Cecal ligation and puncture accelerates development of ventilator-induced lung injury

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Yehya N, Xin Y, Oquendo Y, Cereda M, Rizi RR, Margulies SS. Cecal ligation and puncture accelerates development of ventilator-induced lung injury. Am J Physiol Lung Cell Mol Physiol 308: L443–L451, 2015. First published December 30, 2014; doi:10.1152/ajplung.00312.2014.—Sepsis is a leading cause of respiratory failure requiring mechanical ventilation, but the interaction between sepsis and ventilation is unclear. While prior studies demonstrated a priming role with endotoxin, actual septic animal models have yielded conflicting results regarding the role of preceding sepsis on development of subsequent ventilator-induced lung injury (VILI). Using a rat cecal ligation and puncture (CLP) model of sepsis and subsequent injurious ventilation, we sought to determine if sepsis affects development of VILI. Adult male Sprague-Dawley rats were subject to CLP or sham operation and, after 12 h, underwent injurious mechanical ventilation (tidal volume 30 ml/kg, positive end-expiratory pressure 0 cmH2O) for either 0, 60, or 120 min. Biochemical and physiological measurements, as well as computed tomography, were used to assess injury at 0, 60, and 120 min of ventilation. Before ventilation, CLP rats had higher levels of alveolar neutrophils and interleukin-1β. After 60 min of ventilation, CLP rats had worse injury as evidenced by increased alveolar inflammation, permeability, respiratory static compliance, edema, oxygenation, and computed tomography. By 120 min, CLP and sham rats had comparable levels of lung injury as assessed by many, but not all, of these metrics. CLP rats had an accelerated and worse loss of end-expiratory lung volume relative to sham, and consistently higher levels of alveolar interleukin-1β. Loss of aeration and progression of edema was more pronounced in dependent lung regions. We conclude that CLP initiated pulmonary inflammation in rats, and accelerated the development of subsequent VILI.

sepsis; acute lung injury

NONPULMONARY SEPSIS IS a leading cause of acute respiratory distress syndrome (15); however, the etiology of the lung injury from sepsis is unclear. This is confounded by the fact that many septic patients with respiratory failure require mechanical ventilation, which itself causes injury from regional overdistension and cyclic reopening of atelectatic lung (22). This ventilator-induced lung injury (VILI) is characterized by epithelial (7, 9) and endothelial (12, 37) dysfunction, with barrier failure leading to alveolar flooding. Because sepsis and VILI share similar proinflammatory cytokine profiles (34), it is possible that an initial septic insult predisposes lungs for secondary injury from VILI.

Characterization of potential interaction between sepsis and VILI is required to appropriately animal models that both recapitulate the proinflammatory cascade seen in humans and allow for the testing of a priming septic insult on development of subsequent VILI. The cecal ligation and puncture (CLP) model and its variants (8) induce a systemic inflammatory response from a polymicrobial abdominal infection. CLP, unlike surrogates like endotoxin injection (31), recreates sepsis progression most similarly to humans, with comparable hemodynamic and inflammatory profiles (8).

Exogenous endotoxin administration is well established to predispose lung to further injury (5, 39). However, prior investigations in animal models of systemic sepsis have generated mixed results. Multiple studies suggest that CLP without mechanical ventilation can induce pulmonary neutrophil recruitment (1, 29), unfavorable histology, and endothelial dysfunction (4, 29). The relevance of this inflammatory effect of CLP on the development of subsequent VILI has never been demonstrated. Uematsu et al. (33) reported that CLP mice exposed to high tidal volumes (0 ml/kg VT) and zero positive end-expiratory pressure (0 cmH2O PEEP) to induce VILI had comparable injury to sham-operated mice after 3 h of injurious ventilation, despite elevated serum levels of several proinflammatory cytokines, thereby disassociating cytokine levels with the degree of lung injury. Similarly, brief ventilation of isolated lungs using moderate VT (20 ml/kg VT, 0 cmH2O PEEP, 1 h) did not demonstrate differences in compliance between lungs obtained from CLP or sham rats (25). Others have demonstrated that CLP does not cause appreciable lung injury (14, 18), challenging the hypothesis that the proinflammatory cytokine response seen with CLP predisposes to subsequent VILI, contending that damage from VILI is the primary determinant of lung injury without any priming needed by CLP.

