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EXPERIMENTAL

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Lung overinflation without positive end-expiratory pressure promotes bacteremia after experimental *Klebsiella pneumoniae* inoculation

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Abstract *Objective:* To determine the effect of peak inspiratory pressure (PIP) and positive end-expiratory pressure (PEEP) on the development of bacteremia with *Klebsiella pneumoniae* after mechanical ventilation of intratracheally inoculated rats.
Design: Prospective, randomized, animal study.
Setting: Experimental intensive care unit of a University.
Subjects: Eighty male Sprague Dawley rats.
Interventions: Intratracheal inoculation with 100 µl of saline containing 3.5–5.0 × 10^5 colony forming units (CFUs) *K. pneumoniae*/ml. Pressure-controlled ventilation (frequency 30 bpm; I/E ratio = 1:2; FIO_2 = 1.0) for 180 min at the following settings (PIP/PEEP in cmH_2O): 13/3 (n = 16); 13/0 (n = 16); 30/10 (n = 16) and 30/0 (n = 16), starting 22 h after inoculation. Arterial blood samples were obtained and cultured before and 180 min after mechanical ventilation and immediately before sacrifice in two groups of non-ventilated control animals (n = 8 per group). After sacrifice, the lungs were homogenized to determine the number of CFUs *K. pneumoniae*.
Measurements and results: The number of CFUs recovered from the lungs was comparable in all experimental groups. After 180 min, 11 animals had positive blood cultures for *K. pneumoniae* in group 30/0, whereas only 2, 0 and 2 animals were positive in 13/3, 13/0 and 30/10, respectively ($p < 0.05$ group 30/0 versus all other groups).
Conclusions: These data show that 3 h of mechanical ventilation with a PIP of 30 cmH_2O without PEEP in rats promotes bacteremia with *K. pneumoniae*. The use of 10 cmH_2O PEEP at such PIP reduces ventilation-induced *K. pneumoniae* bacteremia.

Key words *K. pneumoniae* · Bacteremia · Mechanical ventilation · Blood gases · Animal · Rat

Introduction

Patients suffering from acute lung injury (ALI) or acute respiratory distress syndrome (ARDS) who receive mechanical ventilation often develop pneumonia and finally die of septicemia or multiple organ failure (MOF) [1]. The realization is growing that bacterial translocation from the gut into the systemic circulation [2] and the systemic release of inflammatory mediators [3] play a major role in the pathophysiology of MOF.

Recently, it could be demonstrated that using a high peak inspiratory pressure (PIP) and not using positive end-expiratory pressure (PEEP) during mechanical ventilation have a synergistic effect on the release of pro-inflammatory mediators from the lung tissue into the airways [4]. Moreover, use of a high PIP was shown to induce the release of pro-inflammatory mediators

into the systemic circulation [5]. Lowering PIP [4, 5] or increasing the level of PEEP [4] reduced these pro-inflammatory cytokine levels. From these findings it was hypothesized that mechanical ventilation serves to initiate and/or propagate an inflammatory response in the lung that acts as a nidus for the development of a systemic inflammatory response and that MOF could, to a certain degree, therefore be caused by non-optimal mechanical ventilation [4].

Based on the observation that mechanically ventilated ARDS patients often develop pneumonia and septicemia, we raised the question whether mechanical ventilation can promote bacteremia. To test this hypothesis we investigated the role of peak inspiratory pressure (PIP) and the effect of positive end-expiratory pressure (PEEP) on the development of bacteremia with *Klebsiella pneumoniae* after mechanical ventilation of rats inoculated with *K. pneumoniae*.

