Interactive effects of mechanical ventilation and kidney health on lung function in an in vivo mouse model

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Dodd-o JM, Hristopoulos M, Scharfstein D, Brower R, Hassoun P, King LS, Becker P, Liu M, Wang W, Hassoun HT, Rabb H. Interactive effects of mechanical ventilation and kidney health on lung function in an in vivo mouse model. Am J Physiol Lung Cell Mol Physiol 296: L3–L11, 2009. First published October 10, 2008; doi:10.1152/ajplung.00030.2008.—We hypothesized that the influence of acute kidney injury (AKI) on the sensitivity of the lung to an injurious process varies with the severity of the injurious process. Thus, we thought that AKI would exacerbate lung injury from low degrees of lung trauma but attenuate lung injury from higher degrees of lung trauma. C57BL/6 mice underwent AKI (30-min kidney ischemia) or sham surgery, followed at 24 h by 4 h of spontaneous breathing (SB), mechanical ventilation with low tidal volume (7 ml/kg, LTV), or mechanical ventilation with high tidal volume (30 ml/kg, HTV). Compared with LTV, median bronchoalveolar lavage (BAL) protein leak was significantly lower with SB and greater with HTV in both sham and AKI mice. Compared with LTV, median Evans blue dye-labeled albumin extravasation in the lungs (L-EBD) was also significantly lower with SB and greater with HTV. L-EBD showed a significant interaction between ventilatory mode and kidney health, such that AKI attenuated the L-EBD rise seen in HTV vs. LTV sham mice. An interaction between ventilatory mode and kidney health could also be seen in BAL neutrophil number (PMN). Thus, AKI attenuated the BAL PMN rise seen in HTV vs. LTV sham mice. These data support the presence of a complex interaction between mechanical ventilation and AKI in which the sensitivity of the lung to trauma varies with the magnitude of the trauma and may involve a modification of pulmonary neutrophil activity by AKI.

ACUTE LUNG INJURY (ALI) is a bilateral pulmonary inflammatory process characterized by hypoxia in the absence of heart failure (4) and is associated with a 35–40% mortality rate (28). Several investigators (27), including our own group, have now demonstrated that positive pressure ventilation cannot only exacerbate ALI initiated by non-ventilator stimuli (13), but can independently cause ventilation-induced ALI (VILI) in animals with previously uninjured lungs (24). We recently observed that, compared with spontaneously breathing mice, mice ventilated for 2 h with 20 ml/kg tidal volumes demonstrated greater Evans blue-labeled albumin extravasation. This injury, which is not present following 2 h of mechanical ventilation with 7 ml/kg tidal volumes, appears to be mediated through iNOS as it is blocked by aminoguanidine treatment and is not observed in iNOS-deficient mice (24).

In addition to direct lung trauma from inhalation or mechanical ventilation, lung injury can result from extrapulmonary processes. For example, visceral ischemia reperfusion injury has classically been studied as an extrapulmonary cause of ALI (16, 32). Because the incidence of acute kidney injury (AKI) has been reported to be 5–7% in hospitalized patients (15, 30), and because the mortality rate of ALI increases from 40–80% when it is associated with AKI (22), there is a strong interest in exploring the relationship between AKI and lung function. To this end, we previously showed that bilateral kidney ischemia-reperfusion leads to increased pulmonary vascular permeability, lung inflammation, and downregulation of pulmonary epithelial sodium channels and aquaporin-5 water channels in rats without prior lung injury (17, 26). The complexity of AKI effects on ALI was recently demonstrated by Zarbock et al. (35), where bilateral kidney ischemia-reperfusion injury reduces the arterial-alveolar oxygen gradient in mice exposed to intratracheal HCl instillation followed by 2 h of mechanical ventilation (10 ml/kg, FIO2 0.21).

We tested the hypothesis that the influence of AKI on the sensitivity of the lung to an injurious process varies with the severity of the injurious process. To create graded injury in the lungs, we exposed mice to either spontaneous breathing (SB), mechanical ventilation (MV) with 7 ml/kg (LTV), or MV with 30 ml/kg (HTV). We utilized a mouse model of AKI that has been well characterized (3, 6, 7). We compared oxygenation, Evans blue-labeled albumin extravasation in the lung (L-EBD), bronchoalveolar lavage (BAL) protein, and BAL cellularity in spontaneously breathing mice and mice exposed to 4 h of MV with 7 or 30 ml/kg.