In an effort to add clarity to this area of research, the study described below was designed to assess acute interactions between sepsis and VILI using biological and imaging metrics at multiple time points. To characterize the time course of the interaction (if any) between CLP and VILI, we compared lung function, inflammation, and permeability between sham and CLP rats at baseline, after 1 h, and after 2 h of injurious mechanical ventilation. Finally, we used small animal computed tomography (CT) to noninvasively quantify the degree of lung injury after induction of VILI both in the presence and...
absence of CLP. We report that CLP accelerates the development of VILI in rats.

METHODS

This study was approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Adult (8–10 wk old) male Sprague-Dawley rats (Charles River, Boston, MA) weighing 278 ± 6 (SE) grams were used.

CLP. Anesthesia was maintained with 2–3% isoflurane. Rats underwent either CLP or sham surgery. CLP consisted of midline laparotomy, exposure of the cecum, ligation above the ileocecal valve to maintain bowel continuity, two perforations on the antimesenteric side with an 18-gauge needle, and expression of feces. The cecum was then replaced, and the peritoneal layer and abdomen were closed. Sham procedure consisted of laparotomy without cecal manipulation or perforation. All animals received subcutaneous saline (10 ml) and the analgesic buprenorphine (0.1 mg/kg). In our experience, mortality rate 24 h following this CLP model is 10% (6).

VILI model. To avoid the effects of altered hemodynamics on our model (17), rats were subjected to VILI 12 h after CLP or sham surgery. The injurious \( V_T \) initially chosen was 25 ml/kg with PEEP set to 0 cmH2O, but, after 4 h, we saw no significant lung injury on CT. The next test of 30 ml/kg \( V_T \) and 0 cmH2O PEEP demonstrated CT evidence of injury within 2 h, and was chosen for subsequent studies.

Based on this preliminary data, 12 h after CLP or sham surgery, rats were anesthetized with pentobarbital (50 mg/kg ip), underwent tracheostomy with a stiff 14-gauge catheter, and received a second 10 ml saline subcutaneously. Rats were then randomized to injurious ventilation (30 ml/kg \( V_T \), 0 cmH2O PEEP, rate 27 breaths/min, FIO2 1.0) for 0, 60, or 120 min (n = 6 rats/group). All rats survived the VILI protocol.

Rats were ventilated with a custom-built ventilator capable of delivering \( V_T \) ± 100 μl using a square flow waveform, with the CT synchronized to acquire images during end-inspiration and end-expiration. Animals were supine, and anesthesia and paralysis were maintained with boluses of intraperitoneal pentobarbital (25 mg/kg) and pancuronium (0.6 mg/kg). Proximal airway pressure was continuously monitored using a fiber-optic sensor (Samba Sensors). Heart rate and peripheral oxygen saturation levels were monitored noninvasively (Nonin Medical, Plymouth, MN). Body temperature was maintained at 37°C by a water blanket (Gaymar Industries, Orchard Park, NY).

CT and respiratory mechanics. Sham and CLP animals ventilated for 60 or 120 min underwent CT image acquisition at baseline (0 min), and at 60 and 120 min of ventilation. Settings were adjusted for CT acquisition, and all imaging took place on noninjurious settings (10 ml/kg \( V_T \), 5 cmH2O PEEP, rate 53 breaths/min, FIO2 1.0) for 0, 60, or 120 min (n = 6 rats/group). All rats survived the VILI protocol.