Materials and methods

The study protocol was approved by the institutional Animal Investigation Committee. Care and handling of the animals were in accordance with the European Community guidelines. An inoculum of 3.5–5.0 × 10^5 colony forming units (CFU)/ml *K. pneumoniae* (ATCC 43816; serotype 2) was prepared as follows: stationary-phase cultures were prepared by incubation for 16 h at 37 °C in Mueller-Hinton broth (MHB; Difco Laboratories, Detroit, Michigan, USA). After proper dilution and reincubation for 90 min at 37 °C, the culture was washed twice with saline. The inoculum was stored on ice until use. To verify the number of viable bacteria in the inoculum, 100 µl of 10-fold dilution steps in phosphate buffered saline (pH 7.3; Oxoid Ltd., Basingstoke, UK) on ice were plated on Iso-Sensitest agar plates (Oxoid Ltd., Basingstoke, UK). The agar plates were incubated overnight at 37 °C and CFUs were counted the following day.

A total of 85 male Sprague Dawley rats (body weight 270–320 g) was used. Anesthesia was induced with a mixture of nitrous oxide, oxygen and ethrane (66/33/1–2 %). Anesthesia was maintained for approximately half-an-hour by intramuscular injection of a mixture of ketamine (12 mg/kg, Ketalin, Apharmo, Arnhem, The Netherlands) and xylazine (0.8 mg/kg, Xylalin, Apharmo). The rats were then inoculated intratracheally with 100 µl of the *K. pneumoniae* inoculum as previously described, using a Hamilton constant flow syringe [6]. The animals were housed in plastic cages overnight with standard chow (Hope Farms, Woerden, The Netherlands) and water ad libitum.

Twenty-two hours after inoculation, anesthesia was induced by inhalation (see above) and a polyethylene catheter was inserted into one of the carotid arteries for drawing arterial blood samples. Before tracheotomy, the animals received pentobarbital sodium (60 mg/kg BW, i.p., Nembutal, Algin, Maassluis, The Netherlands) and the inhalation of ethrane was decreased by 50 %. A metal cannula was inserted into the trachea and muscle relaxation was induced with pancuronium bromide (2 mg/kg BW, i.m., Pavulon, Organon, Boxtel, The Netherlands). The animals were then mechanically ventilated in parallel in a pressure-controlled mode (Siemens Servo 300 and 900C, Siemens-Elema, Solna, Sweden; frequency = 30 breaths/min; I/E ratio = 1:2; FIO_2 = 1.0). To re-aerate atelectatic lung areas induced by the surgical procedure, the airway pressure was increased to a PIP of 30 cmH_2O at a PEEP level of 3 cmH_2O for 30 s. The animals were then ventilated for 180 min at four different settings (PIP/PEEP): 13/3 (group 13/3); 13/0 (group 13/0); 30/10 (group 30/10) and 30/0 (group 30/0); n = 16 per group. Dead space was adapted to obtain normocapnia at t = 2 min in all groups. Body temperature was kept at 37 °C by means of a heating pad. Two groups of control animals without mechanical ventilation were killed 22 (group control t = 22 h) and 25 (group control t = 25 h) hours after inoculation; n = 8 per group.

Arterial blood gases in the ventilated groups were measured with conventional methods (ABL 505, Radiometer, Copenhagen, Denmark) 2, 60, 120 and 180 min after starting mechanical ventilation. Two milliliters of blood was drawn from the arterial line immediately before and 180 min after starting mechanical ventilation in the ventilated groups, and before sacrifice (overdose pentobarbital) in the non-ventilated groups and cultured undiluted for *K. pneumoniae* on two blood agar plates (Bactim, Breukelen, The Netherlands). To replace blood loss, a bolus of 2 ml Ringer's lactate at 37 °C was given through the arterial line. The blood agar plates were incubated at 37 °C overnight and the number of colonies was determined the next day. Bacteremia was defined as the presence of one or more colonies of *K. pneumoniae* in 2 ml of blood. Colonies were identified by standard microbiological methods. The average colony count per 2 ml of blood in blood positive animals in the different groups was calculated. Animals which had positive blood cultures for *K. pneumoniae* before starting mechanical ventilation were excluded from further analysis.

