in table 2, the lungs of control animals with spontaneously breathing were free of pathological findings. Mechanical ventilation with PEEP 5 cm H\(_2\)O induced alveolar wall disruption and lung inflammation. Acid aspiration–induced ALI increased significantly the proportions of disruption and inflammation and induced lung hemorrhage and alveolar edema.

Mean alveolar area and mean alveolar intercept were significantly greater in animals with ALI than in control animals (table 2).
Table 1. Respiratory Parameters in Pigs with Aspiration-induced Acute Lung Injury

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Baseline Without ALI</th>
<th>PEEP 5</th>
<th>PEEP 10†</th>
<th>PEEP 15†</th>
<th>PEEP 20†</th>
<th>PEEP 15 †</th>
<th>PEEP 10 †</th>
<th>PEEP 5 †</th>
</tr>
</thead>
<tbody>
<tr>
<td>P_{PEAK}, cm H_{2}O</td>
<td>PT</td>
<td>20.0 ± 2.5</td>
<td>29.0 ± 3.4*</td>
<td>34.6 ± 3.6*</td>
<td>39.5 ± 2.5*</td>
<td>43.4 ± 2.0*</td>
<td>43.4 ± 2.0*</td>
<td>31.0 ± 5.8*</td>
<td>33.3 ± 5.9*</td>
</tr>
<tr>
<td></td>
<td>PT + RM</td>
<td>18.2 ± 3.1</td>
<td>28.8 ± 4.0*</td>
<td>32.2 ± 3.8*</td>
<td>37.5 ± 4.7*</td>
<td>44.7 ± 4.6*</td>
<td>33.8 ± 3.8*</td>
<td>29.0 ± 2.6*</td>
<td>28.3 ± 3.1*</td>
</tr>
<tr>
<td>P_{pl}, cm H_{2}O</td>
<td>PT</td>
<td>15.5 ± 1.8</td>
<td>25.8 ± 4.2*</td>
<td>31.0 ± 3.7*</td>
<td>35.8 ± 2.6*</td>
<td>40.7 ± 3.6*</td>
<td>30.7 ± 4.0*</td>
<td>26.8 ± 5.2*</td>
<td>26.3 ± 6.2*</td>
</tr>
<tr>
<td></td>
<td>PT + RM</td>
<td>14.8 ± 1.8</td>
<td>23.2 ± 3.9*</td>
<td>27.8 ± 3.4*</td>
<td>34.3 ± 3.0*</td>
<td>41.0 ± 3.9*</td>
<td>30.5 ± 3.3*</td>
<td>25.3 ± 2.2*</td>
<td>23.8 ± 2.4*</td>
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<tr>
<td>P_{MEAN}, cm H_{2}O</td>
<td>PT</td>
<td>8.1 ± 0.6</td>
<td>9.3 ± 0.9</td>
<td>14.3 ± 0.8*</td>
<td>18.8 ± 1.2*</td>
<td>23.5 ± 1.0*</td>
<td>18.3 ± 0.8*</td>
<td>14.0 ± 1.4*</td>
<td>10.1 ± 1.8*</td>
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<tr>
<td></td>
<td>PT + RM</td>
<td>8.4 ± 1.3</td>
<td>9.9 ± 2.1</td>
<td>14.5 ± 1.4*</td>
<td>19.3 ± 1.0*</td>
<td>24.0 ± 0.9*</td>
<td>18.5 ± 1.0*</td>
<td>14.2 ± 1.2*</td>
<td>10.0 ± 1.5*</td>
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<td>Compliance, ml/cm H_{2}O</td>
<td>PT</td>
<td>25 ± 5</td>
<td>12 ± 3*</td>
<td>12 ± 3*</td>
<td>12 ± 3*</td>
<td>12 ± 2*</td>
<td>17 ± 4*</td>
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<td></td>
<td>PT + RM</td>
<td>24 ± 4</td>
<td>14 ± 5*</td>
<td>14 ± 3*</td>
<td>12 ± 2*</td>
<td>9 ± 3*</td>
<td>15 ± 3*</td>
<td>15 ± 2*</td>
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<td>P_{CO}_{2}, mmHg</td>
<td>PT</td>
<td>44.4 ± 2.5</td>
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<td>PT + RM</td>
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<td>44.2 ± 7.1</td>
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<td>42.9 ± 5.2</td>
<td>43.3 ± 8.7</td>
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<td>P_{O}<em>{2}/F</em>{IO}_{2}</td>
<td>PT</td>
<td>453 ± 44</td>
<td>327 ± 64*</td>
<td>295 ± 84*</td>
<td>357 ± 94*</td>
<td>354 ± 71*</td>
<td>366 ± 102*</td>
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<td></td>
<td>PT + RM</td>
<td>433 ± 29</td>
<td>307 ± 47*</td>
<td>383 ± 17</td>
<td>414 ± 46</td>
<td>360 ± 83</td>
<td>398 ± 82</td>
<td>361 ± 80</td>
<td>278 ± 102*</td>
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<tr>
<td>Qs/Qt, %</td>
<td>PT</td>
<td>7.4 ± 3.0</td>
<td>17.0 ± 5.4*</td>
<td>15.7 ± 5.7*</td>
<td>10.0 ± 4.5</td>
<td>7.9 ± 2.1</td>
<td>8.7 ± 4.3</td>
<td>14.0 ± 6.6</td>
<td>16.7 ± 5.1*</td>
</tr>
<tr>
<td></td>
<td>PT + RM</td>
<td>9.3 ± 3.5</td>
<td>16.0 ± 4.4*</td>
<td>9.3 ± 1.8</td>
<td>6.3 ± 1.9</td>
<td>7.5 ± 2.2</td>
<td>10.6 ± 7.8</td>
<td>11.2 ± 5.0</td>
<td>16.5 ± 4.3*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD.
*P < 0.05 compared with baseline.