METHODS

Mice. The Johns Hopkins University Institutional Animal Care and Use Committee approved all animal protocols. Male C57BL/6 mice (Charles River), 8–10 wk old, were utilized. All animals were maintained under specific pathogen-free conditions.

Animal protocol: mouse AKI and sham. We utilized an established mouse model of AKI (3, 6, 7). Following anesthesia (pentobarbital sodium, 75 mg/kg), a baseline weight was obtained on each mouse. Through a midline abdominal incision, the renal pedicles were exposed by blunt dissection. A microvascular clamp (Roboz Surgical...
Instrument) was placed on each renal pedicle for 30 min. The clamp was then removed, and the wounds were sutured. During the procedure, the animals were hydrated with warm saline, and a heating lamp was used to maintain a constant temperature (36–37°C). Sham-operated mice underwent the same procedure without clamping of the renal pedicle. The animals were then allowed to recover, with free access to food and water.

Animal protocol: mechanical and spontaneous ventilation. Twenty-two hours following the onset of renal reperfusion or sham surgery, mice were anesthetized (pentobarbital sodium, 75 mg/kg), weighed, and endotracheally intubated (20-gauge angiocath). Mice were placed supine, with their legs taped to the surgical surface. A suture tightened around the trachea prevented air leak between the endotracheal tube and trachea. Volume-controlled ventilation (LTV = 7 ml/kg baseline weight, HTV = 30 ml/kg baseline weight) using either a Harvard MiniVent ventilator (LTV group) or a Harvard mouse ventilator model 687 (HTV group) was achieved with fractional inspired oxygen (FiO2) of 0.21 and a rate of 140/min in each group. Dead space was added to the HTV group to achieve a pH near 7.27; the pH achieved by spontaneously breathing mice at the end of 4 h of anesthesia and vascular instrumentation in pilot studies. Airway pressure (minimal, peak, and mean) was measured continuously in the expiratory limb of the breathing circuit. The right internal jugular vein was cannulated for administration of fluid and Evans blue dye-labeled albumin. The left femoral artery was cannulated for arterial blood gas analysis. A rectal temperature probe was placed, and the mice were maintained at a constant temperature (36–37°C).

In spontaneously breathing mice, the protocol differed from that of the mechanically ventilated mice only in that: 1) the spontaneously breathing mice received a smaller dose of anesthetic (pentobarbital sodium, 50 mg/kg); 2) the spontaneously breathing mice were not intubated; and 3) airway pressure was not measured.

Volume resuscitation. Pilot studies demonstrated that survival in the HTV group could be improved with volume resuscitation. All mice except the LTV, AKI group therefore received 16 ml·kg⁻¹·h⁻¹ continuous infusion of normal saline solution for the first 2 h of the ventilation protocol. Lactated Ringer replaced normal saline as the resuscitation fluid in the LTV, AKI group, as pilot studies demonstrated that this allowed the pH of this group to more closely approximate the pH of the other groups. Pilot studies also demonstrated that mice consistently lost weight during the 22 h following the initial surgery (AKI and sham). It was assumed that a large portion of this was from dehydration. In addition to the 16 ml·kg⁻¹·h⁻¹ infusion described above, all mice received additional intravenous resuscitation fluid (1 ml fluid/g weight loss) over the first 1 h of the ventilation protocol to replace volume presumed to have been lost to dehydration.

Microvascular albumin leak. Evans blue dye in 4% albumin was injected (20 mg/kg) into the right internal jugular vein over 10 min beginning 25 min before death. After 4 h of ventilation, the right and left lungs were excised and separately weighed. The left lung was homogenized in formamide (1 ml formamide/100 mg lung, Sigma Chemical) and incubated for 18 h at 60°C. The samples were centrifuged (5,000 rpm × 30 min), and 200-μl aliquots of supernatant were placed in 96-well plates. Optical density, measured with a microplate reader (Organon Teknika, Durham, NC), was reported as “arbitrary units.” We confirmed that the relationship of EBD concentration and optical density at 620 nm was linear (R = 0.999) through the range of absorbance values encompassed by our lung samples by constructing a seven-point standard curve (9, 10, 24).

Determination of BAL protein and total cell counts. Left lung BAL was performed by directed intratracheal injection of 750 μl of PBS solution followed by gentle aspiration. This process was performed twice, with the recovered fluid processed for protein and cell count as previously described (25).

