Impact of the Prone Position in an Animal Model of Unilateral Bacterial Pneumonia Undergoing Mechanical Ventilation

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ABSTRACT

Background: The prone position (PP) has proven beneficial in patients with severe lung injury subjected to mechanical ventilation (MV), especially in those with lobar involvement. We assessed the impact of PP on unilateral pneumonia in rabbits subjected to MV.

Methods: After endobronchial challenge with Enterobacter aerogenes, adult rabbits were subjected to either “adverse” (peak inspiratory pressure = 30 cm H2O, zero end-expiratory pressure; n = 10) or “protective” (tidal volume = 8 ml/kg, 5 cm H2O positive end-expiratory pressure; n = 10) MV and then randomly kept supine or turned to the PP. Pneumonia was assessed 8h later. Data are presented as median (interquartile range).

Results: Compared with the supine position, PP was associated with significantly lower bacterial concentrations within the infected lung, even if a “protective” MV was applied (5.93 vs. 6.66 [0.86] log10 cfu/g, respectively; P = 0.008). Bacterial concentrations in the spleen were also decreased by the PP if the “adverse” MV was used (3.62 [1.74] vs. 6.55 [3.67] log10 cfu/g, respectively; P = 0.038). In addition, the noninfected lung was less severely injured in the PP group. Finally, lung and systemic inflammation as assessed through interleukin-8 and tumor necrosis factor-α measurement was attenuated by the PP.

Conclusions: The PP could be protective if the host is subjected to MV and unilateral bacterial pneumonia. It improves lung injury even if it is utilized after lung injury has occurred and nonprotective ventilation has been administered.

What We Already Know about This Topic

• Prone positioning can improve gas exchange in acute lung injury patients

What This Study Tells Us That Is New

• In anesthetized animals with unilateral bacterial-induced lung injury subjected to nonprotective mechanical ventilation, prone positioning (compared to supine positioning) decreases the bacterial concentration in the infected lungs and attenuates lung and systemic inflammation

Mechanical ventilation (MV) is widely used in critically ill patients with respiratory failure. Cumulative evidence suggests, however, that MV could be harmful for the lung. Thus, both overdistension of the airways and intratidal alveolar cyclic opening and closing are likely to cause tissue damage, called ventilator-induced lung injury (VILI). In addition, lung stretch could lead to the release of inflammatory cytokines, thereby causing additional injury, particularly through the recruitment of polymorphonuclear neutrophils mediated by interleukin (IL)-8. Finally, some experimental findings have increased the possibility that the ability of the host to keep bacterial growth in check could be hampered by the mechanical forces applied to the lung.

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An experimental study was carried out in order to assess the impact of PP on bacterial pneumonia with two main endpoints:

- the severity of the unilateral pneumonia;
- the VILI features within the noninfected contralateral lung.

**Animals**

Male New Zealand white rabbits (body weight, 2.7–3.0 kg) were obtained from Elevage scientifique des Dombes (Romans, France). These animals were not immunosuppressed and were free of both virus antibodies and specific pathogens. They were placed in individual cages and had free access to water and were fed in accordance with current recommendations mentioned in the Guide for the Care and Use of Laboratory Animals, National Institutes of Health No. 92–23, revised 1985. The Dijon Faculty of Medicine Ethical Committee approved the experimental protocol. A central venous catheter was surgically inserted into every rabbit the day before MV.

**Experimental Protocol**

The animals were intubated as previously described. Briefly, during general anaesthesia provided by iterative intravenous injections of propofol, a cuff tube of 3.0 mm was orally inserted into the trachea under view control. The animal was put in the supine position (SP) and connected to a pressure-controlled respirator. During the first 30 min, the VT was set at 8 ml/kg with zero end-expiratory pressure, a respiratory rate of 30 breaths/min and an inspired fraction of oxygen (FIO2) of 0.5 (fig. 1). Throughout the experiment, a continuous infusion of ketamine (1 mg·kg−1·h−1) and pancuronium bromide (0.3 mg·kg−1·h−1), was given. Hydration with isotonic serum was provided intravenously so that the rabbits remained at a constant weight.

*Enterobacter aerogenes* pneumonia was induced as previously described. Briefly, one 10 log10 cfu inoculum of a clinical strain of *E. aerogenes* was instilled through the left stern bronchus. According to previous data, it takes 3 h for histologically proven lobar pneumonia to develop.

