Differential effects of mechanical ventilatory strategy on lung injury and systemic organ inflammation in mice

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Differential effects of mechanical ventilatory strategy on lung injury and systemic organ inflammation in mice. Am J Physiol Lung Cell Mol Physiol 285: L710–L718, 2003. First published May 16, 2003; 10.1152/ajplung.00044.2003.—Patients with acute respiratory distress syndrome are at increased risk for developing multiorgan system dysfunction. The goal of this study was to establish an in vivo murine model to assess the differential effects of ventilation-protective strategies on the development of acute lung injury and systemic organ inflammation. C57B/6 mice were randomized to mechanical ventilation (MV) with conventional, high (17 ml/kg) or protective, low (6 ml/kg) tidal volume (VT) after intratracheal hydrochloric acid or no intervention. Mean arterial pressure was continuously monitored during MV and did not differ between groups. After 4 h, lung injury was assessed by measurement of wet/dry lung weight, lung lavage protein concentration and cell count, and histology. Concentration of IL-6, TNF-α, VEGF, and VEGF receptor-2 (VEGFR2) was measured in lung, liver, kidney, and heart. Results were compared with control, spontaneously breathing mice. Lung injury and altered pulmonary cytokine expression were not detected after MV of healthy mice with low or high VT. Although MV did not significantly alter IL-6 or TNF-α in systemic organs, VEGF concentration significantly increased in liver and kidney. After acid aspiration, mice ventilated with high VT manifested lung injury and increased IL-6 and VEGFR2 in lung, liver, and kidney, whereas VEGF increased only in liver and kidney. MV with low VT after acid aspiration attenuated lung injury, both IL-6 and VEGFR2 expression in lung and systemic organs, and hepatic, but not renal, increased VEGF. Our data suggest that MV strategy has differential effects on systemic inflammatory changes and thus may selectively predispose to systemic organ dysfunction.

multiorgan system dysfunction; interleukin-6; tumor necrosis factor-α; vascular endothelial growth factor; vascular endothelial growth factor receptor-2

EXCESSIVE ALVEOLAR DISTENTION associated with mechanical ventilation can cause acute lung injury, manifested by increased vascular permeability, alterations in lung tissue mechanics, and increased production of inflammatory mediators (3, 10, 14, 49, 50, 54). Although prior results in smaller studies have been controversial (4, 42), a larger, multicenter trial recently demonstrated that mechanical ventilation with lower tidal volumes (VT), more closely approximating spontaneous ventilation, significantly decreased mortality in patients with acute respiratory distress syndrome (ARDS), when compared with ventilation with higher, conventional VT (2).

Many prior animal models evaluating ventilator-associated lung injury have focused on determining the effects of VT higher than those used conventionally to ventilate patients in the intensive care unit (ICU), as these conventional ventilatory strategies do not cause significant injury during short periods of mechanical ventilation of healthy animals, whereas more recent studies have focused on the mechanisms of the protective effects of decreased ventilatory stretch in acute lung injury (15). Several of these prior studies have suggested that proinflammatory cytokines and chemokines are released into the circulation with high VT ventilation (19, 48), although this finding remains controversial (51). Clinically, however, it has also been recognized that patients with ARDS are at increased risk for systemic multiorgan failure (41).

We were therefore interested in determining whether a protective VT strategy attenuates production of mediators that could predispose to both lung injury and remote organ dysfunction and whether systemic organs are differentially susceptible to the effects of VT on inflammation. Our goal in this study was to establish an in vivo murine model of ventilator-associated lung injury, mimicking the protective ventilatory protocols currently in use in the ICU, and to evaluate the effects of mechanical ventilatory strategy on expression of mediators causing vascular leak and/or inflammation, in the lung and in organs remote to the lung. Understanding the molecular and cellular consequences of mechanical ventilation and defining specific pathways mediating the beneficial effects of protective ventilatory protocols will lead to the development of more refined strategies to prevent ventilator-associated injury and associated nonpulmonary organ dysfunction in critically ill patients with respiratory failure.