Blood gas determination. Whole lung obtained from the femoral artery catheter immediately before experiment termination was evaluated by an automated blood gas analyzer (ALB80 FLEX Blood Gas Analyzer; Radiometer America, Westlake, OH).

Statistics. Due to data skewness and zero values for some outcomes, we used robust nonparametric rank-based procedures to perform hypothesis tests and estimate treatment effects. To evaluate whether graded injury to the lungs was induced by ventilatory mode, we tested, within the AKI and sham groups, for an increase in mean arterial pressure among treatment groups throughout the 4-h treatment period. Peak airway pressure and mean airway pressure were higher in the high tidal volume (HTV) group vs. the low tidal volume (LTV) group, although it did not differ between acute kidney injury (AKI) and sham surgery. Neither peak airway pressure nor mean airway pressure changed significantly over the 4-h experimental period; n = 5–14 per group.

Table 1. Effect of ventilatory mode and AKI on mean arterial blood pressure, peak airway pressure, and mean airway pressure

<table>
<thead>
<tr>
<th>Ventilation Strategy</th>
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<tr>
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<tr>
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<td>AKI</td>
<td>56 ± 4</td>
<td>59 ± 2</td>
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<tr>
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There was no significant difference in mean arterial pressure among treatment groups throughout the 4-h treatment period. Peak airway pressure and mean airway pressure were higher in the high tidal volume (HTV) group vs. the low tidal volume (LTV) group, although it did not differ between acute kidney injury (AKI) and sham surgery. Neither peak airway pressure nor mean airway pressure changed significantly over the 4-h experimental period; n = 5–14 per group.
ing trend among the SB, LTV, and HTV arms for the pH, PaO2, HCO3, L-EBD, and BAL protein outcomes, using the Jonckheere-Terpstra test (18). For all outcomes, Akritas’ (2) procedure was used to test for an interaction between surgery (sham vs. AKI) and the ventilatory (SB, LTV, HTV) modes. Since there is no variation in the neutrophil (PMN) and lymphocyte responses for the SB arm, the interaction was evaluated between surgery and the MV modes. When the interaction test yielded a \( P \) value less than or equal to 0.15 (19), we stratified effects by renal health and by ventilatory mode using Wilcoxon rank-sum procedures. To stratify by renal health, differences [along with exact 99% confidence intervals (CI)] in the median values for LTV vs. SB, HTV vs. LTV, and HTV vs. SB, were determined separately in the sham and AKI mice. To stratify by ventilatory mode, differences (along with exact 99% confidence intervals) in the median values for sham vs. AKI mice were determined separately in the SB, LTV, and HTV. If the interaction test yielded a \( P \) value greater than 0.15, then the effects of renal health or of ventilatory mode were determined overall. To determine the overall effect of ventilatory mode, pairwise differences in medians (along with 99% confidence intervals) were estimated between LTV and SB, between LTV and HTV, and between HTV and SB, collapsed over surgery groups. To determine the overall effect of kidney health, pairwise differences in medians (along with 99% confidence intervals) were determined between sham and AKI, collapsed over ventilatory models. For interpretation purposes, we will consider \( P \) values that are less than 0.01, between 0.01 and 0.02, and between 0.02 and 0.05 as strong, moderate, and weak evidence, respectively, against the null hypothesis.

RESULTS

Hemodynamics and airway pressures. Mechanically ventilated mice had similar blood pressures regardless of tidal volume administered or prior exposure to AKI. In these mice, AKI did not alter airway pressures, although mice ventilated with high tidal volumes had higher airway pressures (mean and peak) than those ventilated with low tidal volumes (Table 1). In no group did we observe a change over time in peak or mean airway pressure measured.

