Mechanisms of Ventilation-Induced Lung Injury

Role of Surfactant

Mechanisms of Ventilation-Induced Lung Injury: Role of Surfactant
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MECHANISMS OF VENTILATION-INDUCED LUNG INJURY

Role of Surfactant

MECHANISMEN VAN BEADEMINGS-GEÏNDUCEERDE LONGSCHADE

Rol van Surfactant

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CONTENTS

Preface

Every year, worldwide thousands of patients receive ventilatory support during surgery. Mechanical ventilation has also become an important therapy in the treatment of patients with an impaired pulmonary function and, in particular, in patients with the acute respiratory distress syndrome (ARDS), which is characterized by acute respiratory failure with changes to the endogenous surfactant system and permeability changes of the alveolo-capillary membrane.

Already early after the introduction of mechanical ventilation (1950s), it became realized that mechanical ventilation has several potential drawbacks and complications. Modes of mechanical ventilation, which combine high end-inspiratory lung volumes with low end-expiratory lung volumes, have been shown in healthy animals to induce permeability changes comparable to those seen in ARDS. These permeability changes as a result of mechanical ventilation have led to a growing realization that some of the pathophysiological changes seen in ARDS, may be a consequence of our ventilatory interventions, rather than a consequence of the primary disease process.

Ventilation-induced lung injury (VILI) has been extensively investigated, but such studies were largely descriptive. They quantified the permeability changes of the alveolo-capillary barrier to macromolecules and the development of extravascular lung water, and histologically assessed lung damage as a result of overstretching. The use of positive end-expiratory pressure (PEEP) was shown to reduce the permeability changes at the same degree of overstretching as measured by each of these parameters. Such studies have recognized the role of end-inspiratory overstretching of the lung parenchyma in the mechanisms of VILI, and the reduction of capillary filtration pressure in the effect of PEEP on VILI. However, they failed to recognize ventilation-induced changes to the endogenous surfactant system as a mechanism of VILI. Moreover, the effect of PEEP on changes to the endogenous surfactant system, as a reason for the reduction in permeability changes associated with VILI, was not investigated.

Despite ventilatory support measures, the mortality of ARDS has not decreased since it was first described in 1967 and multiple organ failure (MOF) is still the leading cause of death. Although the exact mechanisms of the transition of ARDS into MOF are still largely unclear, bacteremia/endotoxemia and systemically circulating inflammatory mediators are thought to play an important role in the pathogenesis of the disease process. Given the potential harmful effect of mechanical ventilation, one reason for the high mortality of ARDS may be the fact that there is no routinely available early indicator to monitor and recognize metabolic changes to the lung as a result of our ventilatory interventions. Furthermore, mechanical ventilation may play a role in the pathogenesis of the transition of ARDS into MOF. One of the mechanisms could involve ventilation-induced bacterial translocation from

the lung into the bloodstream, especially given the high rate of ventilator-associated pneumonia in patients on mechanical ventilation. Another mechanism could be the release of inflammatory mediators from the lung into the bloodstream as a result of mechanical stretch on the lung parenchyma due to mechanical ventilation.

The first part of this thesis focuses on the changes to the surfactant system resulting from mechanical ventilation, the role of ventilation-induced surfactant changes in the mechanisms of VILI, and the effects of exogenous surfactant therapy on VILI. In addition, an early marker to recognize VILI is discussed. The second part of this work addresses ventilation-induced bacterial translocation and inflammatory mediator release from the lung into the systemic circulation as a result of different forms of mechanical ventilation.

In total, this work provides a more comprehensive understanding of the mechanisms of ventilation-induced lung injury.

Overview of the thesis

Focusing on experimental studies, *Chapter 1* gives an overview of the literature on ventilation-induced lung injury (VILI). It compromises disturbance of the fluid balance over and integrity of both the endothelial and the epithelial layer. Special attention is given to the role of ventilation-induced surfactant changes and the effects of positive end-expiratory pressure (PEEP) on VILI. Moreover, this chapter addresses new insights in the possible mechanisms by which ventilation-induced changes in the lung tissue may exert systemic effects and effects on other organs. A basic physiological rational to prevent or minimize ventilation-induced lung injury and surfactant changes, and future directives on how to optimally use monitoring techniques during mechanical ventilation are presented.