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METHODS

Preparation
Male C57B/6 mice (6–9 wk old) were utilized for these studies. Animals were anesthetized with acepromazine (10 mg/ml)-ketamine (100 mg/ml) (1:1, 0.03 ml ip). After endotracheal intubation, mechanical ventilation was initiated (Harvard Apparatus, Boston, MA), and pancuronium bromide (1 mg/kg ip) was administered. Additional anesthetic was given throughout the protocol as needed. The femoral artery was cannulated for measurement of systemic arterial pressure and arterial blood gas analysis at the termination of the experimental protocol. Heparinized saline was constantly infused (0.4 μl/min) to maintain patency of the femoral arterial line. All of the experiments described in this manuscript were approved by the Animal Care and Use Committee at the Johns Hopkins University School of Medicine.

Protocols

Effects of V T on lung injury in healthy mice. After anesthesia, mice were randomized to mechanical ventilation with either conventional, high (high V T, 17 ml/kg, n = 14) or protective, low (low V T, 6 ml/kg, n = 12) VT. Respiratory frequency was set at 150 or 300 breaths/min for high and low VT groups, respectively. These ventilatory parameters were based on plethysmographic estimates of V T (5–15 ml/kg) and respiratory frequency (120–300 breaths/min) in awake, spontaneously ventilating mice (46, 47). Preliminary studies were performed to ensure that the minute ventilation chosen for each experimental condition would maintain Pco2 in a normal range (25–35 mmHg) (38) during the first 30 min of the mechanical ventilatory period. Because FlO2 requirements are usually increased in mechanically ventilated patients, we chose to study the effects of VT in mice ventilated with FlO2 of 1.0. All of the experimental measurements presented here were performed in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals.

After 4 h, separate groups of lungs were excised for measurement of wet/dry lung weight (n = 4/group), snap-frozen in liquid nitrogen (n = 4/group), and fixed for histological evaluation (n = 4/group), or bronchial lavage was performed (n = 4–6/group). In the groups where lungs were snap-frozen, both kidneys and the liver were also rapidly excised and snap-frozen in liquid nitrogen for measurement of cytokine concentration. Arterial blood gas measurements were made via an automated blood gas analyzer (Instrumentation Laboratories, Lexington, MA) in all mice except those undergoing bronchial lavage or intratracheal fixation. Results were compared with mice spontaneously ventilating room air (control, n = 14).

Effects of V T on lung injury after acid aspiration. In a concurrent, second series of experiments to determine whether the effects of mechanical ventilation were altered by the administration of a concurrent inflammatory stimulus, mice received intratracheal hydrochloric acid (HCl, 0.2 N diluted with PBS to pH = 1.5) instilled in 20-μl increments for a total of 80 μl. The animals were then ventilated for 4–5 min with a standard protocol (V T of 12.5 ml/kg, frequency 150 breaths/min) to achieve similar distribution of HCl. As above, FlO2 was maintained at 1.0 throughout the experiment, and PEEP of 3 cmH2O was added. Animals were randomized to 4 h of mechanical ventilation with high (high V T + HCl, n = 13) or low (Low V T + HCl, n = 12) VT, using the same ventilatory parameters described above. After 4 h of mechanical ventilation, lung injury was assessed in separate groups of mice by measurement of bronchial lavage protein and cell count (n = 4–5/group), lung wet/dry wt ratio (n = 4/group), or histological evaluation (n = 4/group), or the lungs, kidneys, liver, and heart were snap-frozen for measurement of cytokine concentration (n = 4/group). Terminal arterial blood gas measurements were made as described above, and results were compared with mechanically ventilated and spontaneously breathing animals from the first series of experiments.

To evaluate the potential contribution of respiratory acidosis associated with low VT on ventilator-associated injury, an additional group of mice was ventilated with conventional VT following HCl administration, but respiratory frequency was decreased to 90–100/min to match minute ventilation in the low VT + HCl group. After 4 h, animals were killed, and either lung lavage protein concentration and cell count (n = 3) or lung wet/dry weight (n = 3) was determined.

Measurements

Assessment of acute lung injury. Acute lung injury was assessed by analysis of bronchial lavage protein concentration and cell counts, measurement of lung wet/dry wt ratios, and histological evaluation. Bronchial lavage was performed as described by Walters et al. (53). In brief, 0.5 ml of saline (37°C) was instilled through the endotracheal tube, and then the fluid was slowly withdrawn. After the amount of fluid recovered was recorded, an aliquot of lavage fluid was diluted 1:1 with trypan blue (GIBCO-BRL) for estimation of total cell count by a hemocytometer (Hauser Scientific). The remainder of the lavage fluid was centrifuged, and the supernatant was analyzed at −20°C for measurement of protein concentration, which was determined by comparison with BSA standards. TNF-α, IL-6, and VEGF protein expression was determined with commercially available ELISA kits (R&D Systems, Minneapolis, MN). By comparison with BSA standards, TNF-α, IL-6, and VEGF protein expression was determined with commercially available sandwich ELISA kits (R&D Systems, Minneapolis, MN).