**pH, \( \text{PaCO}_2 \), and \( \text{HCO}_3 \).** Figure 1A shows that there is no evidence to suggest trends in pH with mode of MV in sham \((P = 0.21)\) or in AKI \((P = 0.31)\) mice and that there are no significant interactions between ventilation and surgery. However, there is moderate evidence that kidney injury status alters pH, as median pH was significantly higher in sham vs. AKI mice (Table 2). Figure 1B shows that \( \text{PaCO}_2 \) changes as a function of ventilatory mode for both the sham \((P = 0.001)\) and AKI \((P = 0.0009)\) mice. There is no evidence of an interaction between ventilation and surgery. There is strong evidence that median \( \text{PaCO}_2 \) is lower in mice ventilated with LTV vs. SB, and still lower in mice ventilated with HTV vs. LTV (Table 2). With regard to \( \text{HCO}_3 \) (Fig. 1C), there is no evidence of a difference among ventilatory modes for AKI mice \((P = 0.14)\). By contrast, there is some suggestion that, in sham mice, \( \text{HCO}_3 \) is lower in the LTV vs. SB group, and still

![Fig. 1. The effect of ventilatory mode and of kidney health on arterial pH, arterial \( \text{PaCO}_2 \), and arterial \( \text{HCO}_3 \). A: arterial blood pH values. B: arterial blood \( \text{PaCO}_2 \) (Torr) values. C: arterial blood \( \text{HCO}_3 \) (mmol/l) values. Symbols are values of individual mice exposed to 1 of 2 kidney interventions [sham surgery (*) or acute kidney injury (AKI; \( \times \))] and to 1 of 3 ventilatory modes (SB, spontaneous breathing; LTV, low tidal volume mechanical ventilation; HTV, high tidal volume mechanical ventilation). Horizontal line, median value for the group; \( n = 5-14 \) per group.](http://ajplung.physiology.org/doi/10.1152/ajplung.00071.2008)
lower in the HTV vs. LTV group \((P = 0.03)\). When exploring stratified effects (as suggested by an interaction \(P\) value of 0.10 or less, see Table 2), there is strong evidence within the SB and HTV groups that median HCO3 is higher in sham vs. AKI mice. For sham mice, there is strong evidence of a higher median HCO3 in the SB group than LTV and HTV groups; no effect of HCO3 was seen when comparing the HTV and LTV groups.

**L-EBD.** As shown in Fig. 2, the median L-EBD is greater in LTV vs. SB mice and in HTV vs. LTV mice for both the sham \((P < 0.0001)\) and AKI \((P = 0.0001)\) groups. There was moderate evidence of an interaction \((P = 0.016)\) between ventilatory mode and kidney health (see Table 3). For sham mice, there is strong evidence of higher median L-EBD in LTV vs. SB and in HTV vs. LTV mice; the effect is larger in the latter comparison. For AKI mice, there is strong evidence of higher median L-EBD in LTV vs. SB mice, but much weaker evidence of an effect for HTV vs. LTV mice. These data suggest that AKI attenuates the deleterious effect of HTV, and, in contrast, increases injury in the SB and LTV mice.

**Oxygen.** Figure 3 shows that, depending on the absence or presence of AKI, there is a difference in the effect of MV. In sham mice, there is moderate evidence for a trend towards increasing oxygenation when comparing SB vs. LTV vs. HTV \((P = 0.02)\). This is due mainly to the fact that there is strong evidence for higher oxygenation in the HTV mice compared with those ventilated by SB or LTV in these mice. In sham mice, there is no difference in oxygenation between those ventilated with SB and those ventilated with LTV (see Table 3). By contrast, there is strong evidence in AKI mice that oxygenation is lowest in those ventilated with LTV. There is no difference in oxygenation of AKI mice ventilated with SB vs. HTV.

**BAL protein.** Figure 4 and Table 3 depict strong evidence for a general increase in median BAL protein levels in mice ventilated with LTV vs. SB, and a further increase in mice ventilated with HTV vs. LTV. This pattern was evident for both the sham \((P < 0.0001)\) and AKI \((P < 0.0001)\) groups.

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**Table 2. Effect of kidney health and ventilatory mode, overall or (when appropriate) stratified, on pH, PaCO2, and HCO3**

<table>
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<th>Outcome</th>
<th>Figure</th>
<th>Interaction P Value</th>
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<tr>
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</tr>
<tr>
<td>PaCO2</td>
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</tr>
<tr>
<td>HCO3</td>
<td>3</td>
<td>0.103</td>
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</table>

For each outcome parameter, the \(P\) value of the interaction analysis is given. When this \(P\) value >0.15, the effect of kidney health (sham vs. AKI mice) or of ventilatory mode [spontaneously breathing (SB) vs. LTV vs. HTV] on the outcome parameter is given as difference in median value (with 99% confidence intervals and \(P\) value). When the \(P\) value for interaction <0.15, the effect of the interaction of kidney health and of ventilatory mode on the outcome parameter is presented in a stratified fashion and given as difference in median value (with 99% confidence intervals and \(P\) value). This table also indicates the figure in which the raw data for each outcome parameter can be found.
There was no evidence of an interaction between MV and kidney health \((P = 0.55)\).