The study presented in *Chapter 2* describes three mechanisms of ventilation-induced surfactant impairment in a rat model of lung overinflation, which was first described by Webb and Tierney (1974) and later used in studies by Dreyfuss and colleagues (1985). Much of the experimental evidence on the factors contributing to VILI and the effect of PEEP on VILI comes from this animal model. This study also describes the effect of applying 10 cmH_2O of PEEP on ventilation-induced surfactant changes at the same degree of overinflation.

After investigating the mechanisms of surfactant changes during VILI and describing the possible role of such changes in the mechanisms of VILI, *Chapter 3* reports the effect of administration of different doses of exogenous surfactant in the rat model. These investigations address the effect of exogenous surfactant preceding lung overinflation on lung function, lung mechanics and lung permeability as assessed by transfer of Evans blue dye over the alveolo-capillary barrier.

Chapter 4 describes the effect of exogenous surfactant administration on the clearance of the radioactive tracer molecule ^{99m}Tc-human serum albumin from the lung into the systemic circulation in a lavage model of acute lung injury. Using more conventional methods, this study investigates if the surfactant system is rate-limiting for the transfer of solutes over the alveolo-capillary barrier.

Exogenous surfactant is an expensive treatment modality and the effects of mechanical ventilation on exogenous surfactant are still largely unknown. Therefore, *Chapter 5* addresses the use of a pressure-constant time-cycled ventilation mode with high PEEP levels and minimal pressure swings in a mode creating auto-PEEP, as a ventilatory strategy to adequately support gas exchange and minimize surfactant loss and protein infiltration after exogenous surfactant therapy. The effects of such a ventilatory strategy were compared to the effects of strategies combining larger pressure swings with lower PEEP levels.

The realization that mechanical ventilation may, by itself, induce lung injury should initiate a search for early markers of the possible adverse effects of our ventilatory interventions. In *Chapter 6* the breakdown products of adenosine-tri-phosphate are presented

as early markers of VILI. The effects of PEEP application, and the administration of exogenous surfactant on the release of these markers after lung overinflation, are described.

It is becoming increasingly realized that mechanical ventilation leads to the same pathophysiological features as those seen in the acute lung injury (ALI). The fact that the majority of patients with MOF develops ALI as the initial organ failure, indicates that ALI is an important and possibly causative part of an inflammation-induced systemic disease state that can evolve to MOF and that mechanical ventilation is one of the factors that initiates MOF. *Chapter 7* describes the effect of lung overinflation, with or without PEEP, in inducing bacterial translocation from the lung into the systemic circulation compared to low pressure strategies with or without the use of low levels of PEEP. Recent studies have shown the synergistic effect of lung overinflation and not using PEEP on ventilation-induced mediator expression in isolated lungs of rodents. *Chapter 8* addresses the role of alveolar end-expiratory collapse in inducing ventilation-induced inflammatory mediator release from surfactant-deficient lavaged rats lungs *in vivo*. Both ventilation-induced bacterial translocation and inflammatory mediator expression were investigated as possible mechanisms by which mechanical ventilation leads to the transition of ALI/ARDS into MOF.

Chapter 1

Mechanisms of ventilation-induced lung injury: Physiological rationale to prevent it

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Introduction

Acute respiratory distress syndrome (ARDS) has become a well-recognized condition that can result from a number of different causes [1]. The final common pathway results in damage of the alveolar epithelium and endothelium leading to high permeability edema.

The mortality rate from ARDS has not decreased since it was first described [2] and mortality rates range from 10% to as high as 90%, with an average of 50% [3]. Multiple organ failure (MOF) is the leading cause of death in ARDS [4] and the majority of patients with MOF develops ARDS as the initial organ failure [5]. Therefore, ARDS is now being regarded as an important, causative part of an inflammation-induced systemic disease state that can evolve to MOF, rather than as a sole pulmonary disease process [6]. However, new immunological treatment approaches for ARDS and MOF have been subjected to clinical trials without great success [7]. Two recently published clinical studies have shown a decreased mortality in ARDS by protective lung strategies using exogenous surfactant therapy [8] and mechanical ventilation which prevents the overdistension and repeated collapse and reexpansion of alveoli [9,10].