RESULTS

Lung injury. All animals survived the VILI protocol, and there were no differences in heart rate between groups at either the beginning (sham 455 ± 16; CLP 495 ± 19 beats/min; time 0 sham vs. CLP \( P = 0.127 \)) or the end (sham 392 ± 32; CLP 387 ± 33 beats/min; time 120 min sham vs. CLP \( P = 0.900 \)) of the experiment. There were no differences prevention between sham and CLP rats with respect to oxygenation (Fig. 1A), compliance (Fig. 1B), BAL total WBC (Fig. 2A), lung permeability (Fig. 2C), or lung histology (Fig. 2E); however, BAL neutrophils (Fig. 2B) and IL-1β (Fig. 2D) were higher at baseline in CLP. After 60 min of injurious ventilation, CLP rats demonstrated worse oxygenation and static compliance (Fig. 1), increased lung inflammation and permeability (Fig. 2, A–D), and higher histologic lung injury score (Fig. 2F). After 120 min, CLP rats continued to demonstrate worse oxygenation (Fig. 1A), higher BAL WBC (Fig. 2A), and higher BAL IL-1β (Fig. 2D) relative to sham, but demonstrated equally poor static compliance (Fig. 1B), similarly elevated levels of BAL neutrophils (Fig. 2B) and protein (Fig. 2C), and similar histologic injury (Fig. 2E).
very little aerated lung in end-expiration at 5 cmH2O PEEP expiration: after 120 min of VILI, CLP rats demonstrate tissue in CLP rats relative to sham, especially in end-demonstrates progressive and more rapid loss of aerated dimensional reconstruction of gas volume distributions positive Hounsfield units (HU) in CLP rats (Fig. 5). Three-densities demonstrates a shift toward progressively more rapid development of infiltrates in CLP relative to sham. When comparing weights of the lung segments in end-expiration (Fig. 7F), CLP rats demonstrated heavier lungs across all three segments at 60 min of ventilation relative to sham, with the posterior segment heavier than the anterior and middle segments. After 120 min of VILI, sham rats had similarly heavy middle and posterior segments; however, CLP rats continue to have more edema in the anterior segment after 120 min relative to sham. Overall, this suggests that, for both sham and CLP rats, progression of edema occurred initially in posterior (dependent) regions, and then spread anteriorly.

CT evidence of lung injury. With the use of gas volumes calculated by CT (end-inspiration – end-expiration), compliance decreased in CLP rats after 60 min of injurious ventilation, and declined to similarly low levels in both sham and CLP rats after 120 min (Fig. 3A). CLP rats demonstrated more rapid reduction in end-expiratory gas volume (Fig. 3B), despite a similar increase in strain ratio (V5/total end-expiratory volume at PEEP 5 cmH2O; Fig. 3C). Calculated lung weight demonstrated significantly heavier lungs in CLP rats after 60 min of ventilation while sham lungs were relatively unchanged; 120 min after induction of VILI, lung weight had increased in both sham and CLP (Fig. 3D). Representative coronal CT images corroborate the more rapid development of infiltrates in CLP relative to sham (Fig. 4), and quantification of reconstructed lung densities demonstrates a shift toward progressively more positive Hounsfield units (HU) in CLP rats (Fig. 5). Three-dimensional reconstruction of gas volume distributions demonstrates progressive and more rapid loss of aerated tissue in CLP rats relative to sham, especially in end-expiration: after 120 min of VILI, CLP rats demonstrate very little aerated lung in end-expiration at 5 cmH2O PEEP (Fig. 6). More detailed quantification of lung aeration over time (Fig. 7) showed that CLP rats (relative to sham) had less normally aerated lung in both end-inspiration and end-expiration (Fig. 7B) at 60 min, and more nonaerated lung at end-expiration at 60 min (Fig. 7D).

In the setting of progressively increasing lung weight (Fig. 3D), and progressively more positive HU distribution (Fig. 5), loss of aeration in CLP rats predominantly represents worsening edema. To spatially localize this edema, we divided the lung in the coronal plane into three segments: anterior, middle, and posterior (Fig. 7, E and F). At 60 min of VILI, CLP rats had significantly more positive (less aerated) HU in the posterior (dependent) segment (Fig. 7E) in both end-inspiration and end-expiration, relative to sham. When comparing weights of the lung segments in end-expiration (Fig. 7F), CLP rats demonstrated heavier lungs across all three segments at 60 min of ventilation relative to sham, with the posterior segment heavier than the anterior and middle segments. After 120 min of VILI, sham rats had similarly heavy middle and posterior segments; however, CLP rats continue to have more edema in the anterior segment after 120 min relative to sham. Overall, this suggests that, for both sham and CLP rats, progression of edema occurred initially in posterior (dependent) regions, and then spread anteriorly.