Pulmonary edema coming from the ventilatory tube in group 30/0 was collected over the 180 min ventilation period. The other groups had no pulmonary edema coming from the ventilatory tube. After sacrifice, at the end of the 180 min ventilation period and immediately in the control groups, the lungs were taken sterile from the thorax, weighed and homogenized in 20 ml saline for 1 min at 40,000 rpm with a blender (Virtis "23", The Virtis Company Inc., N.Y., USA). The number of viable bacteria in the lung homogenates and in the pulmonary edema of group 30/0 was determined by plating 10-fold dilution steps on Iso-Sensitest agar plates.

Statistical analysis

Intra-group comparisons for pH, PaO_2, $PaCO_2$ and number of CFUs recovered from the lung after logarithmic transformation, were analyzed with an ordinary ANOVA. If ANOVA resulted in a p less than 0.05 a Bonferroni post-test was performed. Inter-group comparisons for pH, PaO_2 and $PaCO_2$ were analyzed with repeated measures ANOVA with a Bonferroni post-test. Data for pH, PaO_2 and $PaCO_2$ are reported as the mean ± standard deviation (SD). Differences in the number of positive blood cultures for *K. pneumoniae* at t = 22 h and t = 25 h in all groups were determined with an exact χ^2-test on a 2 × 5 contingency table. If p was less than 0.05, Fisher's exact post-tests on 2 × 2 contingency tables were performed; statistical significance was accepted at p less than 0.05.

Results

Verification of the number of viable bacteria in the inoculum showed that the rats were inoculated with 100 µl of saline containing 3.5–5.0 × 10^5 CFU *K. pneumoniae*/ml.

The number of CFUs *K. pneumoniae* recovered from the lung homogenates was comparable in all groups

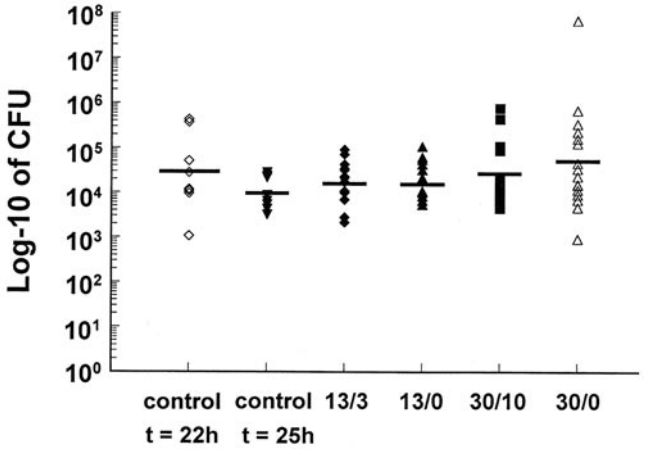

Fig. 1 Quantitative lung bacterial count in four experimental and two control groups. There were no significant differences in the number of bacteria recovered from the lung tissue

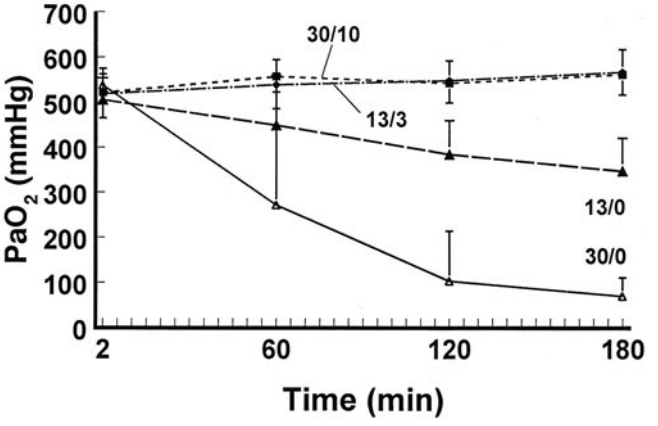

Fig. 3 PaO_2 values (mean ± SD) of the different ventilated groups. Legends indicate peak inspiratory pressure/positive end-expiratory pressure

