Spontaneous Breathing with Biphasic Positive Airway Pressure Attenuates Lung Injury in Hydrochloric Acid–induced Acute Respiratory Distress Syndrome

Jingen Xia, M.S., Heng Zhang, M.S., Bing Sun, M.D., Rui Yang, M.S., Hangyong He, M.D., Qingyuan Zhan, M.D.

ABSTRACT

Background: It has been proved that spontaneous breathing (SB) with biphasic positive airway pressure (BIPAP) can improve lung aeration in acute respiratory distress syndrome compared with controlled mechanical ventilation. The authors hypothesized that SB with BIPAP would attenuate lung injury in acute respiratory distress syndrome compared with pressure-controlled ventilation.

Methods: Twenty male New Zealand white rabbits with hydrochloric acid aspiration–induced acute respiratory distress syndrome were randomly ventilated using the BIPAP either with SB (BIPAP plus SB group) or without SB (BIPAP minus SB group) for 5 h. Inspiration pressure was adjusted to maintain the tidal volume at 6 ml/kg. Both groups received the same positive end-expiratory pressure level at 5 cm H₂O for hemodynamic goals. Eight healthy animals without ventilatory support served as the control group.

Results: The BIPAP plus SB group presented a lower ratio of dead space ventilation to tidal volume, a lower respiratory rate, and lower minute ventilation. No significant difference in the protein levels of interleukin-6 and interleukin-8 in plasma, bronchoalveolar lavage fluid, and lung tissue were measured between the two experimental groups. However, SB resulted in lower messenger ribonucleic acid levels of interleukin-6 (mean ± SD; 1.8 ± 0.7 vs. 2.6 ± 0.5; P = 0.008) and interleukin-8 (2.2 ± 0.5 vs. 2.9 ± 0.6; P = 0.014) in lung tissues. In addition, lung histopathology revealed less injury in the BIPAP plus SB group (lung injury score, 13.8 ± 4.6 vs. 21.8 ± 5.7; P < 0.05).

Conclusion: In hydrochloric acid–induced acute respiratory distress syndrome, SB with BIPAP attenuated lung injury and improved respiratory function compared with controlled ventilation with low tidal volume. (Anesthesiology 2014; 120:1441-9)

Corresponding article on page 1313. The first two authors contributed equally to this article (J.X. and H.Z.).

Submitted for publication May 6, 2013. Accepted for publication January 30, 2014. From the Department of Intensive Care Medicine, China-Japan Friendship Hospital, Beijing, P. R. China (J.X., Q.Z.); and Beijing Institute of Respiratory Medicine, Department of Respiratory and Critical Care Medicine, Beijing Chao-Yang Hospital, Capital Medical University, Beijing, P. R. China (H.Z., B.S., R.Y., H.H.).

Copyright © 2014, the American Society of Anesthesiologists, Inc. Lippincott Williams & Wilkins. Anesthesiology 2014; 120:1441-9

What We Already Know about This Topic

- Smaller tidal volumes decrease the incidence of ventilator-induced lung injury in acute respiratory distress syndrome patients

What This Article Tells Us That Is New

- Anesthetized rabbits with hydrochloric acid–induced lung injury subjected to spontaneous breathing had improved lung injury and improved respiratory function compared with controls which did not spontaneously breathe

BIPHASIC positive airway pressure (BIPAP) is based on an open lung concept and is viewed as time-cycled switching between two levels of continuous positive airway pressure.1,2 Spontaneous breathing (SB) can occur freely during each mechanical cycle phase. When SB disappears, it is equal to the pressure-controlled ventilation mode. A high level of continuous positive airway pressure and prolonged inflation time can efficiently recruit collapsed lung tissue in acute respiratory distress syndrome (ARDS).3 The other advantage of BIPAP is the preservation of unsupported SB.4 Preserved SB with BIPAP has been demonstrated to have meaningful physiological benefits, compared with controlled mechanical ventilation.4,6 However, it remains unknown whether preserved SB with BIPAP has a major impact on ventilator-induced lung injury (VILI). The main mechanisms of VILI are alveolar overdistension (volumtrauma) and/or repetitive cyclic recruitment/derecruitment of lung units (atelectrauma).7 The implications of VILI are not simply referred to the structural insults to lung tissues, but also cover the activation of inflammatory responses in the local pulmonary and systemic circulations (biotrauma),8,9 which can potentially injury the functions of other organs.10–12

Spontaneous breathing could theoretically induce lung injury. Negative pleural pressure induced by SB effort can increase intrathoracic blood volume, worsen pulmonary edema, and cause lung damage.13,14 High transpulmonary pressure15,16 and rapid respiratory rate (RR),17 followed by a strong SB effort, can also aggravate lung injury. However, many experimental18–22 and clinical studies23,24 have...
reported that preserved SB was associated with better aeration and less atelectasis in dependent lung regions, as well as less hyperinflation in nondependent lung regions in ARDS. In addition, Wrigge et al.\textsuperscript{20} and Gama de Abreu et al.\textsuperscript{21} reported that preserved SB countered cyclic alveolar collapse in an experimental model of ARDS, which might reduce the risk of atelectrauma (shear injury).