DISCUSSION

CLP accelerated the development of VILI in rats subject to injurious ventilation, with increased lung inflammation, permeability, and edema after 60 min of ventilation, leading to worse compliance and oxygenation relative to sham. CT imaging demonstrated more rapid and progressive loss of end-expiratory volume in CLP rats relative to sham. After 120 min of injurious ventilation, many of these metrics were not different between CLP and sham rats. Our data support the hypothesis that sepsis primes the lung for subsequent VILI, and suggest that conclusions reached regarding interaction between CLP and VILI are dependent on choices made during study design, including metrics used to assess lung injury and the timing of these measurements.

Mechanisms underlying CLP priming. Central to our conclusion that CLP constitutes an initial insult, thereby accelerating subsequent VILI, is the increased inflammation seen in CLP lungs at baseline. We hypothesize that this inflammation, demonstrated by increased BAL neutrophils and IL-1β prevention in CLP rats (Fig. 2), is necessary for the predisposition to further injury. Although a neutrophil-independent component of experimental lung injury has been described (5), neutrophil activity preceding lung injury is corroborated by other injury models that demonstrate how neutrophil depletion attenuates the degree of injury (21, 30). Consistent with a central role for neutrophil accumulation, pharmacological neutrophilia has been shown to exacerbate lung injury (16).

The increased BAL IL-1β in CLP rats relative to sham prevention, and at both 60 and 120 min of ventilation (Fig. 2D), provides a potential mechanism for the accelerated development of VILI after CLP. Exogenous intratracheal delivery of IL-1β increases BAL neutrophils (20) and lung permeability (19, 20). IL-1β has been postulated to increase permeability by reducing expression of the epithelial sodium channel via activation of p38 MAPK (32) in epithelial cells, possibly acting via...
upregulated neuregulin-1 signaling through human epithelial growth factor receptor-2 (10). In endothelial cells, increased permeability via activation of RhoA and transforming growth factor-β has been implicated (12). Inhibition of IL-1β signaling reduces neutrophil influx in experimental lung injury and mitigates VILI (11). However, the mechanisms leading to increased alveolar IL-1β after CLP are unknown.

CT analyses. We provide for the first time imaging analysis of septic rats subjected to injurious ventilation. This is one of the few studies to radiographically characterize VILI in sham animals (27). CT allows an opportunity to address mechanisms underlying VILI in future studies, both in the presence or absence of sepsis. Similar to what has been reported in non-septic animals (27), VILI occurred predominantly in dependent regions first in both sham and CLP rats (Figs. 5 and 7). The posterior regions being affected first in this model of VILI (30 ml/kg, 0 cmH₂O PEEP) suggests a possible mechanistic role for atelectotrauma affecting more dependent regions first, and thereby allowing propagation of VILI over a smaller lung volume, a phenomenon that may be alterable by higher PEEP.

Given the normal appearance of both sham and CLP CTs at baseline before VILI, the data support the notion that sepsis accelerated the expected progression of VILI, rather than constituting a different category of insult. Clearly, given the

![Fig. 2. Time course of bronchoalveolar lavage (BAL) measurements in sham and CLP rats subject to VILI. A and B: CLP rats demonstrated higher levels of BAL white blood cell (WBC) at 60 and 120 min of ventilation (A) and higher levels of BAL neutrophils at baseline (time 0) and at 60 min of ventilation (B). C: permeability reflected by BAL protein was increased in CLP rats at 60 min of ventilation, and was elevated comparable to sham at 120 min. D: at all time points, BAL levels of interleukin (IL)-1β were higher in CLP rats relative to sham. E: histologic lung injury score (range 0–100) derived from 30 slides/rat is higher at 60 min for CLP rats, with equalization at 120 min of ventilation. Plots are means ± SE. P values compare CLP and sham groups at a given time point using 2-way ANOVA and post hoc Tukey, n = 6 rats/group. *Significant differences (P < 0.05 by Tukey) from baseline (time 0) for CLP or sham at 60 or 120 min.](http://ajplung.physiology.org/)

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similarly poor appearance of CTs and respiratory mechanics at 120 min of VILI, the injurious settings used represent the predominant mechanism of injury in this study. Future studies will further dissect the sensitivity of CLP lungs to smaller VT in an attempt to better delineate differences between sham and CLP rats. Also, CT imaging of a larger septic animal subject to VILI will allow better resolution of the heterogeneity of lung injury, potentially allowing lobar-specific sampling by BAL.