Baseline without acute lung injury (ALI) = animal without ALI and ventilated with positive end-expiratory pressure (PEEP) 5 cm H_{2}O for 1 h; F_{IO}_{2} = oxygen inspired fraction; PEEP = ventilation with PEEP 5 cm H_{2}O; PT = incremental and decremental PEEP titration; PT + RM = PEEP titration associated with three consecutive recruitment maneuvers at each PEEP level; P_{pl} = peak airway pressure; P_{pl} = plateau pressure; P_{MEAN} = mean airway pressure; P_{CO}_{2} = partial pressure arterial carbon dioxide; Qs/Qt = right to left intrapulmonary shunt.

Effects of PEEP Titration and Recruitment Maneuvers on Lung Injury

In animals without ALI, significant lung inflammation was detected in pigs submitted to PEEP titration. Recruitment maneuvers did not induce additional lung inflammation (fig. 4). Mechanical ventilation–induced alveolar wall disruption was not aggravated by PEEP titration and PEEP titration + RM. Lung hemorrhage and edema were not observed after PEEP titration and PEEP titration+ RM.

In animals with hydrochloric acid-induced ALI, lung inflammation was significantly greater in pigs after PEEP titration. Recruitment maneuvers during PEEP titration did not induce further increase in lung inflammation (fig. 4). Alveolar wall disruption, edema, and hemorrhage were similar in the three groups of pigs ventilated with PEEP alone, PEEP titration and PEEP + RM. Lung inflammation was observed predominantly in diaphragmatic lobes (fig. 5).

Effects of PEEP Titration and Recruitment Maneuvers on Air-space Enlargement

In pigs with and without ALI, mean alveolar area and mean linear intercept significantly decreased after PEEP titration and PEEP titration + RM compared with PEEP 5 cm H_{2}O (fig. 6). They were smaller than in control animals. The decrease of mean alveolar area was more important in diaphragmatic lobes (fig. 5).

Discussion

The major findings are: (1) in the absence of lung injury, a 1-h period of mechanical ventilation with PEEP 5 cm H_{2}O induces significant alveolar wall disruption and inflammation, without producing hyperinflation; (2) acid aspiration produces hyperinflation, alveolar edema, hemorrhage, and inflammation predominating in diaphragmatic lung regions; (3) in the presence of acid aspiration–induced lung injury, incremental and decremental PEEP titration improves arterial oxygenation, aggravates lung inflammation, and reverses hyperinflation; and (4) recruitment maneuvers performed during PEEP titration further improve arterial oxygenation and have no additional effects on inflammation and hyperinflation.

Characteristics of the Experimental Model

Acid aspiration is one of the animal models most consistently approaching the feature of human ALI.26 As previously described,22,26,27 tracheal instillation of hydrochloric acid induces lung injury, predominantly distributed in dependent diaphragmatic lobes and characterized by lung inflammation, edema, hemorrhage, and focal loss of lung aeration where normally aerated lung regions coexist with poorly and nonaerated lung areas. When increasing transpulmonary pressure, injured areas are feebly recruited whereas aerated lung regions are hyperinflated.