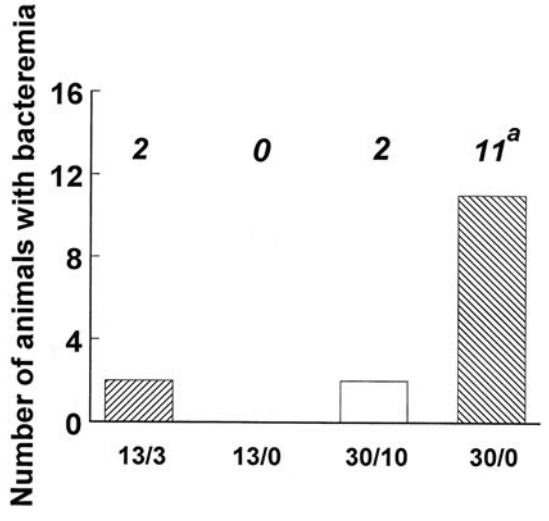

Fig. 2 Number of animals with positive blood cultures for *K. pneumoniae* in 2 ml of blood at t = 180 min, that were not bacteremic at t = 0 min ($n = 16$ in each group). [a] $p < 0.05$ versus all other groups

(Fig. 1). The number of CFUs *K. pneumoniae* recovered in the pulmonary edema of group 30/0 (2.5 ± 1.5 ml) was on average 17% of the number recovered from the lungs.

Five animals were excluded from analysis because they had bacteremia before starting mechanical ventilation (1, 1, 1 and 2 animals in groups 13/3, 13/0, 30/0 and 30/0, respectively). Figure 2 shows the number of animals with positive blood cultures for *K. pneumoniae* in the ventilated groups in the animals that did not have bacteremia before starting mechanical ventilation ($n = 16$ per group). Significantly more animals had positive blood cultures at t = 25 h in group 30/0 than in the other groups. The average colony count in positive animals was 1.0, 1.3 and 5.0 CFUs *K. pneumoniae*/2 ml in groups 13/3, 30/10 and 30/0, respectively. None of the animals in either control group were bacteremic.

Arterial oxygenation over time is given in Fig. 3. There were no decreases in oxygenation over time in groups 13/3 and 30/10, whereas oxygenation slowly decreased over time in group 13/0 and dropped significantly over time in group 30/0. All four groups were normocapnic and had normal pH values at t = 2 min (Table 1). The animals ventilated with PEEP remained normocapnic and retained normal pH values during the whole study period, whereas the animals in the groups ventilated without PEEP became hypercapnic and acidemic.

The lung weight (mean ± SD) was 1.5 ± 0.3, 1.5 ± 0.2, 1.8 ± 0.2, 3.0 ± 0.3, 1.6 ± 0.1 and 1.5 ± 0.1 in groups 13/3, 13/0, 30/10, 30/0, control t = 22 h and control t = 25 h, respectively and was significantly higher in group 30/0 compared to all other groups.

Discussion

The present study shows that mechanical ventilation with a combination of a high PIP of 30 cmH_2O without PEEP induces *K. pneumoniae* bacteremia after a ventilation period of 180 min. The use of 10 cmH_2O PEEP at the same PIP reduces *K. pneumoniae* bacteremia. A low PIP of 13 cmH_2O without PEEP or in combination with 3 cmH_2O of PEEP could not induce *K. pneumoniae* bacteremia in the given time period.

To exclude inter-group differences before starting mechanical ventilation, animals with positive blood cultures before starting mechanical ventilation were excluded from analysis. We chose to start mechanical ventilation 22 h after inoculation. Inoculation just before

Table 1 Data on arterial carbon dioxide tension and pH over time (mean ± SD) in the ventilated groups