In a previous study, we reported that preserved SB could reduce lung inflammatory responses in ventilated healthy lungs, compared with controlled mechanical ventilation.\textsuperscript{25} However, it remains unknown whether SB can affect VILI in ARDS. ARDS is common in critically ill patients admitted to intensive care units. Despite the use of low tidal volume ventilation, the overall intensive care units and hospital mortality of ARDS patients remain higher than 40%.\textsuperscript{26} Because of some pathophysiological changes in ARDS lungs, such as reduced functional residual capacity (baby lung), more alveolar collapse, and consolidation in dependent lung regions and surfactant deficiency,\textsuperscript{27} lung injury is prone to aggravation during mechanical ventilation. In this study, we hypothesized that preserved unsupported SB effort with BIPAP could further reduce VILI in a hydrochloric acid aspiration-induced ARDS model, compared with pressure-controlled ventilation with a low tidal volume.

In the experimental protocol, the infusion of pipecuronium bromide was stopped, and the dose of pentobarbital sodium was gradually reduced. Based on previous studies,\textsuperscript{4,18–20} to guarantee the physiological advantages of preserved SB effort, the mandatory RR was adjusted to maintain a VT of 6 ml/kg, the mandatory RR was gradually increased to maintain a PaCO\textsubscript{2} level of 45 to 60 mmHg, positive end-expiratory pressure was set at 5 cm H\textsubscript{2}O, FIO\textsubscript{2} was set at 0.5, and inspiratory-to-expiratory ratio was set at 1:1.

In the BIPAP plus SB group, to retain SB, the infusion of pipecuronium bromide was stopped, and the dose of pentobarbital sodium was gradually reduced. Based on previous studies,\textsuperscript{4,18–20} to guarantee the physiological advantages of preserved SB during BIPAP and to avoid too strong an SB effort, the mandatory RR was adjusted to maintain MV of unsupported SB at 10 to 50% of total MV (fig. 1). The other ventilator settings were maintained the same as in the BIPAP minus SB group.

The other eight healthy rabbits comprised the control group. The control group was not mechanically ventilated and was immediately sampled after surgical intervention and sedation.

At the end of experiment, all of the animals were exsanguinated via a carotid artery, and lung tissues and hearts were harvested. Bronchoalveolar lavage (BAL) was performed in the left lower lobes. The left lung tissue was stored in liquid nitrogen for later measurement of the protein levels and messenger RNA (mRNA) expression of selected cytokines. Samples from the dorsal and ventral sections of the right lung were obtained separately. These samples were immediately fixed in 10% buffered formalin for histological analysis. The remaining right lower lung lobe was used for lung wet-to-dry weight ratio determination.

**Materials and Methods**

This study was approved by the Animal Care Committee of Capital Medical University (Beijing, China). The animals were cared for in accordance with the University standards for the care and use of laboratory animals.

**Animal Preparation**

Twenty adult male New Zealand white rabbits (1.9 to 2.5 kg) were anesthetized with 3% pentobarbital sodium (Sigma Chemical Co., St. Louis, MO) at 25 mg/kg, followed by continuous infusion at 5 to 10 mg kg\textsuperscript{-1} h\textsuperscript{-1}. Pipercuronium bromide (0.2 mg kg\textsuperscript{-1} h\textsuperscript{-1}) (Gedeon Richter Plc., Budapest, Hungary) was infused for muscle relaxation. Tracheotomies were performed, and the animals were ventilated in BIPAP mode using a Drager Evita 2dura ventilator (Drager Medical AG & Co., Lubeck, Germany). The initial ventilator settings were as follows: inspiration pressure resulting in a tidal volume (VT) of 6 ml/kg; mandatory RR was adjusted to maintain PaCO\textsubscript{2} within 35 to 45 mmHg; FIO\textsubscript{2} of 0.3; positive end-expiratory pressure of 2 cm H\textsubscript{2}O; and an inspiratory-to-expiratory ratio of 1:1. Intravenous fluid (normal saline; 8 ml kg\textsuperscript{-1} h\textsuperscript{-1}) administration remained constant to maintain a mean arterial pressure greater than 80 mmHg, and vasoactive agents were not used during the experiment.

**Experimental Protocol**

Hydrochloric acid (pH 1.0) was instilled intratracheally in each lateral position (1.5 ml/kg per side), followed by an inspiratory pause at a plateau pressure of 25 cm H\textsubscript{2}O for 5 s. Thirty minutes thereafter, if PaO\textsubscript{2}/FIO\textsubscript{2} less than 200, the ARDS model was considered stable. Otherwise, the procedure would be repeated until PaO\textsubscript{2}/FIO\textsubscript{2} reached the predefined standard.