Ambrosio et al.
Effects of Acid Aspiration, Mechanical Ventilation, and PEEP Titration on Lung Inflammation

Incremental and decremental PEEP trial induced a moderate increase in $\text{PaO}_2/\text{FiO}_2$ ratio, which remained below pre-ALI values associated with a significant increase in pulmonary inflammation and lung weight. To our knowledge, this is the first time that lung inflammation is quantitatively measured on histological samples representative of apical, middle, and diaphragmatic lobes. These findings confirm a previous study performed in sheep with lung lavage.\textsuperscript{24} Increasing PEEP from 2 cm H$_2$O above the lower inflection point of the inflation pressure-volume curve to the point of

Fig. 3. Gross examination of lungs in the seven groups of pigs. A, pig of the control group. B, pig without acid instillation and mechanically ventilated with positive end-expiratory pressure (PEEP) 5 cm H$_2$O for 1 h. C, pig without acid instillation and treated with PEEP titration. D, pig without acid instillation and treated with PEEP titration and three consecutive recruitment maneuvers (RMs) at each PEEP level. E, pig with acid instillation and mechanically ventilated with PEEP 5 cm H$_2$O for 1 h. F, pig with acid instillation and treated with PEEP titration. G, pig with acid instillation and treated with PEEP titration and three consecutive RMs at each PEEP level.
maximum curvature of the deflation pressure–volume curve was associated with histological inflammation predominating in dependant lung regions. Other studies have shown that high PEEP and recruitment maneuvers augment alveolar epithelium injury and decrease alveolar fluid clearance in ALI characterized by focal lung morphology. Our results strongly support the concept of applying high PEEP with caution and according to lung morphology. Interestingly, mean alveolar areas measured in aerated lung regions were smaller in diaphragmatic lobes than in apical and middle lobes (fig. 5). The predomiance of lung inflammation in diaphragmatic lobes likely explains increased lung elastance in regions remaining aerated.

Interestingly, lung inflammation was also observed in pigs without ALI submitted to mechanical ventilation with PEEP 5 cm H₂O. Five previous experimental studies have reported mechanical ventilation–induced lung inflammation in healthy animals. In mice, mechanical ventilation using a tidal volume of 8 ml/kg and a PEEP of 4 cm H₂O induced a significant lung inflammation increasing with the duration of mechanical ventilation. Increasing tidal volume to 16 ml/kg induced lung hyperinflation associated with loss of septal walls and injury of type I pneumocytes. The implication of Toll-like receptors 2 and 4 in lung inflammation was subsequently demonstrated.

In healthy piglets mechanically ventilated for 60 h using a tidal volume of 15 ml/kg without PEEP, significant increase in lung weight caused by extensive lung inflammation was observed. In healthy sheep mechanically ventilated for 2 h using tidal volumes ranging between 16 and 24 ml/kg without PEEP, lung inflammation, alveolar edema, alveolar hemorrhage, and vascular congestion were observed in dorsal lung regions. In healthy pigs mechanically ventilated for 8 h using tidal volume of 15 ml/kg and a PEEP of 3 cm H₂O, mild lung inflammation and alveolar edema were observed. When tidal volume was decreased to 6 ml/kg and PEEP increased to 10 cm H₂O, lung inflammation and alveolar edema markedly increased and were associated with extensive alveolar hemorrhage and vascular congestion.

The current study demonstrates that mechanical ventilation–induced lung inflammation was markedly aggravated by PEEP titration. Quantitatively, lung inflammation produced by PEEP titration in animals with normal lungs was greater than inflammation resulting from acid aspiration. This result indicates that PEEP titration by itself is able to injure normal lung. Recently, Hong et al. demonstrated in healthy pigs, that a tidal volume of 6 ml/kg with a PEEP of 10 cm H₂O was associated with increased inflammatory cytokines in bronchoalveolar lavage and substantial histological inflammation.