Fig. 3. Computed tomography (CT)-derived parameters of sham and CLP rats subject to VILI. All imaging took place on noninjurious settings [10 ml/kg tidal volumes (V̇ₜ), 5 cmH₂O PEEP, rate 53 breaths/min, FIO₂ 1.0]. A: CLP rats demonstrate worse static compliance using volumes derived from CT (end-inspiratory – end-expiratory airspace volumes) at 60 min, with similar levels after 120 min. B: both sham and CLP rats demonstrate decreasing end-expiratory volumes (EEV) with progressive mechanical ventilation, with CLP rats demonstrating significantly lower EEV after 60 min relative to sham. C: the strain ratio (Vₜ/EEV) is nonsignificantly higher at 60 min in CLP rats. D: lung weight (normalized to initial weight at time 0) as measured by CT demonstrates a 50% increase in CLP rats at 60 min relative to sham, which is essentially unchanged from baseline until 120 min of ventilation. Plots are means ± SE, P values compare CLP and sham groups at a given time point using 2-way ANOVA and post hoc Tukey, n = 6 rats/group. #Significant differences (P < 0.05 by Tukey) from baseline (time 0) for CLP or sham at 60 or 120 min.

Fig. 4. Representative end-expiratory coronal CT images of sham and CLP rats subject to VILI. CLP rats demonstrate increased infiltrates at 60 min relative to sham, with near-complete opacification after 120 min of ventilation.
and thus characterize the regional heterogeneity of the inflammation in VILI both in the presence and absence of sepsis, and allow correlation between regional imaging and regional physiology.

Comparison with other studies. There is a paucity of literature investigating whether CLP increases susceptibility to VILI. Many studies using rodent lungs “primed” by CLP do not use a control group, which would allow differentiation of the effects of sepsis from the effects of high VT (35, 36). Recently, Uematsu et al. (33) demonstrated that CLP mice ventilated with injurious settings (40 ml/kg VT, 0 cmH2O PEEP) had comparable lung injury to nonseptic mice at the end of 3 h of ventilation. Our results are entirely consistent with these findings, since sham and CLP rats in our study had comparable levels of inflammation, permeability, compliance, and edema after 120 min of ventilation. However, the acute end points measured at 60 min demonstrated increased susceptibility to VILI in CLP in our study.

Fig. 5. Representative 3-dimensional (3D) densitometry reconstructions with associated frequency distribution of Hounsfield units (HU) of sham and CLP rats subject to VILI. Images are the posterior view in both end-inspiration (EI) and end-expiration (EE). Gray, −1,000 to −500 HU; yellow, −500 to −100 HU; magenta, −100 to +100 HU. For reference, −1,000 HU are typical for air and 0 HU is typical for water. CLP lungs demonstrate more rapid and progressive pulmonary edema relative to sham, and a more rapid rightward shift of HU distribution.