Samples were then resolved by SDS polyacrylamide gel electrophoresis as previously described by Laemmli (22). After electrophoresis, the proteins were transferred onto polyvinylidene difluoride membranes, and membranes were stained with the rabbit anti-human antibodies against VEGF receptor-2 (VEGFR2/KDR; Santa Cruz Biotechnology, Santa Cruz, CA). This antibody recognizes 195- and 235-kDa forms of VEGFR2. After washing away the primary antibody, we used horseradish-conjugated avidin secondary antibody for visualization. To control for differences in protein concentration between samples or loading errors, we stripped and reprobed blots for β-actin expression.

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Statistical Analysis

Results presented represent means ± SE. Differences between groups in arterial blood gas parameters, lung wet/dry weight, lung lavage protein concentration and cell count, and IL-6, VEGF, and TNF-α concentration were compared by one-way analysis of variance. Repeated-measures analysis of variance was used for the comparison of mean arterial and peak airway pressure data. When significant variance ratios were obtained, least-significant differences were calculated to allow comparison of individual group means. Differences were considered significant for \( P \leq 0.05 \).

RESULTS

Effects of VT on Lung Injury in Healthy Mice

As shown in Fig. 1A, peak airway pressure averaged 18.4 ± 4.5 and 6.9 ± 1.0 mmHg in the high and low VT groups, respectively. There were no significant differences in mean arterial pressure between the groups throughout 4 h of mechanical ventilation (Fig. 1B). Similarly, pulmonary vascular permeability, assessed by measurement of lung wet/dry wt ratio (Fig. 2A), did not differ between healthy mice ventilated with high or low VT and was not increased over values obtained from spontaneously ventilating control mice. Although bronchoalveolar lavage (BAL) protein concentration did not change significantly after 4 h of high or low VT ventilation of healthy animals, however, lavage protein concentration increased significantly with high VT ventilation after acid aspiration, and this increase was completely attenuated by low VT ventilation in acid-injured mice. Values represent means ± SE.

Effects of VT on Lung Injury After Acid Aspiration

Shown in Fig. 1A, peak airway pressure during the first 30–45 min of mechanical ventilation was slightly higher in acid-injured lungs compared with healthy mice, regardless of VT. As in healthy mice, VT did not
alter mean arterial pressure during 4 h of mechanical ventilation (Fig. 1B). However, in contrast to healthy mice, VT did alter manifestations of lung injury following acid aspiration. Pulmonary vascular permeability, evaluated by estimation of lung wet/dry wt ratio (Fig. 2A) and lung lavage protein concentration (Fig. 2B), increased significantly after acid aspiration in mice ventilated with high VT, compared with control animals or healthy mice ventilated with high VT. Shown also in Fig. 2, low VT ventilation after acid aspiration completely attenuated the increased wet/dry lung weight and lavage protein concentration seen in the high VT + HCl group. Total cell count in bronchial lavage fluid was also significantly lower in the low VT + HCl group compared with high VT + HCl (Fig. 3B). Absolute cell counts in lung lavage were lower in all mice mechanically ventilated after acid aspiration compared with control, spontaneously ventilated mice, either secondary to dilutional effects or HCl-mediated cellular degradation. Histological evaluation confirmed the presence of pulmonary edema following acid aspiration in mice ventilated with high, but not low, VT. As shown in Fig. 4 microscopically, this injury was manifest by the accumulation of fluid in the perivascular space and proteinaceous material in air spaces.

Arterial blood gas analysis demonstrated a significant respiratory acidosis in mice ventilated with low VT for 4 h after acid aspiration, compared with healthy mice ventilated with low VT, as well as mice ventilated with high VT following HCl administration (Table 1). Also shown in Table 1, PO2 did not differ after 4 h of mechanical ventilation in the low VT + HCl group. All anesthetized, mechanically ventilated mice exhibited a comparable, mild metabolic acidosis independently of VT or the administration of HCl.