**BAL total cell count, macrophages, and lymphocytes.** Based on the interaction effect \((P = 0.10)\) for BAL total cell count (Fig. 5A and Table 4), we explored stratified effects. In sham mice, but not in AKI mice, we observed strong evidence of higher median BAL total cell count for HTV vs. LTV groups. For BAL macrophages (Fig. 5B and Table 4) and BAL lymphocytes (Fig. 5C and Table 4), there was no evidence of interaction or main effects for ventilation or surgery for any of the six combinations of kidney health and ventilatory mode.

**BAL PMNs.** Figure 6 and Table 4 show that, whereas SB mice demonstrate no BAL PMNs regardless of the presence or absence of AKI, the use of MV is associated with the presence of BAL PMNs. There is evidence of an interaction between MV and AKI \((P = 0.03)\). Within the LTV group, there is no evidence of a difference in medians between sham and AKI mice. Within the HTV group, however, there is strong evidence that sham mice have a higher median BAL PMN than AKI mice.

**DISCUSSION**

Using a murine model with careful hemodynamic and metabolic control, our study demonstrates that L-EBD, arterial oxygenation, BAL protein, and BAL PMNs are each altered by MV (vs. SB) alone. Furthermore, AKI (vs. sham surgery) interacts with ventilatory mode to further influence L-EBD, arterial oxygenation, and BAL PMNs in this model. Thus, AKI (vs. sham surgery) tends to increase L-EBD in SB and LTV mice while decreasing it in HTV mice, decreases oxygenation most dramatically in LTV mice, and increases BAL PMNs in LTV mice while decreasing PMNs in HTV mice. These findings are consistent with the concept that MV alters lung microvascular barrier function and inflammation, and that the influence of kidney health status on the lung varies with the mode of ventilation in this model.

Mechanical ventilation alone leads to L-EBD and BAL protein leak in our model. The effect of MV is graded with tidal volume, that is, as the magnitude of tidal volume increases, a more profound L-EBD and more profound leak of BAL protein are seen. Interestingly, injury is observed after MV with tidal volumes as little as 7 ml/kg for 4 h. This 7 ml/kg tidal volume is equal to the tidal volume of spontaneously breathing C57BL/6 mice (31), suggesting that MV is intrinsically injurious. The mechanism for this intrinsically injurious effect of MV on the lungs is unclear. We observed more BAL PMNs in MV mice than SB mice. The BAL neutrophil content was not graded with magnitude of tidal volume, however, suggesting that a pathway in addition to lung neutrophils plays a role in the injurious effect of MV on the lungs. The literature suggests a possible influence of surfactant. When rats are mechanically ventilated with 100% oxygen, surfactant phosphorous becomes inactivated after only 20 min (34). Although not statistically significant until the tidal volume is 48 ml/kg, trends can be seen after 20 min in rats ventilated with 7 ml/kg. Rats have a spontaneous tidal volume of 8 ml/kg (11).
AKI alone (compared with sham surgery) did not alter L-EBD. AKI did decrease the number of BAL PMNs in mice exposed to HTV. In our hands, this renal insult (30-min ischemia, 24-h reperfusion) results in sublethal kidney injury with renal dysfunction. Serum creatinine, normally under 0.4 mg/dl, rises rapidly by 24 h to the 1.5–2.5 mg/dl range (5, 12). Histologically, most of the injury occurs in the cortico-medullary junction with tubular epithelial necrosis, tubular dilatation, and cast formation. Ischemia reperfusion also induces renal inflammation at both the cellular and molecular level, with upregulation of proinflammatory cytokines and chemokines, plus evidence of apoptosis. Cellular inflammation includes infiltration of neutrophils, macrophages, and T lymphocytes into the postischemic kidney. Others have reported that the pulmonary sequestration of neutrophils normally prompted by lung injury is attenuated when this injury is combined with AKI (35). In that model, acid aspiration resulted in both an increase in lung PMN sequestration and decrease in oxygenation in mice ventilated for 2 h with FIO₂/10 ml/kg tidal volumes. The increase in lung PMN sequestration and decrease in oxygenation were attenuated in mice subjected to prior AKI (ischemia 32 min, reperfusion 22 h) and reestablished if these AKI mice were given neutrophils from uninjured mice before induction of acid aspiration. In the current studies, we used 30 min of ischemia and found moderate evidence that AKI decreases oxygenation in mice exposed to 7 ml/kg tidal volumes for 4 h. This deleterious effect of AKI on oxygenation was specific for our LTV group. Surprisingly, we did not find that AKI altered BAL PMN numbers in our LTV group.