### Table 2. Histomorphometrical Characteristics of Pigs with and without Acid Aspiration-induced Acute Lung Injury

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PEEP 5</th>
<th>ALI – PEEP 5 + ALI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 6)</td>
<td>(n = 7)</td>
</tr>
<tr>
<td>No. of fields analyzed</td>
<td>1585</td>
<td>1687</td>
<td>1705</td>
</tr>
<tr>
<td>Disruption, %</td>
<td>0 (0–4.8)</td>
<td>13.7 (0–34.8)‡</td>
<td>24.5 (7.5–39.8)§</td>
</tr>
<tr>
<td>Hemorrhage, %</td>
<td>0 (0–0)</td>
<td>0 (0–5.4)</td>
<td>15.4 (7.7–28.5)‡</td>
</tr>
<tr>
<td>Edema, %</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–15.5)‡</td>
</tr>
<tr>
<td>Inflammation, %</td>
<td>0 (0–0)</td>
<td>0.4 (0–15.6)‡</td>
<td>11.3 (0–34.9)‡</td>
</tr>
<tr>
<td>MAA, µm²</td>
<td>8998 (7591–10439)</td>
<td>7890 (6365–9607)</td>
<td>10149 (8693–11922)‡</td>
</tr>
<tr>
<td>MLI, µm</td>
<td>80 (72–86)</td>
<td>76 (71–84)</td>
<td>88 (78–98)‡</td>
</tr>
</tbody>
</table>

Data are expressed as median and 25–75% interquartile. Numbers presented in the table represent the values of all microscopic fields present in histological sections of tissue blocks sampled in apical, middle and diaphragmatic lobes of all animals in each group (n = 4,978) and characterized by presence of inflammatory cells infiltrate, hemorrhage, alveolar edema and alveolar wall disruption.

* P < 0.05 vs. control. † P < 0.05 vs. PEEP 5. ‡ P < 0.05 vs. PEEP 5.

ALI – PEEP 5 = Pigs with aspiration-induced acute lung injury and submitted to 1 h mechanical ventilation with positive end-expiratory pressure (PEEP) 5 cm H₂O; Control = pigs with normal lung without mechanical ventilation; MAA= mean alveolar area; MLI = mean linear intercept; n = number of pigs in each group; PEEP 5 = pigs submitted to 1 h mechanical ventilation with PEEP 5 cm H₂O.

### Effects of Acid Aspiration and Mechanical Ventilation on Lung Hyperinflation

In healthy pigs, a 1-h period of mechanical ventilation produced alveolar wall disruption, the magnitude of which was not big enough to induce hyperinflation. After acid aspiration, the same type of mechanical ventilation (tidal volume ≤ 8 ml/kg and PEEP = 5 cm H₂O) aggravated alveolar wall disruption and significantly increased mean alveolar area in apical, middle, and diaphragmatic lobes, suggesting lung hyperinflation. The resulting air-space enlargement was entirely reversed by PEEP titration with or without recruitment maneuver. Such results are not observed in lung lavage-induced surfactant depletion. When increasing transpulmonary pressure, the collapsed lung is massively recruited and lung volume progressively returns to control values without pulmonary hyperinflation.

In a piglet model of bronchial inoculation pneumonia, a nonprotective ventilatory strategy (tidal volume = 15 ml/kg and PEEP = 0 cm H₂O) induced multiple confluent air wall disruptions and significant air-space enlargement. In the current study performed in a different model, we...
provide evidence that reducing tidal volume and increasing PEEP with or without RM, may partially or entirely prevent ventilator-induced lung hyperinflation. A similar result was previously reported in anesthetized sheep with ALI caused by bilateral lung lavage, an experimental model providing greater lung recruitability than the acid aspiration model.\textsuperscript{24}

However, the “protective effect” of PEEP titration and RM on ventilator-induced lung hyperinflation should be interpreted with caution. It was recently shown in a pig model of acid aspiration–induced lung injury,\textsuperscript{18} that a slight but significant end-expiratory lung hyperinflation and a marked and significant end-inspiratory lung hyperinflation could be detected using computed tomography after a 6-h period of protective ventilatory strategy (tidal volume = 6 ml/kg and PEEP = 14 cm H\textsubscript{2}O), preceded itself by a lung recruitment maneuver (continuous positive airway pressure