After induction of lung injury, 20 animals were randomly ventilated in BIPAP mode, either without SB (the BIPAP minus SB group, n = 10) or with SB (the BIPAP plus SB group, n = 10) for 5 h.

In the BIPAP minus SB group, because of deterioration of lung elastance after ARDS induction, the inspiratory pressure was adjusted to maintain a VT of 6 ml/kg, the mandatory RR was gradually increased to maintain a PaCO\textsubscript{2} level of 45 to 60 mmHg, positive end-expiratory pressure was set at 5 cm H\textsubscript{2}O, FIO\textsubscript{2} was set at 0.5, and inspiratory-to-expiratory ratio was set at 1:1.

In the BIPAP plus SB group, to retain SB, the infusion of pipecuronium bromide was stopped, and the dose of pentobarbital sodium was gradually reduced. Based on previous studies,\textsuperscript{4,18–20} to guarantee the physiological advantages of preserved SB during BIPAP and to avoid too strong an SB effort, the mandatory RR was adjusted to maintain MV of unsupported SB at 10 to 50% of total MV (fig. 1). The other ventilator settings were maintained the same as in the BIPAP minus SB group.

The other eight healthy rabbits comprised the control group. The control group was not mechanically ventilated and was immediately sampled after surgical intervention and sedation.

At the end of experiment, all of the animals were exsanguinated via a carotid artery, and lung tissues and hearts were harvested. Bronchoalveolar lavage (BAL) was performed in the left lower lobes. The left lung tissue was stored in liquid nitrogen for later measurement of the protein levels and messenger RNA (mRNA) expression of selected cytokines. Samples from the dorsal and ventral sections of the right lung were obtained separately. These samples were immediately fixed in 10% buffered formalin for histological analysis. The remaining right lower lung lobe was used for lung wet-to-dry weight ratio determination.

**Measurements**

Hemodynamic, ventilatory, and blood gas variables were recorded every hour. Arterial blood gas variables were determined with an ABL 725 analyzer (Radiometer, Copenhagen, Denmark); variable measurements included pH, Pa\textsubscript{CO\textsubscript{2}}, Pa\textsubscript{O\textsubscript{2}}, HCO\textsubscript{3}{\textsuperscript{−}}, and lactic acid. An in-line pressure differential pneumotachometer (CO\textsubscript{2}SMO Plus; Novametrix Medical Systems, Wallingford, CT) was used to measure end-tidal carbon dioxide (ET\textsubscript{CO\textsubscript{2}}), gas flow, and airway pressure at the proximal end of the tracheotomy tube. MV was derived from the integrated gas flow signal. In BIPAP plus SB group,
VTs were expressed by VT of nonsupported SB (VTspont) and mandatory VT (VTmand). To compare the tidal volume between both groups, we calculated average VT (VTave), which in the case of BIPAP minus SB group equaled the VTmand; whereas for the BIPAP plus SB groups, it was the total MV divided by total RR. The ratio of alveolar dead space to tidal volume (VD/VT) was calculated by: VD/VT = (PaCO2 − ETCO2)/PaCO2.28 Static lung compliance (Cstatic) was calculated at healthy baseline, at ARDS baseline, and at the end of the experiment.25

Protein and mRNA Expression Levels of Inflammatory Mediators
Sterile normal saline (10 ml) was used to lavage the left lower lobes. After 5 s, the lavage liquid was recycled. The percentage of the return volume was 50 to 60%. Plasma was collected before ARDS induction, 2 h thereafter, and at the end of experiment. BAL and plasma samples were immediately centrifuged at 3,000 to 4,000 rpm for 15 min. Supernatant aliquots were frozen at −80°C for subsequent analysis. Interleukin (IL)-6 and IL-8 were selected. The protein level measurements of IL-6 and IL-8 were obtained using a commercial enzyme-linked immunosorbent assay kit for rabbits (BlueGene, Shanghai, China). All of the enzyme-linked immunosorbent assay procedures were performed according to manufacturer protocol. The mRNA expression levels of IL-6 and IL-8 were measured using quantitative real-time reverse transcription polymerase chain reaction, as previously described.25 As an internal control, glyceraldehyde-3-phosphate dehydrogenase primers were used for RNA template normalization.

Lung Wet-to-dry Ratio
The lungs were weighed (wet weight) and subsequently dried in a microwave at 80°C for 48 h. The final weight measurement represented the dry weight.

Lung Histopathology
The lung histopathological injury for each sample was evaluated by an independent pathologist, using the lung injury histopathology scoring system.29 Four lung injury pathomorphological changes (alveolar congestion, hemorrhage, infiltration, and aggregation of neutrophils in the airspace or vessel wall and thickness of the alveolar wall/hyaline membrane formation) were evaluated and graded on a scale from 0 to 4. The grading system was as follows: 0, minimal damage; 1, mild damage; 2, moderate damage; 3, severe damage; and 4, maximal damage. The total score for each sample was the sum of these four pathomorphological changes, and it ranged from 0 to 16. The total score of each animal was the sum of the histopathological injury score of the dorsal and ventral samples.