Because it has been suggested that respiratory acidosis may attenuate ventilator-associated lung injury (5), we ventilated an additional group of HCl-treated mice with high VT but decreased respiratory frequency (F = 90–100 breaths/min) to allow the development of hypercapnia. Although matching minute ventilation did not achieve as significant a degree of respiratory acidosis in this group (pH 7.15 ± 0.03, PCO2 47 ± 7 mmHg) as in low VT + HCl mice, values for lung wet/dry weight (5.55 ± 0.05) and lung lavage protein concentration (1.39 ± 0.33 mg/ml) and cell count (3.2 ± 1.1* 104 cells/ml) were comparable with those seen in the high VT + HCl group and remained significantly elevated (P ≤ 0.05 for all three parameters) compared with low VT + HCl animals.

**Cytokine Concentration**

Shown in Table 2, concentrations of IL-6 in the lung, liver, or kidney were not significantly altered by high or low VT ventilation in healthy mice, compared with control, spontaneously ventilating animals. In contrast, IL-6 was significantly increased in the lung, liver, and kidney of mice ventilated with high VT after HCl administration, whereas low VT ventilation after acid aspiration significantly attenuated increased IL-6 concentrations in all three of these organs. Unlike hepatic and renal measurements, cardiac IL-6 concentrations in acid-injured mice were not significantly altered by mechanical ventilation with either high or low VT.

Also shown in Table 2, VEGF concentration tended to increase in lungs from mechanically ventilated animals compared with control but did not appear to vary as a function of delivered VT, and differences did not achieve statistical significance (P = 0.09). Interestingly, there was a suggestion of a negative correlation between lung VEGF concentration and arterial PCO2 (R2 = 0.58, P = 0.08) following 4 h of mechanical ventilation in mice receiving intratracheal HCl, such that VEGF concentration tended to decrease as PCO2 increased, but this relationship did not achieve statistical significance.

<table>
<thead>
<tr>
<th>Condition</th>
<th>pH</th>
<th>PCO2</th>
<th>PO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>High VT</td>
<td>7.28 ± 0.03</td>
<td>28.4 ± 2.8</td>
<td>276 ± 43</td>
</tr>
<tr>
<td>Low VT</td>
<td>7.18 ± 0.07</td>
<td>52.9 ± 12</td>
<td>228 ± 39</td>
</tr>
<tr>
<td>High VT + HCl</td>
<td>7.29 ± 0.04</td>
<td>23.4 ± 4.5</td>
<td>301 ± 47</td>
</tr>
<tr>
<td>Low VT + HCl</td>
<td>7.00 ± 0.07*</td>
<td>85.8 ± 12.6†</td>
<td>189 ± 42</td>
</tr>
<tr>
<td>P value</td>
<td>0.006</td>
<td>0.002</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Values are means ± SE. VT, tidal volume. Significant differences in individual group means for arterial blood gas parameters were determined by calculation of least significant differences when significant variance ratios were obtained. Post hoc analysis demonstrated significant differences in individual group means for the Low VT + HCl group with all other groups for both pH(*) and PCO2(†).
In contrast to the lung, VEGF protein concentration increased significantly in the liver and kidney of healthy mice after both high and low VT ventilation, compared with control. As in healthy animals, VEGF concentration in kidney was significantly increased after 4 h of either high or low VT ventilation in acid-injured lungs. In contrast, low VT attenuated the increase in liver VEGF protein concentration seen in animals ventilated with high VT following acid aspiration. Mechanical ventilation with either high or low VT did not alter cardiac VEGF concentration following acid aspiration. Unlike the lung, VEGF expression in the liver or kidney did not correlate with arterial PCO2 ($R^2 = 0.15$, $P = 0.45$ for liver; $R^2 = 0.14$, $P = 0.48$ for kidney). However, there was a significant positive correlation between VEGF and IL-6 expression in both the liver and kidney after mechanical ventilation of acid-injured mice ($R^2 = 0.61$, $P = 0.003$ for liver; $R^2 = 0.32$, $P = 0.05$ for kidney). Mechanical ventilation with either high or low VT after acid aspiration had no significant effect on cardiac VEGF concentration.