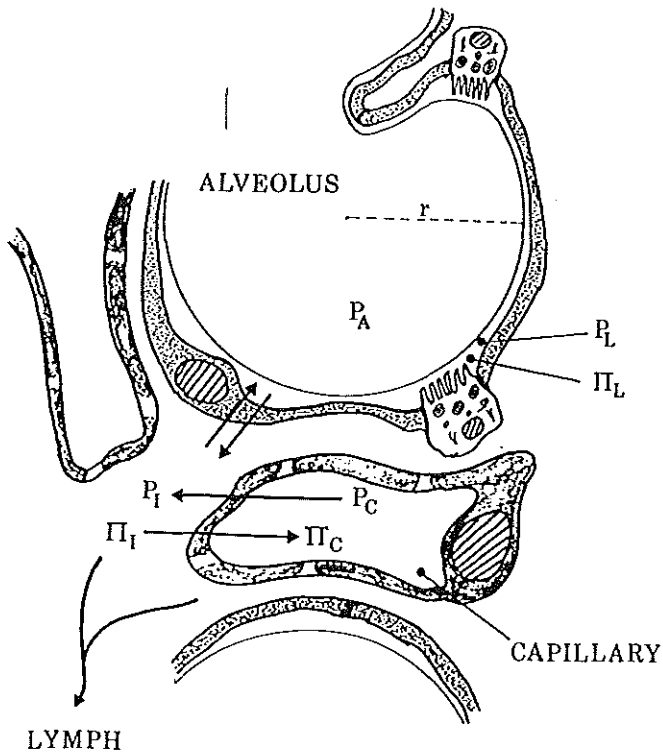


Figure 1 Diagram of the alveolus showing the various forces determining the fluid balance between the three liquid compartments of the alveolo-capillary barrier. *(Used with permission from Reference 12)*

In this review we will present an overview of recent studies on the mechanisms of ventilation-induced lung injury which provide an explanation for the reduced mortality from ARDS due to protective lung strategies, and give directions to prevent complications in healthy patients and patients with respiratory failure on ventilatory support.

Physiology of the fluid balance across the alveolo-capillary membrane

The alveolo-capillary barrier compromises three extracellular liquid compartments: 1) the vascular space, 2) the interstitial space and 3) the liquid in the lumen of the alveoli, which are separated by the capillary endothelium and the alveolar epithelium, respectively.

a Fluid balance across the capillary barrier

The driving force for exchange of hydrophillic solutions across the pulmonary capillary through 'open' intracellular junctions is determined by several factors [11, 12] (Figure 1).

1) The outward directing capillary hydrostatic pressure (p_c), which increases with the amount of filling of the capillary and is the highest in the most dependent parts of the lung.

2) The inward directing oncotic pressure difference across the capillary, which is determined by the positive difference of the plasma colloid oncotic pressure (π_c) and the interstitial colloid oncotic pressure (π_i) [13].

3) The alveolar surface tension, determined by the attractive forces of the molecules at the air-liquid interface of the alveolus. These forces result in suctioning where the curvature of the alveolar wall at the border of a capillary is sharp; they decrease filtration where the pulmonary capillary bulges into the alveolus, supporting the capillaries like the hoops of a barrel [14, 15] (Figure 2). The presence of surface tension at the air-liquid interface of the lung is believed to generate most of the observed negative pressure in the interstitial space (p_i) at those places where the capillary does not protrude into the alveolus.

4) The resulting small net filtration is balanced by lymphatic drainage of the interstitium, which also contributes to the subatmospheric pressure in the interstitial spaces, favoring fluid filtration [16].

b Fluid balance over the epithelial barrier

Exchange of fluid also takes place over the alveolar epithelium between the interstitial and alveolar fluid compartment [12] (Figure 1).

1) The hydrostatic pressure (p_L) of the fluid compartment in the alveolus lining the epithelial layer is equal to the pressure of gas in the airspace (p_A), minus a certain amount of pressure necessary to compensate the collapse tendency of the alveolus ($p_{Collapse}$) caused by the retractive forces at the air-liquid interface of the alveolus. This collapse pressure is given by the law of LaPlace, $p_{Collapse} = 2\gamma/r$ (γ = surface tension at the air-liquid interface; r = radius of the alveolus) and is very low in the normal alveolus. Depending on the status of the alveolus, inflating or deflating, p_L directs a fluid stream into or out of the alveolus.