Time (min)	2′		60′		120′		180′	
	pH	$PaCO_2$	pH	$PaCO_2$	pH	$PaCO_2$	pH	$PaCO_2$
Group								
30/0	7.41 ± 0.07	32.4 ± 6.1	7.36 ± 0.13[b,c]	43.4 ± 11.2[a,c]	7.18 ± 0.17[a]	54.1 ± 10.9[a,c]	7.08 ± 0.12[a]	77.0 ± 17.5[a]
30/10	7.35 ± 0.08	37.9 ± 5.9[d]	7.38 ± 0.04	43.8 ± 4.4[a,b,c]	7.39 ± 0.06[d,e]	39.3 ± 5.2[d,e]	7.42 ± 0.07[a,d,e]	37.8 ± 6.1[d,e]
13/3	7.35 ± 0.07[d]	37.8 ± 4.8[a,d]	7.40 ± 0.07	42.8 ± 7.3[a,e]	7.40 ± 0.05[d,e]	44.8 ± 6.1[a,e]	7.40 ± 0.06[d,e]	45.1 ± 4.3[a,d,e]
13/0	7.41 ± 0.05	35.8 ± 5.0	7.34 ± 0.06[a,b,c]	50.7 ± 8.4[a,b,c]	7.25 ± 0.07[a]	66.1 ± 16.1[a,c,d]	7.18 ± 0.08[a,b,f]	81.0 ± 14.3[a]

abc: intra-group comparisons over time; $p < 0.05$ vs [a] t = 2 min; [b] t = 120 min; [c] t = 180 min (Repeated measures ANOVA with Bonferroni post test if $p < 0.05$)
def: inter-group comparisons; $p < 0.05$ [d] group 30/0; [e] group 13/0; [f] group 30/10 (Ordinary ANOVA with Bonferroni post test if $p < 0.05$)

starting mechanical ventilation resulted in a significantly lower bacterial recovery from lungs that developed edema, which was attributed to a washout effect of edema on bacteria from the lungs into the small airways. Studies by Roosendaal et al. have shown that after the intratracheal inoculation of healthy rats with 8×10^4 CFUs *K. pneumoniae*, bacteremia does not develop in the first 24 h after inoculation [7]. Our pilot experiments showed that inoculation with a higher count of *K. pneumoniae* (3.5×10^5 CFUs) in healthy non-ventilated animals induced bacteremia in five out of eight animals after 22 h. Inoculation with a lower count of *K. pneumoniae* (3.5×10^3 CFUs) did not induce bacteremia 22 h after inoculation. These data showed a relationship between the concentration of the bacterial inoculum and the presence or absence of bacteremia. Mechanical ventilation at a PIP of 30 cmH_2O without PEEP 22 h after inoculation in the group inoculated with 3.5×10^3 CFUs induced bacteremia in three out of seven animals only. Therefore, the highest possible inoculum was used for the experiments, one comparable to the experiments of Roosendaal et al. [7].

It was first demonstrated by Webb and Tierney that mechanical ventilation can be injurious to intact animals [8]. Mechanical ventilation in rats at a PIP of 30 cmH_2O resulted in pulmonary perivascular edema, whereas a PIP of 45 cmH_2O without PEEP also induced intra-alveolar edema [8]. Subsequent studies in the same animal model have shown that ultrastructural changes to the lung parenchyma include damage to endothelial and epithelial cells with denudement of their base membranes [9]. The use of 10 cmH_2O PEEP at a high PIP has been shown to prevent permeability edema partially and almost completely prevent histologically assessed lung injury [8, 10].

The development of pulmonary edema in group 30/0 is probably the key factor for the increase in bacteremia in this group. Pulmonary edema may mediate bacteremia by several mechanisms. It has been shown that pulmonary edema dose-dependently impairs bactericidal activity of the alveolar macrophage [11], which is essential in the pulmonary defense against *K. pneumoniae* [12]. Moreover, it is a well-known fact that pulmonary edema, as seen in group 30/0, also results in a dose-dependent inhibition of pulmonary surfactant [13]. Surfactant impairment with a resulting impaired gas exchange with hypoxemia (Fig. 1) and acidosis (Table 1) as seen in group 30/0, are all factors that may be associated with a reduced efficacy of lung antibacterial defense [14, 15]. However, the average PaO_2 value of 70.1 mm Hg in group 30/0 is well above those currently regarded as safe (50 mm Hg) during artificial ventilation of ARDS patients [16]. Moreover, the animals in group 13/0 also had acidosis but did not develop bacteremia. Therefore, although hypoxemia and acidosis are factors which may contribute to the development of bacteremia, they are probably not the main cause of bacteremia in this study.