TNF-α levels were below the limits of detection for the assay in all organs from spontaneously ventilating mice. Similarly, no TNF-α was detected in lungs from healthy mice ventilated with either high or low VT for 4 h or from the liver, kidney, or heart in any mechan-

![Fig. 4. Ventilation with high VT after acid aspiration led to accumulation of proteinaceous material in air spaces (A, bottom) and formation of perivascular edema, marked by arrows (B, bottom). These changes were not seen in control lungs or lungs from mice ventilated with low VT following HCl. Results are representative of 4 experiments for each condition (hematoxylin and eosin stain, magnification $\times 200$, bar = 50 µm).](image)

Table 2. Expression of inflammatory mediators following 4 h of mechanical ventilation

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Condition</th>
<th>Lung</th>
<th>Liver</th>
<th>Kidney</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6, pg/mg protein</td>
<td>Control</td>
<td>9 ± 2</td>
<td>1.3 ± 0.9</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>High VT</td>
<td>47 ± 11</td>
<td>14 ± 3</td>
<td>24 ± 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low VT</td>
<td>22 ± 8</td>
<td>17 ± 8</td>
<td>17 ± 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High VT + HCl</td>
<td>209 ± 26*</td>
<td>49 ± 16*</td>
<td>78 ± 35*</td>
<td>12 ± 9</td>
</tr>
<tr>
<td></td>
<td>Low VT + HCl</td>
<td>100 ± 25*</td>
<td>9 ± 5</td>
<td>10 ± 11</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$P &lt; 0.001$</td>
<td>$P = 0.004$</td>
<td>$P = 0.017$</td>
<td>$P = 0.16$</td>
</tr>
<tr>
<td>VEGF, pg/mg protein</td>
<td>Control</td>
<td>104 ± 8</td>
<td>11 ± 2</td>
<td>31 ± 7</td>
<td>35 ± 1</td>
</tr>
<tr>
<td></td>
<td>High VT</td>
<td>193 ± 60</td>
<td>23 ± 1*</td>
<td>41 ± 1*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low VT</td>
<td>190 ± 42</td>
<td>35 ± 6*</td>
<td>46 ± 5*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High VT + HCl</td>
<td>141 ± 30</td>
<td>23 ± 3*</td>
<td>41 ± 5*</td>
<td>39 ± 10</td>
</tr>
<tr>
<td></td>
<td>Low VT + HCl</td>
<td>140 ± 20</td>
<td>18 ± 2</td>
<td>42 ± 3*</td>
<td>25 ± 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$P = 0.09$</td>
<td>$P = 0.0004$</td>
<td>$P = 0.05$</td>
<td>$P = 0.39$</td>
</tr>
<tr>
<td>TNF-α, pg/mg protein</td>
<td>Control</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>High VT</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td></td>
<td>Low VT</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High VT + HCl</td>
<td>16 ± 9</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low VT + HCl</td>
<td>8 ± 10</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$P = 0.27$</td>
<td></td>
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</tbody>
</table>

Values are means ± SE. *$P < 0.05$ vs. other groups; †$P = 0.05$ vs. low VT + HCl; ND, none detected.
ically ventilated animals. Three of four mice ventilated with high VT following HCl administration had detectable levels of TNF-α in lung homogenate (mean concentration 16 ± 9 pg/mg protein), whereas only one of four mice ventilated with low VT after acid aspiration had measurable amounts of pulmonary TNF-α (mean concentration 8 ± 10 pg/mg protein).

Although VT did not appear to influence VEGF expression, Western blot analysis suggests that VEGFR2 expression did vary with delivered VT after acid aspiration. Shown in Fig. 5, VEGFR2 was upregulated in the lung, liver, and kidney of mice ventilated with high VT after HCl administration, compared with the low VT + HCl and control groups. As with VEGF, changes in VEGFR2 expression were not seen in the heart. No differences in VEGFR2 expression were seen following mechanical ventilation of healthy mice (data not shown).

**DISCUSSION**

Several prior studies have suggested that excessive alveolar distention leads to the development of acute lung injury (3, 10, 14, 49, 50, 54) and may promote a proinflammatory state that causes a predisposition to multiple organ system failure (19, 41, 48). Most of these earlier studies demonstrated lung injury in healthy animals only if VT was significantly higher than that considered conventional in clinical ICUs. On the other hand, a recent multicenter clinical trial reported that mechanical ventilation of patients with ARDS with a low VT strategy (4–8 ml/kg) significantly reduced mortality and increased ventilator-free days when compared with ventilation with traditional, high VT strategy. Low VT ventilation under these circumstances significantly attenuated evidence of both increased pulmonary vascular permeability and alveolar inflammation. These findings are similar to a recent report that ventilation with a VT of 6 or 3 ml/kg offered significant protection from acid aspiration lung injury in rats, compared with a traditional, 12 ml/kg VT strategy (15). In this prior study, acid aspiration lung injury was allowed to mature for 2 h before the initiation of mechanical ventilation, leading to a more pronounced degree of acute lung injury, and circulating markers of both endothelial and epithelial injury were reduced in the low VT groups.