Statistical Analysis
Results are expressed as the means ± SDs, except for cytokine levels in BAL, lung tissue, and plasma, which are presented
**Table 1. Hemodynamics and Respiratory Measurements**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group (n = 10 per Group)</th>
<th>Before ARDS</th>
<th>After Induction of ARDS</th>
<th>Group Effect</th>
<th>Time × Group Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIPAP minus SB</td>
<td>269 ± 19</td>
<td>234 ± 28</td>
<td>227 ± 19</td>
<td>217 ± 20</td>
<td>207 ± 21# 197 ± 25# 193 ± 27# 0.702 0.021</td>
</tr>
<tr>
<td>BIPAP plus SB</td>
<td>247 ± 40</td>
<td>228 ± 30</td>
<td>224 ± 24</td>
<td>221 ± 23</td>
<td>225 ± 24 219 ± 26 211 ± 21</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIPAP minus SB</td>
<td>93 ± 7</td>
<td>84 ± 12</td>
<td>83 ± 9</td>
<td>90 ± 9</td>
<td>95 ± 11# 93 ± 10</td>
</tr>
<tr>
<td>BIPAP plus SB</td>
<td>88 ± 10</td>
<td>91 ± 7</td>
<td>89 ± 10</td>
<td>86 ± 11</td>
<td>88 ± 14 90 ± 13 88 ± 14 0.767 0.002</td>
</tr>
<tr>
<td>Plateau pressure (cm H₂O)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIPAP minus SB</td>
<td>8.7 ± 1.0</td>
<td>15.4 ± 1.2</td>
<td>16.2 ± 1.7</td>
<td>15.7 ± 1.3</td>
<td>15.8 ± 1.2 15.9 ± 1.2 16.0 ± 1.1 0.316 0.841</td>
</tr>
<tr>
<td>BIPAP plus SB</td>
<td>8.5 ± 1.0</td>
<td>15.2 ± 2.0</td>
<td>15.3 ± 1.3</td>
<td>15.3 ± 1.2</td>
<td>15.1 ± 1.1 15.1 ± 1.2 15.2 ± 1.1</td>
</tr>
<tr>
<td>Mean airway pressure (cm H₂O)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIPAP minus SB</td>
<td>5.3 ± 0.50</td>
<td>10.7 ± 0.6</td>
<td>10.6 ± 0.8</td>
<td>10.3 ± 0.7</td>
<td>10.4 ± 0.6 10.5 ± 0.6 10.5 ± 0.5 0.361 0.841</td>
</tr>
<tr>
<td>BIPAP plus SB</td>
<td>5.3 ± 0.5</td>
<td>10.5 ± 1.0</td>
<td>10.2 ± 0.6</td>
<td>10.1 ± 0.6</td>
<td>10.1 ± 0.6 10.1 ± 0.6 10.1 ± 0.6</td>
</tr>
<tr>
<td>VTave (ml/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIPAP minus SB</td>
<td>6.2 ± 0.8</td>
<td>6.5 ± 0.9</td>
<td>6.2 ± 0.6</td>
<td>6.1 ± 0.2</td>
<td>6.2 ± 0.3 6.1 ± 0.3 5.8 ± 0.3 0.994 0.519</td>
</tr>
<tr>
<td>BIPAP plus SB</td>
<td>5.9 ± 0.9</td>
<td>6.1 ± 0.9</td>
<td>6.4 ± 0.8</td>
<td>6.1 ± 1.1</td>
<td>6.0 ± 1.5 6.1 ± 1.3</td>
</tr>
<tr>
<td>VTspont (ml/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIPAP minus SB</td>
<td>— — — — —</td>
<td>2.7 ± 1.9</td>
<td>4.2 ± 0.7</td>
<td>4.4 ± 1.0</td>
<td>4.4 ± 0.5 4.5 ± 1.2</td>
</tr>
<tr>
<td>BIPAP plus SB</td>
<td>— — — — —</td>
<td>— — — —</td>
<td>— — — —</td>
<td>— — — —</td>
<td>— — — —</td>
</tr>
<tr>
<td>Total RR (breaths/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIPAP minus SB</td>