Studies in hamsters in paraquat-induced lung injury with prior *Pseudomonas aeruginosa* challenge suggest that the effect of this type of lung injury in facilitating bacteremia can be due to changes in both lung and systemic defense against this organism and not to the lowering of the threshold value for bacterial translocation from the lung into the bloodstream [17]. In a preliminary study in dogs inoculated with *Pseudomonas aeruginosa* and ventilated for 24 h (15 ml/kg) with or without the use of 10 cmH_2O PEEP it was shown that, without PEEP, lung defense was reduced and histologically assessed lung injury was increased; two out of four non-PEEP treated dogs showed positive blood cultures whereas none of four PEEP treated animals were positive [18]. In the present study, however, the number of viable bacteria recovered from the lung homogenates was the same in all experimental groups, which indicates that differences in lung defense due to pulmonary edema are not likely to be the main explanation for the difference in bacteremia.

It is conceivable that bacteria more readily gain access to the circulation from damaged lung parenchyma than from previously normal lung tissue [17, 19]. Pulmonary edema contributes to a great extent to ventilation-induced lung injury, although the exact mechanisms remain a point of discussion [20]. Peak inspiratory overstretching alone can not explain ventilation-in-

duced lung edema because the use of PEEP at the same high PIP prevents pulmonary edema [8, 21] (group 30/0 versus 30/10). This lack of pulmonary edema with PEEP has been attributed to reductions in capillary hydrostatic pressure, which will reduce filtration over the alveolo-capillary barrier [21]. However, such a mechanism can not explain the protective effect of PEEP on lung parenchymal changes [21]. Recently, it was shown that even mild surfactant changes predispose the lung to ventilation-induced lung parenchymal damage by promoting repeated opening and closure of alveolar units which create intrapulmonary shear forces [22]. It might be speculated that, in the present study, ventilation-induced surfactant inhibition by pulmonary edema [13] may have interacted with *K. pneumoniae*-induced surfactant changes through endotoxins or a direct effect of these bacteria on type II cells [14]. The rapid decrease in oxygenation in group 13/0, probably due to atelectasis, suggests that such surfactant changes have occurred. Ventilation-induced lung parenchymal changes may well be responsible for the lowering of the threshold for *K. pneumoniae* translocation in group 30/0, whereas such changes did not occur in the other groups.

Finally, pulmonary edema results in an increased lymph flow, which promotes drainage of bacteria from the lymphatics into the bloodstream [23], that may have been a contributing factor for increased bacteremia.

It can not be excluded that bacteremia originated from the splanchnic area, due to liver or splenic septic metastisis, which might have been present prior to starting mechanical ventilation, as demonstrated in other animal models of lung infection [17]. However, it has been shown that PEEP results in a reduced blood flow to the liver [24], which would increase the likelihood of the development of bacteremia and not result in a reduction in bacteremia, as shown in our study. Therefore, we believe that the effect of PEEP in reducing the development of bacteremia is mediated by a direct effect of the prevention of lung injury, as shown by others [8, 10], and not to secondary effects on other organs. Further studies are needed to fully elucidate the mechanisms of ventilation-induced bacteremia and the effect of PEEP.

The lung is a potential source of bacterema in intubated patients with *K. pneumoniae*. This study in rats shows that (1) mechanical ventilation with a high PIP of 30 cmH_2O without PEEP induces *K. pneumoniae* bacteremia; (2) the use of 10 cmH_2O PEEP at the same PIP reduces *K. pneumoniae* bacteremia. We suggest that such results are probably attributable to ventilation-induced damage of the alveolo-capillary barrier, which results in lowering of the threshold for bacterial translocation. When applied to a clinical setting, our data advocate the use of a level of PEEP sufficiently high to prevent bacteremia in intubated patients receiving mechanical ventilation.

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