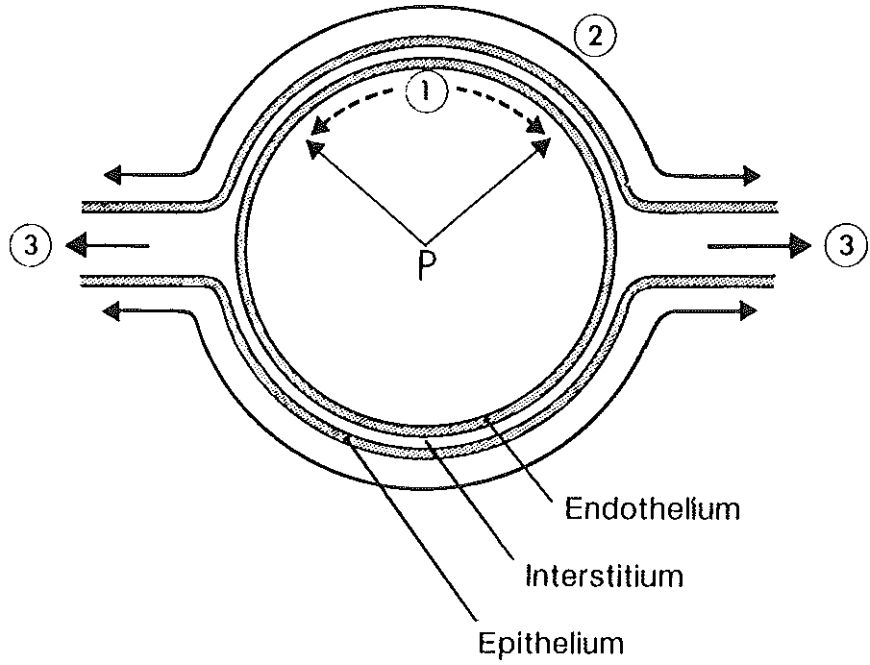


Figure 2 Pulmonary capillary in an alveolar wall showing the three principal forces to which the vessel is exposed. Both an increase in capillary pressure (1) and an increase in lung inflation (3) stretch the capillary wall. The surface tension of the alveolar lining layer (2) is thought to be protective. *(Used with permission from Reference 15).*

2) The interstitial pressure (p_i) is negative, which favors a fluid stream from the alveolus into the interstitial spaces (see above).

3) The epithelium is impermeable for proteins. Therefore, a large oncotic pressure gradient may be created between the alveolar (π_A) and interstitial (π_i) fluid compartment. The magnitude is unknown, but a net oncotic pressure gradient favoring absorption has been reported [12].

4) Finally, there is an active transport of sodium by the epithelium out of the lung lumen, which leads to active lung water transport out of the lung lumen into the interstitium.

Disturbance of the fluid balance due to mechanical ventilation

Despite initial controversy about the role of mechanical ventilation in inducing lung injury [17] it has now been undisputably demonstrated in different animal models that

mechanical ventilation at high peak inspiratory lung volumes can cause lung injury and edema, which does not fundamentally differ from that seen in human acute respiratory distress syndrome (ARDS) [18]. Pulmonary edema is considered hydrostatic when due to increased hydrostatic pressures and/or filtration and when the permeability of the endothelial barrier to protein is intact; it is considered high permeability edema when it is caused by permeability of the endothelial barrier to protein. The distinction between the two is a grey area because increased permeability makes the lung more susceptible to increased hydrostatic pressures/filtration [19] and, on the other hand, high capillary circumferential tensions eventually lead to permeability changes [15].

a Surfactant changes due to mechanical ventilation

Pioneering work of Mead in 1959 showed that mechanically ventilated dogs had a progressive fall in pulmonary compliance [20]; such mechanical changes were related to the pulmonary surfactant system as shown by Greenfield and coworkers who demonstrated increased surface tensions of lung extracts in dogs ventilated at peak inspiratory pressures of 28-32 cmH_2O for 1 to 2 hours [21]. A subsequent report by Sladen et al. showed that also patients ventilated for long periods suffered from an increased alveolar-arterial oxygen gradient, and a fall in respiratory system compliance [22].