<td>55.4 ± 10.5</td>
<td>75.0 ± 10.8</td>
<td>75.0 ± 13.0</td>
<td>75.4 ± 11.1* 76.0 ± 11.1* 76.0 ± 11.1* 76.0 ± 11.1* 0.031 0.181</td>
<td></td>
</tr>
<tr>
<td>BIPAP plus SB</td>
<td>48.4 ± 11.3</td>
<td>72.8 ± 9.9</td>
<td>69.4 ± 12.1</td>
<td>65.0 ± 11.4 64.2 ± 7.8 61.8 ± 8.5 63.6 ± 6.3</td>
<td></td>
</tr>
<tr>
<td>RRspont (breaths/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIPAP minus SB</td>
<td>— — — — —</td>
<td>16.6 ± 14.6</td>
<td>22.8 ± 9.1</td>
<td>23.0 ± 6.7</td>
<td>20.6 ± 8.5 21.1 ± 7.3</td>
</tr>
<tr>
<td>BIPAP plus SB</td>
<td>— — — — —</td>
<td>— — — —</td>
<td>— — — —</td>
<td>— — — —</td>
<td>— — — —</td>
</tr>
<tr>
<td>MVtot(L/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIPAP minus SB</td>
<td>1.06 ± 0.19</td>
<td>1.52 ± 0.25*</td>
<td>1.67 ± 0.34*</td>
<td>1.67 ± 0.37* 1.65 ± 0.37* 1.62 ± 0.37</td>
<td></td>
</tr>
<tr>
<td>BIPAP plus SB</td>
<td>1.02 ± 0.26</td>
<td>1.49 ± 0.26</td>
<td>1.48 ± 0.3</td>
<td>1.34 ± 0.23 1.40 ± 0.30# 1.29 ± 0.30# 1.35 ± 0.25#</td>
<td></td>
</tr>
<tr>
<td>Cstastic(ml/cm H₂O)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIPAP minus SB</td>
<td>2.94 ± 0.35</td>
<td>1.68 ± 0.28</td>
<td>— — —</td>
<td>— — — —</td>
<td>1.60 ± 0.27</td>
</tr>
<tr>
<td>BIPAP plus SB</td>
<td>3.09 ± 0.57</td>
<td>1.69 ± 0.39</td>
<td>— — —</td>
<td>— — — —</td>
<td>2.10 ± 0.64</td>
</tr>
<tr>
<td>Arterial pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIPAP minus SB</td>
<td>7.37 ± 0.08</td>
<td>7.29 ± 0.08</td>
<td>7.26 ± 0.06</td>
<td>7.26 ± 0.05 7.26 ± 0.03 7.25 ± 0.03 7.24 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>BIPAP plus SB</td>
<td>7.38 ± 0.08</td>
<td>7.29 ± 0.06</td>
<td>7.28 ± 0.07</td>
<td>7.29 ± 0.08 7.27 ± 0.08 7.27 ± 0.06 7.24 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>Paco₂ (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIPAP minus SB</td>
<td>44.6 ± 5.3</td>
<td>58.6 ± 11.7</td>
<td>55.6 ± 9.9</td>
<td>54.9 ± 8.1   54.6 ± 8.0 57.2 ± 6.4 59.0 ± 5.2</td>
<td></td>
</tr>
<tr>
<td>BIPAP plus SB</td>
<td>43.6 ± 8.4</td>
<td>55.7 ± 12.1</td>
<td>50.9 ± 8.0</td>
<td>47.5 ± 12.3 49.4 ± 8.0 51.4 ± 9.3 54.4 ± 11.0</td>
<td></td>
</tr>
<tr>
<td>PaO₂/FIO₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIPAP minus SB</td>
<td>394 ± 71</td>
<td>176 ± 54</td>
<td>235 ± 55</td>
<td>246 ± 65     233 ± 60 218 ± 67 204 ± 64 0.330 0.721</td>
<td></td>
</tr>
<tr>
<td>BIPAP plus SB</td>
<td>408 ± 57</td>
<td>166 ± 28</td>
<td>284 ± 75</td>
<td>292 ± 98     286 ± 59 284 ± 98 261 ± 93 0.135 0.241</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD.