**Fig. 4.** Percentages of all microscopic fields present in histological sections of tissue blocks sampled from apical, middle, and diaphragmatic lobes of all animals in each of the six groups (n = 6,267) and characterized by inflammation, alveolar wall disruption, hemorrhage, and edema. Lung samples were obtained in three groups of pigs without ALI (A, C, E, G) and three groups of pigs with acid aspiration ALI (B, D, F, H). PEEP 5 = animals ventilated with PEEP 5 cm H\textsubscript{2}O for 1 h, PT = PEEP titration, pigs treated with incremental and decremental values of PEEP, PT + RM = pigs treated with PEEP titration plus three consecutive recruitment maneuvers during each PEEP level. ALI – PEEP 5 = pigs with ALI and ventilated with PEEP 5 cm H\textsubscript{2}O for 1 h. ALI – PT = pigs with ALI and treated with PEEP titration. ALI – PT + RM = pigs with ALI and treated with PEEP titration plus three consecutive recruitment maneuvers during each PEEP level. P value less than 0.001 at the top of the figures indicates statistically significant difference existing among the three groups using linear mixed models. Comparisons between two groups were performed using Tukey tests. *P < 0.01, PEEP 5 versus PT, § P < 0.01, PEEP 5 versus PT + RM. ALI = acute lung injury; PEEP = positive end-expiratory pressure; RM = recruitment maneuver.
of 42 cm H\textsubscript{2}O during 40–60 s). Therefore, the protective effect of PEEP titration and RM reported in our experimental study after a short period of mechanical ventilation may not persist after a longer period if end-inspiratory hyperinflation occurs at each respiratory cycle.

**Limitations**

First, the results obtained in an acid aspiration–induced experimental model may not be extrapolated to other experimental models of ALI or to critically ill patients with various causes of ALI/acute respiratory distress syndrome other than aspiration pneumonia. Second, the duration of mechanical ventilation was limited to 60 min except in group 1 (control). Detecting a significant effect on lung inflammation and hyperinflation after a short period of mechanical ventilation indicates a powerful effect. In the PEEP titration and PEEP titration + RM groups, PEEP titration and RM needed additional 2 h of mechanical ventilation. The possibility that this 2-h period of mechanical ventilation for PEEP titration and RM may induce more lung inflammation than a 1-h period cannot be ruled out. Another potential aggravating factor on lung inflammation could be that inspiratory plateau pressure was not limited during PEEP titration and PEEP titration + RM. Such a design was adopted to facilitate lung recruitment with the potential drawback of inducing volutrauma and additional lung inflammation. Furthermore, biochemical inflammatory response was not assessed. Previous experimental studies have clearly suggested a link between biochemical response and histological inflammation.\textsuperscript{23,24}

The technique used for lung fixation is a factor that could have influenced postmortem morphometry results. Although the lungs were slowly instilled 50 ml by 50 ml to reach a pulmonary volume close to the actual disease-related end-expiratory lung volume,\textsuperscript{7,25} the artifactual hyperinflation of noninjured lung areas cannot be totally ruled out. After acid...
aspiration, a part of the lung was injured, producing a complete loss of aeration, whereas other lung regions remained normally or partially aerated. The latter regions were therefore exposed to the risk of hyperinflation during the postmortem filling procedure. The causative mechanism, however, is exactly similar to the one producing mechanical ventilation–induced hyperinflation. Therefore, it seems reasonable to hypothesize that postmortem measurements of mean alveolar area and mean linear intercept were representative of \textit{in vivo} mechanical ventilation–induced lung hyperinflation.

**Clinical Consequences**

In an experimental model of acid aspiration, PEEP titration and recruitment maneuvers markedly improved arterial oxygenation. This beneficial effect was, however, associated with lung inflammation, a detrimental effect previously reported. In other words, implementing a respiratory strategy exclusively directed to improve arterial oxygenation might be associated with serious and undiagnosed deleterious effects. As a consequence, randomized multicenter studies reporting the impact of “high” PEEP versus “low” PEEP on acute respiratory distress syndrome mortality and based exclusively on the best oxygen response should be interpreted with caution: a beneficial effect of PEEP-induced alveolar recruitment might have been offset by concomitant increase in lung inflammation and hyperinflation. Finally, the current study supports the concept of best PEEP defined as a compromise between alveolar recruitment, lung inflammation, and hyperinflation, particularly in patients with a focal loss of lung aeration.

In conclusion, in this experimental model of ALI caused by acid aspiration, incremental and decremental PEEP titration aimed at optimizing arterial oxygenation, substantially increased lung inflammation. Recruitment maneuvers further improved arterial oxygenation without additional effects on inflammation and hyperinflation.

**References**


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