We found that the BAL PMN count, which rises directly with the tidal volume size in sham-operated mice ventilated mechanically, decreases in AKI mice mechanically ventilated with HTV (compared to AKI mice mechanically ventilated with LTV). This AKI-induced decrease in BAL PMN of the HTV group paralleled the AKI-related increase in oxygenation and decrease in EBD-labeled albumin leak of the HTV group. This is consistent with renal health interacting with ventilation mode to influence PMN activity as a means of modifying lung function. This influence on PMN activity may, in part, explain the bimodal effect of AKI on lung function. Thus, some aspect of AKI, which exacerbates VILI at LTV, may be counterbalanced in the HTV group because AKI inhibition of neutrophil accumulation attenuates the amplification of VILI which would otherwise be seen in the HTV group.

HTV ventilation resulted in significantly higher PaO₂ compared with LTV ventilation. This difference was most prominent in the AKI mice. The PaO₂ difference between LTV and HTV groups could not be completely explained by a difference in PaCO₂ in either sham mice or AKI mice. The difference in median PaCO₂ between LTV and HTV ventilatory modes was −8.5 [99% CI −18, 1] and similar in both sham and AKI mice. According to the Alveolar Gas Equation, these PaCO₂ differences would contribute a PaO₂ difference of only 10.6 [99% CI −1.4, 20.3] Torr. By contrast, we observed median PaO₂ differences between LTV and HTV ventilatory modes of 33.5 [99% CI 2, 57] Torr in sham mice and 63.5 [99% CI 41, 78] Torr in AKI mice. This observation of injurious effects in spite of a higher ratio of partial pressure of arterial oxygen to fraction of inspired oxygen in the HTV group is reminiscent of the findings grouping a clinical trial of higher vs. lower tidal volume ventilation. Although the higher tidal volume strategy ultimately led to a higher mortality rate in their study, oxygenation in the early period following initiation of MV was better in the higher tidal volume group than in the lower tidal volume group (1). In fact, PEEP and FIO₂ had to be increased in their lower tidal volume group to achieve adequate oxygenation. Although the reason for this discrepancy between early oxy-

![Fig. 3. The effect of ventilatory mode and of kidney health on arterial PaO₂ (Torr). Arterial blood PaO₂ (Torr) values of individual mice exposed to 1 of 2 kidney interventions [sham surgery (*) or acute kidney injury (×)] and to 1 of 3 ventilatory modes. Horizontal line, median value for the group; n = 4–14 per combination of ventilatory mode and kidney health.](http://ajplung.physiology.org/)

![Fig. 4. The effect of ventilatory mode and of kidney health on lung bronchoalveolar lavage (BAL) protein. BAL protein (units = mg protein/ml BAL) of individual mice following exposure to 1 of 2 kidney interventions [sham surgery (*) or acute kidney injury (×)] and to 1 of 3 ventilatory modes. Horizontal line, median value for the group; n = 6–14 per combination of ventilatory mode and kidney health.](http://ajplung.physiology.org/)
generation and long-term outcome was attributed to greater atelectasis in the lower tidal volume group, the actual etiology was not pursued. It nevertheless suggests that oxygenation in the early hours following the initiation of MV may not be reflective of the potential injury being caused.

We do not find complete correlation between indices of injury such as BAL protein concentration and L-EBD. These parameters, however, are often used to evaluate function at different sites. Thus, BAL protein reflects alveolar epithelial integrity and has been attributed to altered capillary/alveolar membrane permeability, increased protein contamination of alveolar fluid due to local inflammation, or condensation due to removal of alveolar fluid (20, 21). By contrast, EBD extravasation is a popular parameter of microvascular endothelial permeability (23, 33). More recently, the specificity of EBD extravasation has been questioned. Dallal and Chang (8), as well as our own prior studies (9), have suggested that Evans blue dye can dissociate from albumin and bind to lung tissue proteins. If a treatment causes an increase in endothelial cell surface proteins, the unbound Evans blue dye would then adhere to these proteins. Utilizing the albumin-bound EBD technique would then overestimate permeability of the lung to albumin. Regardless of the specific etiology of the differences in Evans blue dye content, the differences in lung tissue Evans blue dye content, BAL protein content, and BAL cellularity among the different protocols suggest a complex interaction between ventilatory mode and kidney function on lung vascular endothelial and alveolar epithelial integrity.