*P < 0.05 BIPAP plus SB group vs. BIPAP minus SB group at the same time; #P < 0.05 vs. baseline in the same group.

ARDS = acute respiratory distress syndrome; BIPAP = biphasic positive airway pressure; Cstastic = static lung compliance; MVtot = total minute ventilation; Paco₂ = partial pressure of carbon dioxide; PaO₂/FIO₂ = ratio of partial pressure of arterial oxygen to faction of inspired oxygen concentration; RR = respiratory rate; RRspont = respiratory rate of unsupported spontaneous breathing; SB = spontaneous breathing; VTave = average tidal volume; VTspont = tidal volume of unsupported spontaneous breathing.
as medians and interquartile ranges. The Kolmogorov–Smirnov test was used to assess normal distribution of the data. Comparison of continuous data between two experimental groups with each other was performed using the two-tailed Student t test. Paired t tests were used to evaluate differences of continuous data within the same group toward the baseline. Differences among groups were analyzed using one-way ANOVA. Changes in the measurement of hemodynamics, ventilatory parameters, static lung compliance, and blood gas were analyzed using two-way repeated measures ANOVA with group and time. Multiple comparisons were adjusted by the post hoc multiple Bonferroni procedure.

To compare cytokine levels in BAL, lung tissue and plasma among different groups, the Kruskal–Wallis test was applied. A P value less than 0.05 level of significance was set. All of the analyses were performed with SPSS software (SPSS Inc., Chicago, IL), version 13.0.

Results

Hemodynamics, Ventilatory, Gas Exchange, and Respiratory Mechanics

The heart rate, mean arterial blood pressure, and lactic acid levels were similar between the groups during the entire experiment (table 1). There were also comparable plateau pressure and average VT between the experimental groups (table 1). The average percentage of MV of unsupported SB relative to total MV in the BIPAP plus SB group was 36.5%. The PaCO₂ level in all of the animals was determined to be less than 60 mmHg. Moreover, the BIPAP plus SB group presented a lower total RR (RRtot) and lower total MV (MVtot) compared with the BIPAP minus SB group (table 1). At the same time, the BIPAP plus SB group showed a lower VD/VT after randomization \((P = 0.018; \text{fig. 2})\). SB showed a trend toward improving PaO₂/FİO₂ in the BIPAP plus SB group; however, the difference in PaO₂/FİO₂ between the groups was not statistically significant \((P = 0.160; \text{table 1})\). After induction of ARDS, the static lung compliance (Cstatic) was decreased by approximately 50% in the experimental groups, and the difference of Cstatic between BIPAP plus SB group and BIPAP minus SB group was not statistically significant after 5 h of ventilation (table 1).

Assessment of Inflammatory Mediators in Plasma, BAL Fluid, and Lung Tissue

The levels of IL-6 and IL-8 in plasma did not differ significantly between the BIPAP plus SB group and the BIPAP minus SB group over the course of the experiment \((P > 0.05)\). The levels of IL-6 and IL-8 in BAL fluid and lung tissue were significantly higher in the experimental groups than in the control group. We did not find a significant difference between the two experimental groups (table 2).

Table 2. Protein Levels of Inflammatory in Plasma, BALF, and Lung Tissue

<table>
<thead>
<tr>
<th>Inflammatory Mediators</th>
<th>Group</th>
<th>Plasma (pg/ml)</th>
<th>Lung Tissue (pg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline 2 h 5 h</td>
<td>Nondependent Lung Region</td>
</tr>
<tr>
<td>IL-6</td>
<td>Control (n = 8)</td>
<td>— — —</td>
<td>28.4 (26.1, 34.4)</td>
</tr>
<tr>
<td></td>
<td>BIPAP plus SB (n = 10)</td>
<td>82.9 (73.1, 108.1)</td>
<td>79.0 (59.7, 113.4)</td>
</tr>
<tr>
<td></td>
<td>BIPAP minus SB (n = 10)</td>
<td>112.6 (88.1, 121.8)</td>
<td>105.2 (74.6, 120.6)</td>
</tr>
<tr>
<td>IL-8</td>
<td>Control (n = 8)</td>
<td>— — —</td>
<td>213.6 (201.0, 229.6)</td>
</tr>
<tr>
<td></td>
<td>BIPAP plus SB (n = 10)</td>
<td>213.6 (201.0, 229.6)</td>
<td>219.3 (187.7, 260.6)</td>
</tr>
<tr>
<td></td>
<td>BIPAP minus SB (n = 10)</td>
<td>237.6 (216.2, 272.2)</td>
<td>259.0 (212.5, 268.8)</td>
</tr>
</tbody>
</table>

Values are median (quartiles).

BALF = bronchoalveolar lavage fluid; BIPAP = biphasic positive airway pressure; IL = interleukin; SB = spontaneous breathing.
However, the BIPAP minus SB group showed higher mRNA expression levels of IL-6 and IL-8 than the BIPAP plus SB group (IL-6, 1.8 ± 0.7 vs. 2.6 ± 0.5, \( P = 0.008 \); IL-8, 2.2 ± 0.5 vs. 2.9 ± 0.6, \( P = 0.014 \)), and both experimental groups had higher mRNA expression levels of inflammatory mediators than the control group (fig. 3).

**Lung Wet-to-dry Ratio**

The wet-to-dry ratios in the BIPAP minus SB group (6.8 ± 1.0) and in the BIPAP plus SB group (6.3 ± 0.5) were significantly higher compared with the healthy control group (5.0 ± 0.3). The difference between the two experimental groups was not statistically significant \( (P = 0.149) \).

**Lung Histopathological Injury**

When compared with the BIPAP minus SB group, the BIPAP plus SB group presented with less lung damage, less alveolar hemorrhage, less congestion, and less infiltration of neutrophils. The BIPAP minus SB group showed more alveolar collapse, more inflammatory cell infiltration, greater thickness of the alveolar wall, more alveolar congestion, and greater interstitial edema with hyaline membrane formation (fig. 4). The histopathological lung injury scores for the nondependent and dependent lung regions were increased in both experimental groups compared with healthy control group. The histopathological lung injury scores for the nondependent lung regions were comparable between two experimental groups; however, the histopathological lung injury scores for the dependent lung regions were higher in the BIPAP minus SB group compared with the BIPAP plus SB group. The total lung injury score was also higher in the BIPAP minus SB group than BIPAP plus SB group and control group (fig. 5).