Fig. 6. Representative 3D reconstructions of coronal CT gas volumes of sham and CLP rats subject to VILI. Coronal slices were segmented by thresholding (less than −300 HU) and reconstructed in 3 dimensions (see METHODS for details). EI and EE images in both anterior (A) and posterior (P) views are shown for sham and CLP rats. CLP rats demonstrate decreased gas volume at both 60 and 120 min relative to sham, in both EI and EE, with near-complete absence of alveolar airspace in EE at 120 min.
Variability in this body of literature may be the result of discrepancies in the time between CLP surgery and ventilation. Nakamura et al. (25) ventilated ex vivo lungs for 1 h (20 ml/kg VT, 0 cmH2O PEEP) and found no differences in compliance between lungs obtained from CLP or sham rats. Unlike our study, however, Nakamura et al. found no difference in BAL IL-1β at baseline, possibly because lungs were extracted 22–23 h after sham or CLP surgery, and serum IL-1β and lung myeloperoxidase activity is known to peak as early as 4 h after CLP, but return to baseline by 24 h (1). Similarly, Kuiper et al. (18) ventilated rats 24 h after surgery and did not find worsening of lung compliance, oxygenation, or edema in CLP rats (15 ml/kg VT, 0 cmH2O PEEP, 4 h). By comparison, we ventilated rats 12 h after sham or CLP surgery, suggesting that lungs may be more susceptible to subsequent injury 12 h after CLP, rather than 24 h.
sufficiently overdistend rodent lungs to cause VILI (38). The abovementioned study by Kuiper et al. (18) used even lower VT ventilation (15 ml/kg VT, 0 cmH2O PEEP).

Taking these studies together, we conclude that CLP can constitute the first “hit” predisposing the lung to further injury from VILI, and we postulate that differences in conclusions between our study and others’ can be explained by differences in the timing of measurements of lung injury (33), the timing after CLP surgery (18, 25), and the ventilator settings used to induce VILI (18, 25). Future investigations using different ventilator settings at different time points after CLP will be needed to test this hypothesis explicitly.

Limitations. Several limitations may influence our conclusions. CLP and sham rats were compared with respect to their susceptibility to VILI only at a single time point: specifically, 12 h after surgery. While this was a time point with confirmed lung inflammation in CLP prevention (Fig. 2), it restricts our ability to conclude the necessity of BAL neutrophils or IL-1β as a requisite hit from CLP predisposing lungs to subsequent VILI. The time course of BAL and serum IL-1β after CLP surgery will need to be better defined, and future studies will need to subject rats to ventilation at different times after surgery to test whether the increased susceptibility to VILI seen in our study is dependent on timing after surgery and the degree of pulmonary inflammation evident at that time point. Also, because our metrics of lung inflammation are limited to BAL WBC, neutrophils, and IL-1β, we are limited in the conclusions we can draw regarding which cytokines may be involved in any potential “priming” of septic lungs for further injury. Future studies will require measurements of several more potential inflammatory mediators.

While we demonstrated increased susceptibility to VILI in septic rats, the mechanisms underlying this remain unclear. Future investigations will focus on inhibition of IL-1β in septic rats, with subsequent exposure to VILI, hypothesizing that this may mitigate the predisposition to VILI. Human trials investigating IL-1β inhibition in human sepsis failed to demonstrate mortality benefit (28); however, the (more focused) outcome of preventing VILI in septic patients requiring mechanical ventilation has not been investigated.

The VT used (30 ml/kg) in this study is approximately four- to fivefold larger than volumes used in humans, which may limit translatability to human VILI. Given that rodent lungs demonstrate significantly different responses to increasing pressure than humans (26, 38, 40), we selected a VT of 30 ml/kg, which is within the range used to generate VILI in rats in the literature; in a recent publication, a range of 18.8 to 51.5 [mean 36.2 ± 2.2 (SE)] ml/kg was reported (2). It is possible that ventilation with lower VT for longer periods of time would confirm the conclusions presented here; however, the VT of 30 ml/kg was chosen because of evidence of lung damage on CT, one of the metrics used to quantify injury in this study. Future studies testing incrementally lower VT are warranted to validate our findings. If confirmed, predisposition to lung injury in septic animals using lower VT may allow better delineation and temporal resolution of the initial priming induced by CLP, and subsequent damage induced by the ventilator.

In conclusion, CLP accelerated the development of VILI in rats subject to injurious mechanical ventilation as evidenced by multiple measures of lung inflammation, permeability, imaging, histology, and functional physiology. Conclusions reached regarding the interaction between CLP and VILI are sensitive to the metrics used to assess lung injury and the timing of these measurements. Research must account for the substantial influence of these factors if it is to achieve a better mechanistic understanding of VILI in healthy and diseased lungs.

REFERENCES