In the first set of experiments, the impact of PP was tested in animals subjected to “adverse” MV strategy. Thus, 1 h after bacterial challenge, the peak inspiratory pressure was set at 30 cm H2O and the animals were randomly kept in the SP (n = 4) or turned to the PP (n = 5). The animals were subjected to MV for 8 h before being killed by an overdose of thiopental.

![Timeline representation of the experimental protocol. MV = mechanical ventilation; PP = prone position; SP = supine position.](image)
Assessment of Respiratory System Compliance and Other Physiological Measurements

Inspiratory pressure–volume curves were constructed post-mortem according to the supersyringe method. Respiratory system compliance (CRS) was deduced from the slope of the linear portion of the pressure–volume curve. An arterial catheter was inserted in most of the animals subjected to “adverse” MV for blood sampling and blood pressure monitoring to ascertain the safety of our “adverse” MV.

It is worth noting that none of the animals subjected to “protective” MV was thus monitored. Actually, we had previously checked the safety of these settings as well as the lack of hypoxemia and hyperlactatemia in mechanically ventilated rabbits with this type of pneumonia.5,24

Evaluation of Unilateral Pneumonia

The animals were exsanguinated by venous puncture. Autopsies were carried out and the lungs and spleen were harvested aseptically.

Each lung was isolated and homogenized in sterile water. Tenfold dilution cultures were then performed. The mean bacterial concentration of the infected lung was calculated according to the lung weight. The spleen of each rabbit was also crushed and cultured since a positive E. aerogenes spleen culture was considered a marker of bacteremia.

The remaining lung and spleen homogenates were then frozen, batched, and stored at −80°C until tissue concentrations of cytokines were measured. Accordingly, IL-8 and tumor necrosis factor-α were assessed using a rabbit-specific enzyme-linked immunosorbent assay following the manufacturer’s instructions (Euromedex®, Strasbourg, France).

Blood samples were obtained before bacterial inoculation (H0), at H1 and H8 only in animals in the second set of experiments (i.e., “protective” MV), to assess systemic inflammation according to the position. Thus, IL-8 and tumor necrosis factor-α blood concentrations were measured using the above-mentioned enzyme-linked immunosorbent assay test.

Assessment of VILI and Inflammation within the Noninfected Lung

For microscopic examination, approximately 1 cm³ of tissue was fixed in formalin and embedded in paraffin. Four-micro meter sections were obtained and stained with hematoxylin–eosin. A pulmonary pathologist blinded to the treatment group examined 10 fields of each section and an injury score was calculated as previously described.28 Briefly, lung injury assessment was based on the degree of neutrophil infiltration, hemorrhage, and oedema. Lung injury was considered absent (0), mild (1), moderate (2), or severe (3). In addition, the presence or absence of hyaline membranes as well as emphysema-like lesions was systematically sought.

Another lung sample was harvested for RNA extraction using the GenElute kit (Sigma®, Dorset, United Kingdom). RNA extraction was performed using the RNA GenElute kit accordingly. Complementary DNA was obtained by reverse transcription using random primers, RNAs in treatment, and ImProm II reverse transcriptase (Promega®, Madison, WI). Quantitative polymerase chain reaction was performed using the IQ5 thermocycler (Biorad®, Hercules, CA) and the IQ Sybergreen Supermix (Biorad®) and rabbit-specific primers, designed using Primer3 (version 0.4.0),** and the rabbit (Oryctolagus cuniculus) sequence database.†† Melting curves were plotted to check the specificity of the amplifications. The following primers were used: rGapdh forward: 5′-ATG TTT GTG ATG GGC GTG AAC C-3′, reverse: 5′-CCC AGC ATC GAA GGT AGA GGA-3′; rIl-8 forward: 5′-AAC CTT CCT GCT GCT TCT GA-3′, reverse: 5′-TCT GCA CCC ACT TTT TCC TTG-3′. The results were expressed as expression levels normalized to a reference gene (rGapdh). The corresponding protein assessment could not however be achieved since all of the remaining tissue had to be used for gravimetric evaluation of the lung as described below.

The remaining fresh tissue was weighed to measure the lung wet weight (WW) and warmed to 37°C until desiccation before recording the dry weight (DW). The WW to DW ratio (WW/DW) was then calculated as a surrogate for lung permeability oedema. Previously, healthy rabbits with normal lung tissue (n = 4) were killed and used as controls.

Statistical Analysis

Data are presented as median (interquartile range) except otherwise stated. The Mann–Whitney U test was used to compare continuous variables between groups. All tests were two-tailed. A P ≤ 0.05 was considered significant. The Statview software was used (SAS Institute, Cary, NC) for all analysis.