**Discussion**

In this experimental model of ARDS, we demonstrated that preserved SB with BIPAP not only improved gas exchange, but it also significantly reduced the mRNA expression levels of selected inflammatory mediators in lung tissue, and it attenuated lung histopathological injury compared with controlled protective mechanical ventilation.

We selected a hydrochloric acid aspiration–induced lung injury model to mimic ARDS. This model is regarded as a form of direct lung injury, and it is characterized by epithelial barrier disruption, pulmonary hypertension, and increased lung elastance induced by alveolar collapse, flooding, and reduced production of surfactant, and it is similar to that observed in human ARDS, induced by the aspiration of gastric contents.\(^3\)\(^0\) The main feature of BIPAP is that SB can be allowed during any phase of the mechanical cycle, so it is easy to maintain comparable levels of ventilator support between BIPAP with SB and without SB.\(^3\)\(^1\) We selected positive end-expiratory pressure at 5 cm H\(_2\)O in both group because the hemodynamic goal was difficult to maintain with higher positive end-expiratory pressure in our preliminary observations.

**SB, Gas Exchange, and Lung Elastance**

In agreement with other experimental\(^1\)\(^8\)\(^–\)\(^2\)\(^2\) and clinical reports,\(^4\)\(^,\)\(^2\)\(^3\)\(^,\)\(^2\)\(^4\) we found that BIPAP plus SB was associated with better gas exchange compared with BIPAP minus SB group, with comparable VT and plateau pressure. In addition, both groups had similar \( \text{PaCO}_2 \) levels; however, SB was associated with decreased MV, lower total RR, lower the ratio of dead space to tidal volume. Different mechanisms have been postulated to explain the observed improvement in gas exchange: (1) SB increases lung aeration to dependent regions, recruits atelectasis in dependent lung regions,\(^1\)\(^9\)\(^–\)\(^2\)\(^2\) and reduces hyperinflation in nondependent lung regions;\(^2\)\(^2\) and (2) SB is associated with better blood perfusion and cardiac output to nondependent lung regions, resulting in greater homogeneity of lung ventilation to perfusion.\(^4\)\(^,\)\(^2\)\(^1\)\(^,\)\(^3\)\(^2\) Unfortunately, we did not find that SB significantly improved oxygenation as in other articles. The reason for this difference might be the different experimental ARDS models and lung injury levels.

---

Fig. 3. The messenger RNA (mRNA) expression levels of interleukin (IL)-6 and IL-8 in the lung tissue after 5-h mechanical ventilation (control group, \( n = 8 \); biphasic positive airway pressure [BIPAP] plus spontaneous breathing [SB] group, \( n = 10 \); BIPAP minus SB group, \( n = 10 \)). *\( P < 0.05 \), versus control group; #\( P < 0.05 \) BIPAP minus SB group versus BIPAP plus SB group.
Low tidal volume ventilation in ARDS patients was reported to attenuate VILI and decrease mortality significantly.33 Unfortunately, during low tidal volume–controlled ventilation, lung hyperinflation34 and atelectasis35 have still been observed. It has been proved that SB can reduce atelectasis and hyperinflation, compared with controlled mechanical ventilation; therefore, preserved SB might further affect VILI in ARDS lungs ventilated with low tidal volumes.

Few studies have explored the relationship between SB and VILI in ARDS. In hydrochloric acid aspiration–induced ARDS, we showed that SB with BIPAP was associated with lower mRNA levels of IL-6 and IL-8 in lung tissue and less evidence of lung histopathological injury. These findings were similar to the observations in other studies with indirect lung injury models. Spieth et al.36 found that pressure support ventilation and noisy ventilation could attenuate lung inflammatory responses in surfactant depletion–induced lung injury, compared with pressure-controlled ventilation. Saddy et al.37 also showed that assisted modes (assisted pressure-controlled ventilation and BIPAP) reduced VILI in araquat-induced lung injury. However, the lung injury was less significantly severe than in our study. The average ratio of arterial oxygen partial pressure to fraction of inspired oxygen was only 302 mm Hg in the study by Saddy et al. It is important to note that different mechanisms might be associated with these findings. These mechanisms include some of the following. First, increased transpulmonary pressure, induced by spontaneous negative pleural pressure, in dependent lung regions favored more aeration to dorsal lung tissue, recruited less aerated lung tissue, and attenuated lung tissue open and collapse cycling.20,22 Second, SB distributed more tidal ventilation to dependent lung regions,19 which could have reduced hyperinflation in nondependent lung regions.22 Third, there were improved end-expiratory lung volume20,24 and improved lung mechanical stress distribution. Fourth, SB could increase end-expiratory lung volume in ARDS lung,20,24 therefore, lung strain (the ratio of tidal volume to end-expiratory lung volume), a main determinant of VILI,38–40 might be also reduced. Fifth, negative pleural pressure increased lymphatic drainage.41 Sixth, SB improved the redistribution of pulmonary blood flow.21,32