In our study, differences in lung injury between high and low VT acid-injured mice were not explained by altered hemodynamics, as mean arterial pressure did not differ between the groups. Both decreased cyclic stretch (49) and acidosis (5) have been suggested as mediators of protection from acute lung injury induced by mechanical ventilation. Low VT ventilation following acid aspiration was associated with a significant respiratory acidosis compared with the high VT + HCl and low VT groups. Pco2 also increased significantly in mice mechanically ventilated after acid aspiration with a high VT strategy but decreased respiratory frequency, compared with the high VT + HCl group, without concomitant attenuation of lung injury. However, because we were unable to achieve as severe a degree of respiratory acidosis in this latter group, we cannot exclude contributions of both acidosis and decreased ventilatory stretch to the protective effects of low VT ventilation in this model. All of the mechanically ventilated mice in this study developed a comparable, mild metabolic acidosis, perhaps related to relative hypotension, induced by the anesthetic agents and/or increased pleural pressures secondary to positive pressure ventilation.

**Fig. 5.** Western blot analysis demonstrated increased expression of VEGF receptor-2 (VEGFR2) in lung, liver, and kidney of mice ventilated with high VT following acid aspiration. These differences were significantly attenuated by low VT ventilation. Results are representative of 4 experiments for each condition.
The mechanisms by which increased cyclic stretch generate injury, or decreased cyclic stretch affords protection from injury, have recently been the focus of intensive investigation (3, 5, 11, 15, 19, 50). Our results suggest that lung concentration of IL-6 increased significantly if mice were ventilated with a high V_T following acid aspiration, compared with healthy mice subjected to high V_T ventilation, or low tidal ventilation of animals following HCl aspiration. This change in IL-6 expression was mirrored by a trend toward increased lung expression of TNF-α. IL-6 is generally considered a proinflammatory, injurious cytokine, although its role may depend on the type of lung injury (39). In vitro studies have demonstrated that IL-6 causes increased endothelial permeability (13, 25), and several investigators have noted a correlation between plasma IL-6 and outcome in ARDS (55) and sepsis (1, 56). In addition, IL-6 may alter neutrophil deformability (43) and surface L-selectin expression (44), thereby potentially increasing both pulmonary neutrophil sequestration and demargination of circulating neutrophils.

The present study confirms the findings of several previous studies in isolated lungs from healthy animals, which demonstrated increased release of multiple cytokines and chemokines, including TNF-α and IL-6, into lung perfusate or bronchoalveolar lavage (BAL) fluid (19, 48, 50) as V_T was increased. On the other hand, other investigators could not find increased proinflammatory cytokines in BAL fluid or plasma following high V_T ventilation of healthy or injured lungs (35, 51). We did not measure increased IL-6 or TNF-α in lungs from healthy mice ventilated with high or low V_T, suggesting that acid aspiration in some way primed the lung to increase cytokine expression in response to increased ventilatory stretch.

No significant differences in pulmonary concentrations of VEGF, a potent mediator of increased vascular permeability, were seen as a function of V_T or acid aspiration. VEGF protein concentration in lung tissue homogenates tended to increase in all mechanically ventilated animals compared with spontaneously breathing, control mice. Because lung epithelial cells represent the primary source of VEGF production in the lung, we might predict VEGF concentration would decrease following acid aspiration, as a result of epithelial injury. Interestingly, there was a suggestion of a negative correlation between VEGF protein expression in lung homogenates and P_{CO_2}, such that VEGF expression decreased as P_{CO_2} increased. This is in contrast to a recent study by D’Arcangelo and colleagues (12), which demonstrated evidence of increased expression of VEGF in response to acidosis in bovine aortic endothelial cells in vitro. A relationship between VEGF expression and acidosis was not seen for liver or kidney VEGF expression from the same mice. Because VEGF was measured in whole tissue homogenates, this may reflect differing volumes of cell compartments expressing VEGF in these organs (27). Alternatively, it is possible that acidosis elicits tissue-specific responses. Because VEGF is a significant mediator of increased vascular permeability, any attenuation of pulmonary VEGF expression by acidosis suggests a potential mechanism by which low V_T ventilation might limit ventilator-associated lung injury.