Results

C_Rs, Gas Exchange and Hemodynamics

C_Rs was measured postmortem in all rabbits with pneumonia (table 1). C_Rs was greater in animals that had undergone “protective” MV than in the others but remained unchanged by the body position. In addition, C_Rs was improved by PP

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if “adverse” MV strategy was applied (2.8 [1.4] vs 5.2 [1.1] ml/cm H₂O; \( P = 0.001 \)).

Differences were also observed regarding gas exchange in the animals subjected to “adverse” MV in which blood gases could be obtained. An increase of 235 (244) mmHg in the \( \text{PaO}_2/\text{FiO}_2 \) ratio was recorded in the PP group, whereas a decrease of 69 (77) mmHg was measured in the supine animals (\( P = 0.04 \)). No difference was seen regarding blood arterial pressure and heart rate measured just before sacrifice. However, the blood lactate concentration in the SP increased throughout the experiment while it remained within normal range in the PP (\( \Delta \text{Lactate} = 2.1 [3.1] \) vs. \(-0.4 [0.7] \) mmol/l, respectively; \( P = 0.034 \)) (table 1).

### Assessment of Unilateral Pneumonia

All of the animals but one (SP) survived until they were killed 8 h after having been turned prone or kept supine. The size of the \( E. \) aerogenes inoculum in the SP and PP groups was found to be comparable, regardless of the MV strategy (data not shown).

Quantitative lung culture results showed high mean concentrations of \( E. \) aerogenes 8 h after inoculation (fig. 2). Lower concentrations were found in the PP group than in the SP group (8.38 [0.91] vs. 9.81 [0.52] log₁₀ cfu/g of tissue, respectively; \( P = 0.002 \)) when an “adverse” MV strategy was used. Although “protective” MV decreased the bacterial pulmonary burden regardless of the position, the lowest bacterial concentrations within this group were found in animals in the PP (5.93 [0.34] vs. 6.66 [0.86] log₁₀ cfu/g, respectively; \( P = 0.008 \)). When the lung inflammatory response was considered, the PP was associated with the release of smaller amounts of IL-8, regardless of the MV strategy (fig. 3). In contrast, although a “protective” MV significantly decreased pulmonary concentrations of tumor necrosis factor-\( \alpha \) regardless of the position (2155 [830] vs. 3955 [1412] pg/g of tissue, respectively; \( P = 0.014 \)), the PP did not decrease cytokine release further.

The extrapulmonary impact of pneumonia was also assessed. Like the lung, spleen culture results showed

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<th>Table 1. Physiological Parameters Recorded in Rabbits with Unilateral Enterobacter aerogenes Pneumonia Submitted to Adverse MV in either SP or PP</th>
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Results are presented as median ± IQR. The delta values mean variation from H₀ (randomization to the SP or the PP group) to H₈ (sacrifice).

*\( P < 0.05 \) between SP and PP.

\( C_{RS} \) = respiratory system compliance; \( \text{FiO}_2 \) = inspired fraction of oxygen; IQR = interquartile range; MAP = mean arterial pressure; MV = mechanical ventilation; PP = prone position; SP = supine position.

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**Fig. 2.** Bacterial burden (infected lung, [A]; spleen, [B]) in animals with Enterobacter aerogenes pneumonia submitted to an adverse (\( n = 9 \)) or a protective (\( n = 10 \)) mechanical ventilation (MV) in supine position (SP), or turned from supine to prone position (PP). There is a bacterial overgrowth in the lung of the animals from the SP group, whenever the MV is protective or not. The pulmonary-to-systemic translocation is increased by SP in the only animals submitted to an adverse MV strategy, as reflected by the larger amount of bacteria recovered from the spleen culture in this group. Results are expressed as median ± IQR.

*\( P < 0.05 \) between SP and PP groups. IQR = interquartile range.
lower bacterial concentrations in the animals turned to the PP than in those kept supine within the “adverse” MV group (3.62 [1.74] vs. 6.55 [3.67] log$_{10}$ cfu/g, respectively; $P = 0.038$). However, pulmonary-to-systemic translocation of *E. aerogenes* was not lower in prone than in supine animals subjected to “protective” MV (2.93 [1.59] vs. 3.24 [1.51] log$_{10}$ cfu/g, respectively; $P = 0.684$). The systemic inflammatory response was evaluated by the measurement of two of its key mediators within the blood compartment (fig. 3). Although concentrations of tumor necrosis factor-α in the spleen were similar in both groups regardless of the MV strategy, lower amounts of IL-8 were found in the PP than in the SP group when the rabbits underwent “adverse” MV (1910 [784] vs. 3005 [1290] pg/g of tissue, respectively; $P = 0.038$). In the animals subjected to “protective” MV, a statistically nonsignificant lower IL-8 release was found in the PP group (1561 [274] vs. 1930 [1022] pg/g of tissue, respectively; $P = 0.124$). Similarly, blood concentrations of IL-8 rose more slowly with time when the rabbits were turned to the PP than when they were kept supine (fig. 4).