In contrast to our study, some authors13,42,43 have reported that preserved SB contributed to lung edema and lung
damage. The discrepancies in SB efforts and experimental models across different studies should be considered. Strong SB effort can significantly increase transpulmonary pressure, which is the main determinant of lung inflation, at end-inspiration and end-expiration, a consequence of which is lung tissue overstretching.\(^4\)\(^{,}\)\(^8\)-\(^{20}\) Moreover, strong SB efforts can increase the RR and MV, which can, in turn, exacerbate lung injury.\(^17\) Yoshida \(\text{et al.}\)\(^{12}\) found that even when plateau pressure was limited to less than 30 cm H\(_2\)O, strong SB could worsen lung injury because of greater transpulmonary pressure (>33 cm H\(_2\)O), more MV and a higher RR. In this study, to avoid strong SB efforts, we strictly limited the MV of unsupported SB to less than 50% of total MV, based on previous experimental and clinical studies.\(^4\)\(^{,}\)\(^8\)-\(^{20}\) In addition, SB can result in additional increases in transpulmonary pressure and inspiratory volume, so we limited tidal volume to less than 8 ml/kg to minimize lung overstretching during our whole experiment. It is worth mentioning that preserved SB aggravated VILI in an intra-abdominal hypertension model,\(^43\) perhaps because abdominal hypertension affected diaphragm movement and decreased transpulmonary pressure.

Our study had several limitations. First, because we used an ARDS model induced by hydrochloric acid, we cannot extend our data to other ARDS models or to more complex clinical scenarios. Second, the level of SB effort that is best for lung protection remains unknown. We arbitrarily limited the MV of unsupported SB to less than 30 cm H\(_2\)O, strong SB could worsen lung injury because of greater transpulmonary pressure (>33 cm H\(_2\)O), more MV and a higher RR. In this study, to avoid strong SB efforts, we strictly limited the MV of unsupported SB to less than 50% of total MV, based on previous experimental and clinical studies.\(^4\)\(^{,}\)\(^8\)-\(^{20}\) Third, SB is allowed in many modes of ventilation and triggers assisted or supported breaths. In our study, only unsupported SB in BIPAP was studied. Therefore, the results of this study cannot be extended to other modes of assisted mechanical ventilation. Fourth, although the average VT was comparable between both groups, unsupported SB in BIPAP plus SB group was associated with lower VT (VTs-point) than mandatory VT in BIPAP minus SB group. Accordingly, we cannot exclude the low VT of unsupported SB reduced VILI in BIPAP plus SB group. Fifth, we did not directly measure the intensity of breathing efforts, such as transpulmonary pressure, which could perhaps clearly explain the difference in ventilation between the two experimental groups. Sixth, we obtained BAL samples after exsanguination. The levels of inflammatory mediators might have been affected by the physiologic changes caused by exsanguination. Finally, in the controlled mechanical ventilation group, we used more muscle relaxants (pipercuronium bromide) and deeper levels of sedation (pentobarbital sodium). We cannot exclude the possibility that these drugs affected the lung inflammatory response.

In conclusion, in this hydrochloric acid aspiration-induced lung injury model, we found that preserved SB during BIPAP attenuated lung inflammation responses and lung histopathological injury, as well as improved gas exchange compared with controlled protective mechanical ventilation. Clinical studies are necessary to investigate the effects of preserved SB on lung injury during lung-protective ventilation.

**Acknowledgments**
The authors thank Xiaoji Guo, B.S., and Zhenhong Liu, B.S. (both from Beijing Chao-Yang Hospital, Capital Medical University, Beijing, China), for their skillful technological assistance during the entire experiment.

This work was supported by grant number 30971311 from the National Natural Science Foundation of China (Beijing, China) and by grant number 2009C13522105 from the National Basic Research Program of China (Beijing, China; to Dr. Zhan).

**Competing Interests**
The authors declare no competing interests.

**Correspondence**
Address correspondence to Dr. Zhan: Department of Intensive Care Medicine, China-Japan Friendship Hospital, 2 Yinghua Dongjie, Hepingli, Chaoyang District, Beijing 100029, P. R. China. dr.zhan@163.com. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue.

**References**
10. Ranieri VM, Giunta F, Suter PM, Slutsky AS: Mechanical ventilation as a mediator of multisystem organ failure in acute respiratory distress syndrome. JAMA 2000; 284:43–4

Anesthesiology 2014; 120:1441-9


1449 Xia et al.