Although we were unable to detect an effect of V_T on lung VEGF concentration, pulmonary VEGFR2 expression did increase with high V_T ventilation after acid aspiration. Although expression of IL-6 (31, 32) and TNF-α (31, 32, 52, 57) was not consistently upregulated by cyclic stretch in vitro, expression of both VEGF (18, 24, 28, 34, 45, 58) and VEGFR2 (45) increased in response to stretch in multiple cell types both in vitro and in vivo. In addition, in vitro studies suggest that IL-6 and TNF-α both upregulate VEGF expression (9, 16), and TNF-α may upregulate (17) or downregulate (33) VEGFR expression.

In addition to demonstrating that ventilatory strategy altered pulmonary cytokine concentrations, our results suggest that V_T delivered during mechanical ventilation following an inflammatory lung insult altered cytokine expression in systemic organs. IL-6 concentration significantly increased in both the liver and kidney of mice ventilated with a high V_T strategy following acid aspiration, whereas low V_T ventilation completely attenuated systemic IL-6 expression. Similarly, increased hepatic and renal VEGFR2 expression was attenuated by low V_T ventilation in acutely injured lungs. This was also true of hepatic VEGF expression, whereas kidney VEGF expression increased comparably with both high and low V_T ventilation. These findings suggest the release of humoral factor(s) from inflamed lungs subjected to increased ventilatory stretch, which selectively activate proinflammatory pathways at sites remote to the lung. Interestingly, mechanical ventilation of healthy mice led to increased VEGF concentration in both the liver and kidney, even in the absence of lung injury. The mechanism of this response is not known and will be investigated in future studies. Our data are supported by the recent report by Choi et al. (6), which demonstrates increased serum VEGF concentration in response to high (20 ml/kg) V_T ventilation in healthy rats, although serum VEGF levels in spontaneously ventilating controls are not presented in that study. Also of note, no significant changes in cardiac IL-6, VEGF, or VEGFR2 expression were found in mechanically ventilated mice, suggesting that systemic tissues may respond differentially to the effects of mechanical ventilation. Because of its anatomic location in the thorax, the heart is exposed to different mechanical forces during positive pressure ventilation, and it is interesting to speculate as to whether this may account for our findings.

Our data also suggest that expression of VEGF correlated with IL-6 expression in both liver and kidney of mice mechanically ventilated after acid aspiration. We suspect this may represent a common stimulus regulating the expression of these proteins, rather than a causal relationship between increased IL-6 and VEGF expression. IL-6 and VEGF have both been suggested as mediators of increased permeability and/or vascular...
remodeling in a number of disorders, including metastatic cancer (30, 37), ovarian hyperstimulation syndrome (36), Castleman’s disease (20), and POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes) syndrome (23). In many of these studies, IL-6 and VEGF expressions were significantly linked. Both IL-6 and VEGF may promote a procoagulant state, by increasing expression of tissue factor on endothelial cells and/or monocytes (7, 8, 21, 26, 29, 40). Increased tissue factor expression is thought to play a significant role in the development of multiorgan system failure in sepsis and acute lung injury (1, 55, 56). Of note, prior studies suggest that IL-6 and VEGF regulate both endothelial barrier dysfunction and tissue factor expression by different mechanisms. This suggests the possibility that IL-6 and VEGF might act synergistically to potentiate lung injury and/or systemic organ dysfunction.

In summary, we have established a model of ventilator-associated lung injury in intact mice and demonstrated that a short period of mechanical ventilation with a conventional, high VT strategy causes both increased pulmonary vascular permeability and hepatic and renal, but not cardiac, inflammation. This effect was dependent on presence of a localized pulmonary inflammatory stimulus (acid aspiration) and did not occur after mechanical ventilation of healthy mice. Low VT ventilation after acid aspiration significantly attenuated lung injury and pulmonary IL-6 and VEGF expression but had no effect on pulmonary VEGF. In addition, ventilation of acid-injured lungs with a protective VT strategy attenuated increased hepatic and renal IL-6 and VEGF expression but reduced VEGF expression only in the liver. These data suggest that mechanical ventilatory strategy may differentially mediate inflammation in systemic organs, raising the possibility of discrepant end-organ susceptibility to the harmful effects of mechanical ventilation.

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DISCLOSURES

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REFERENCES


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