Early studies by Benzer have demonstrated that rabbits ventilated with an open thorax at a peak inspiratory pressure of 30 cmH_2O with 5 cmH_2O of PEEP or with a closed thorax without PEEP had a better preserved surfactant system at the end of a 75-minute ventilation period than animals ventilated with an open thorax without PEEP [23]; these observations were extended in a subsequent report by Woo and Hedley-Whyte who observed pulmonary edema foam in the airways of open-chest dogs ventilated with large tidal volumes, whereas the same ventilator settings in closed-chest animals induced no such abnormalities [24].

Two primary mechanisms of surfactant inactivation by mechanical ventilation have been described. First, mechanical ventilation was shown to enhance surfactant release from the pneumocyte type II into the alveolus [25-28]. This material is subsequently lost into the small airways as a result of compression of the surfactant film when the surface of the alveolus becomes smaller than the surface occupied by the surfactant molecules [29, 30] (Figure 3).

A second mechanism to describe the surfactant changes associated with mechanical ventilation is based on the observation that the alveolar surface area changes associated with mechanical ventilation, result in the conversion of surface active large surfactant aggregates into non-surface active small surfactant aggregates [31-33] (Figure 4).

Surfactant changes due to mechanical ventilation are reversible as a result of a metabolically active process, involving de novo production of surfactant [34]. It probably involves a balance between secretion and production of large aggregates, and uptake clearance and reconversion of small aggregates in the pneumocyte type II [35].

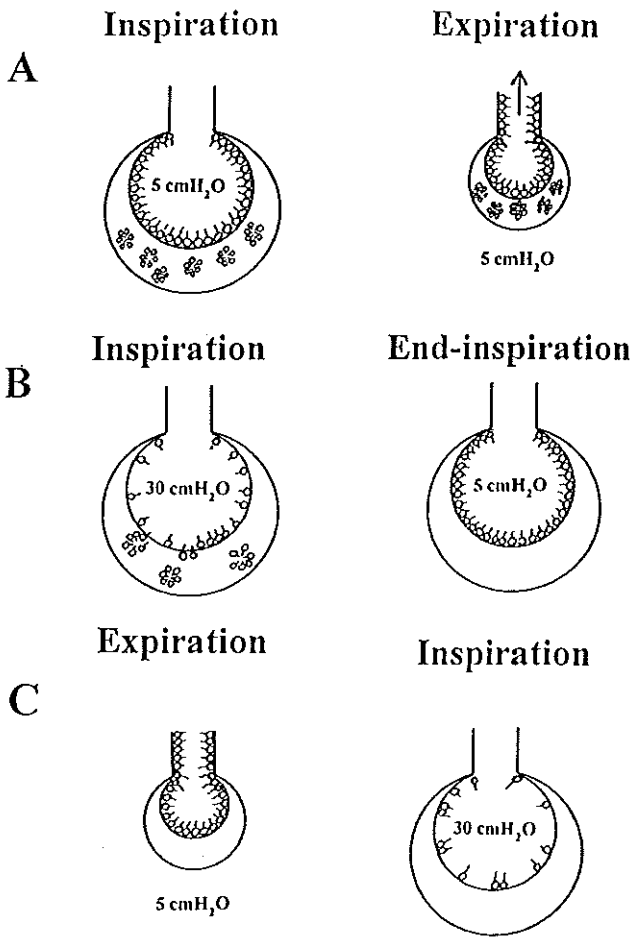


Figure 3 (A) Balance between synthesis, release and consumption of surfactant in the healthy lung. The pressure values given represent the intrapulmonary pressure needed to open up the alveolus. At the surface and the hypophase (micelles), there are sufficient molecules of surfactant. These micelles deliver the surfactant necessary to replace the molecules squeezed out during expiration.

(B) Imbalance between synthesis, release and consumption of surfactant due to artificial ventilation. At the beginning of inspiration, there is an apparent deficiency of surfactant molecules but there is a respreading of molecules stored in the hypophase of the surfactant layer. At the end of inspiration there is, in principle, enough surfactant on the surface.

(C) With the next expiration, surface active molecules are squeezed out and no surface active molecules are left in the hypophase for respreading, creating the situation where a serious surfactant deficiency follows.