**Assessment of Lung Injury and Inflammation in the Noninfected Lung**

Microscopic examination revealed that lung injury within the noninfected lung depended on both the MV strategy and body position (fig. 5). As expected, “protective” MV was associated with less tissue damage. The histologic scores tended to be greater in animals from the SP group than in those from the PP group, regardless of the MV strategy (table 2). Thus, there was an obvious loss of aeration within the lower lobe in the animals kept supine while the airspaces within the upper lobe appeared enlarged, especially if the MV was “adverse.” In contrast, lung aeration in the upper and lower lobes of the animals ventilated in the PP was quite similar. In addition, features like hyaline membranes and emphysema-like lesions were mainly seen in the animals kept supine.

However, no difference was found according to the body position regarding the WW/DW ratio when the animals were ventilated adversely. This suggests the formation of comparable amounts of permeability oedema in the two groups since far lower values were measured in the healthy rabbits (table 2). In contrast, the WW/DW ratio was significantly lower in the PP than in the SP when “protective” MV was applied.

**Fig. 3.** Interleukin (IL)-8 (A) and tumor necrosis factor-α (TNF-α) (B) concentrations in the infected lung (a), and the spleen (b) of rabbits with *Enterobacter aerogenes* unilateral pneumonia according to the mechanical ventilation strategy (i.e., “adverse” or “protective”), and the position (i.e., supine position [SP] or prone position [PP]). Results are expressed as median ± IQR. It is worth noting that IL-8 release was decreased within the pulmonary and the systemic compartment in the animals turned into PP. In contrast, no significant difference was seen regarding TNF-α. *P < 0.05 between SP and PP groups. IQR = interquartile range.*
The evaluation of pulmonary inflammation within the noninfected lung was based on IL-8 gene expression. We observed that there was a stronger induction of IL-8 gene expression in the noninfected lung from SP animals than in those from PP animals regardless of the MV strategy (fig. 6).

**Discussion**

In the current study, we showed that turning animals from the supine to the PP was likely to improve the features of *E. aerogenes* unilateral pneumonia regardless of the MV strategy (i.e., “adverse” or “protective”). Most of all, bacterial concentrations within both the instilled lung and the spleen were found to be lower in rabbits from the PP group than in those kept supine. In addition, IL-8 concentrations, a powerful chemoattractant for polymorphonuclears, were greater in the infected lung of the supine animals, as well as in an extrapulmonary organ, the spleen, suggesting that both lung and systemic inflammation were blunted by the PP. Taken together, these findings suggest that in our model, the PP improved lung bacterial clearance, reduced pulmonary-to-systemic translocation of bacteria, and mitigated the host inflammatory response. In addition, we showed that in this model of lobar lung injury, the PP was likely to diminish the damage inflicted by MV to the noninfected lung and to modulate inflammation as well. Moreover, our findings illustrate the hypothesis that although pneumonia is less severe when using clinically relevant ventilator settings, further improvements could be obtained by turning the animals to the PP.

The best way to ventilate patients with lung injury remains a matter of concern. Improving gas exchange and optimizing alveolar recruitment are key issues in this setting. However, animal models of ventilator-associated pneumonia similar to ours have shown that pneumonia could induce airway overdistension within the infected as well as the “healthy” lung in animals subjected to MV.\(^1^6,\!^3^2\) This deleterious effect of MV has been attributed to the loss of aeration within the infected pulmonary area, which could not be easily recruited by positive pressure. The magnitude of the local inflammatory response could account for this decrease in lung compliance.\(^3^3\) As a result, although protective if set at a moderate level, PEEP may become harmful if higher levels are reached since it could create lung overdistension.\(^2^5,\!^3^4\)