Our VILI model was not compounded by aspiration, toxin inhalation, or systemic endotoxin administration. We employed this “pure” VILI model in an effort to minimize potential confounders to our interpretation of an interaction between AKI and VILI. In this model, we aimed our mechanical ventilation to achieve blood pH of 7.28 ± 0.05. This was the pH observed in pilot studies using SB mice instrumented and sedated for 4 h, similar to our MV mice. We utilized SB mice as a control group, and MV with 7 ml/kg as a LTV group and 30 ml/kg as a HTV group.

Occasional blood samples gave PaO2 in the 120–150 Torr range. Given our FIO2 of 0.21, such a PaO2 likely represents the influence of entrapped air in the blood sample. The small blood volume of the mouse limits the volume of the arterial blood sample. This increases the influence of minute volumes of entrapped room air on the composition of the blood sample. Given the uniform appearance of all utilized blood samples, any blood sample has equal potential to have unrecognized entrapped air. Since samples were not selectively deleted, the relationship between groups is likely accurate.

In summary, we found that in a tightly controlled murine model, MV alters lung inflammation and lung function in a graded fashion depending on tidal volume. While AKI alone causes inflammatory changes in the lung, no associated impairment in oxygenation or barrier function could be detected as a result of moderate AKI alone. However, MV can interact with AKI to cause changes in pulmonary function through mechanisms that may include PMN activity. Highly controlled mod-

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Fig. 5. The effect of ventilatory mode and of kidney health on lung BAL total cell count, lung BAL macrophage count, and lung BAL lymphocyte count. A: lung BAL total cell count. B: lung BAL macrophage count. C: lung BAL lymphocyte count. Symbols indicate individual mouse values of cell count (units = cell counts × 10⁴/ml BAL) in mice following exposure to 1 of 2 kidney interventions [sham surgery (*) or acute kidney injury (×)] and to 1 of 3 ventilatory modes. Horizontal line, median value for the group; n = 5–14 per combination of ventilatory mode and kidney health.
el allow the elucidation of inter-organ links observed in critically ill patients and reveal the complexity of these interactions. Further studies are needed to examine how MV and AKI interact to simultaneously change function in either organ.

GRANTS

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REFERENCES


Table 4. Effect of kidney health and ventilatory mode, overall or (when appropriate) stratified, on BAL total cell, BAL macrophages, BAL lymphocytes, and BAL PMN

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Figure</th>
<th>Interaction P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL total cell</td>
<td>7</td>
<td>0.10</td>
</tr>
<tr>
<td>BAL macrophages</td>
<td>8</td>
<td>0.29</td>
</tr>
<tr>
<td>BAL lymphocytes</td>
<td>9</td>
<td>0.39</td>
</tr>
<tr>
<td>BAL PMN</td>
<td>10</td>
<td>0.03</td>
</tr>
</tbody>
</table>

For each outcome parameter, the P value of the interaction analysis is given. When this P value >0.15, the effect of kidney health (sham vs. AKI mice) or of ventilatory mode (SB vs. LTV vs. HTV) on the outcome parameter is given as difference in median value (with 99% confidence intervals and P value). When the P value for interaction < 0.15, the effect of the interaction of kidney health and of ventilatory mode on the outcome parameter is presented in a stratified fashion and given as difference in median value (with 99% confidence intervals and P value). This table also indicates the figure in which the raw data for each outcome parameter can be found.

Fig. 6. The effect of ventilatory mode and of kidney health on lung BAL PMN count. Individual mouse values of BAL PMN count (units = cell counts × 10⁴/ml BAL) in mice following exposure to 1 of 2 kidney interventions [sham surgery (*) or acute kidney injury (×)] and to 1 of 3 ventilatory modes. Horizontal line, median value for the group; n = 5–14 per combination of ventilatory mode and kidney health.
INTERACTIVE EFFECTS OF MECHANICAL VENTILATION AND KIDNEY HEALTH