Prone positioning has been proposed primarily as an efficient way to improve gas exchange in patients with the
most severe forms of acute lung injury. More recently, it has been shown that the PP allows better distribution of lung inflation along the craniocaudal axis through improvement in respiratory system compliance, together with lung recruitment. Interestingly, some authors have shown that those patients in whom compliance of the respiratory system improved once turned to the PP were more likely to present with lobar lung injury including pneumonia. In addition, some recently published clinical studies showed that PP could either prevent ventilator-associated pneumonia or improve the outcome of existing ventilator-associated pneumonia, whenever patients had lung injury before pneumonia. Altogether, these findings suggest that PP could be of particular interest in the setting of lobar lung injury including bacterial pneumonia. Our results provide additional insights that are likely to improve our understanding of such data since they draw a link between the well-known beneficial effects of the PP on lung mechanics, and a possible improvement in the host response against bacterial infection. We were, however, unable to determine the underlying mechanisms as far as the host innate immunity is concerned. Further studies are necessary since as shown by our group and others the toll-like receptors pathway could be altered by the unusual mechanical stretch applied to the lung subjected to MV. One could only speculate that the PP attenuates lung distension leading in turn to the release of smaller amount of IL-8, thereby protecting the host from the detrimental effects of an overwhelming inflammatory response.

In addition to such beneficial effects of the PP on the infected lung regarding bacterial clearance and the inflammatory response, we showed that the PP was likely to reduce lung injury within the noninfected lung. The decrease in PCO₂ subsequent to the increase in tidal ventilation (i.e., when peak inspiratory pressure was set from 12 to 30 cm H₂O) was the same regardless of the body position, indicates that the PP did not necessarily increase the end-expiratory volume, as reported previously in acute respiratory pressure patients. However, strikingly, the distribution of lung aeration appeared to be different in the SP and the PP groups. In the SP group, there was a marked loss of aeration within the lower lobe whereas the airspaces of the upper lobe were enlarged. This resulted in the presence of emphysema-like lesions. In addition, the presence of hyaline membranes, one of the hallmarks of VILI, was encountered exclusively in the animals that were ventilated supine. Similar findings were obtained when the “protective” ventilator settings were applied, although tissue injury was less severe. Interestingly, IL-8 gene expression was markedly higher in the noninfected lung of the supine animals than in those turned to the PP, and this was independent of the MV strategy. This could be considered a surrogate for biotrauma induced by MV, thereby indicating a greater level of lung distension in the SP group. Surprisingly, there was no difference between SP and PP animals subjected to the “adverse” MV regarding the formation of lung permeability oedema within the noninfected lung as assessed by gravimetric measurements. We can only hypothesize that its distribution within the lung was different between groups since our approach (i.e., WW/DW measurement) provided only an overall assessment. However, as expected, VT reduction together with PEEP probably decreased the WW/DW ratio.

In addition, we showed that further improvement was achieved by turning the animals in the PP. Altogether, these findings suggest that the PP improves VT distribution...
within the lung and in turn reduces airspace overdistension thus preventing VILI, as already demonstrated in animal models of diffuse lung injury.28–30

Our study has several limitations. First, any extrapolation of our findings should be done very cautiously since small animals are known to be more prone to VILI than larger ones. Moreover, the MV settings used in the “adverse MV” group is far from the clinical practice. The way pneumonia was induced as well as the short duration of MV were also specific to our model and further experimental studies are necessary before our findings can be generalized. Second, one could argue that bacterial growth was better controlled in the PP because of improved drainage of bronchial secretions regardless of any alteration of lung strain.44 However, this could hardly account for the worsening of the lung injury within the contralateral lung. Third, the hemodynamic assessment was not performed extensively. As a result, we cannot exclude the possibility that the lower pulmonary-to-systemic translocation of both bacteria and mediators was subsequent to a drop in cardiac output in the PP group. However, previous clinical and experimental studies failed to show any difference regarding this point.26,30,36,45 Finally, we should acknowledge the small size of our experimental groups and the lack of any a priori calculation. Our study is therefore underpowered, making statistics difficult to interpret.

**Conclusion**

In a model of lobar lung injury, the PP may not only improve lung mechanics and blood oxygenation, but also enhance antibacterial defences and mitigate inflammation while protecting the contralateral lung.

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**References**


ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

Statikil Sparked Paul Wood’s Interest

Fifty years in the wake of losing Dr. Paul Meyer Wood, we celebrate the semicentennial of his namesake library-museum in Park Ridge, Illinois. Among the many odd items Dr. Wood collected was this bottle of Statikil, which was distributed by a firm located just 1.5 miles southwest of the New York brownstone that once harbored the Wood Library-Museum. Product labeling claimed that any garment immersed in Statikil (and then squeezed out and hung out to dry) would be rendered “spark-proof.” Statikil was yet another weapon against static electrical ignition of flammable anesthetic gases in the operating room. (Copyright © the American Society of Anesthesiologists, Inc.)

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