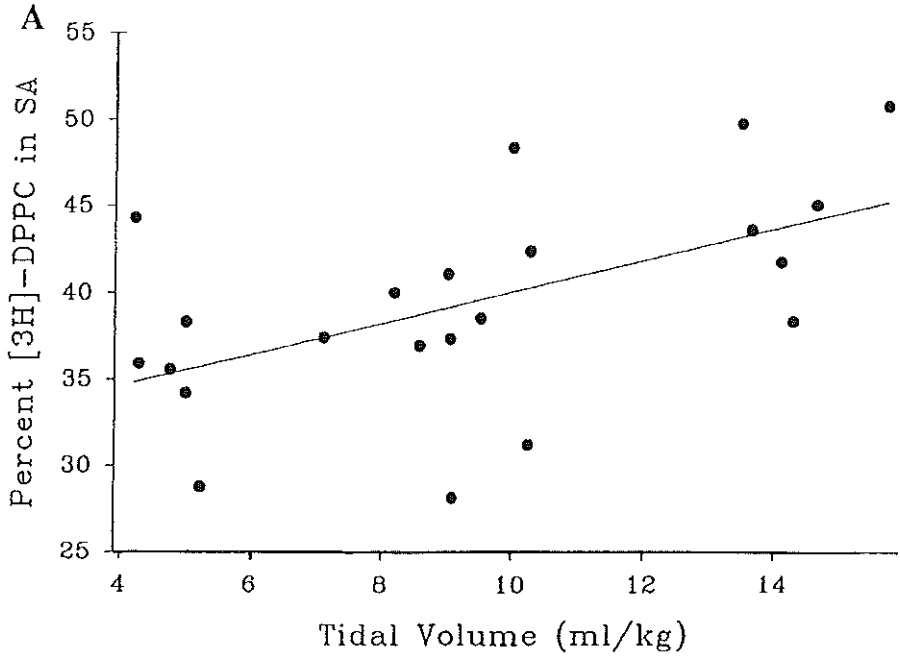


Figure 4. Effects of varying tidal volumes (from 4-6 mL/kg to 13-16 mL/kg) on surfactant aggregate conversion in vivo expressed as the amount of [^{3}H]DPPC recovered in the small aggregate fraction as % total [^{3}H]DPPC recovered in the alveolar wash after 1 h of ventilation. Aggregate conversion correlated significantly with increasing tidal volumes ($p < 0.01$, $r = 0.54$). *(Used with permission from Reference 31)*

Consequences of surfactant changes for fluid balance and solute permeability of the alveolo-capillary barrier

The hypothesis proposed by Pattle and Clements [36, 37] that surfactant inactivation with an increase in alveolar surface tension results in a decrease in pericapillary pressure was proven by demonstrating increased transmural filtration pressure, in experiments in which surfactant impairment was induced by cooling the lung and ventilation with large tidal volume [38] or by aerosolation of detergent [39].

However, surfactant dysfunction has also been shown to increase the permeability of the alveolo-capillary barrier to small solutes e.g. technetium-99m-labelled diethylene triamine pentaacetic acid (^{99m}Tc-DTPA) in the absence of other substantial changes in the function of

the alveolocapillary unit [40], and increased surfactant content in healthy ventilated rabbits was shown to reduce the permeability for the same molecule [41]. However, surfactant is not only rate-limiting for the transfer of small solutes; studies in both premature animals [42, 43] and adult surfactant-depleted animals [44] have shown that surfactant treatment before starting mechanical ventilation substantially reduces the transfer of albumin over the alveolo-capillary barrier. Studies in smokers compared to healthy controls have shown that a reduction in the amount of phosphatidyl choline in broncho-alveolar lavage of smokers is associated with changes in the permeability of the alveolo-capillary barrier to ^{99m}Tc-DTPA [45].

These studies indicate that surfactant has a primary role in the regulation of the permeability of the alveolo-capillary barrier to small solutes and protein. This may be due to both a direct action of surfactant on the alveolo-capillary barrier and a reduction of the structural damage caused by mechanical ventilation due to the presence of surfactant (see below).

b Disturbed fluid balance over the capillary barrier due to mechanical ventilation

Functional integrity of both the endothelium and epithelium is a prerequisite for maintaining a normal fluid balance at the alveolo-capillary membrane.

Both increased capillary filtration pressure and altered microvascular protein permeability have been shown to contribute to pulmonary edema after lung overinflation. Studies in open-chest large animals, which indirectly calculated the capillary filtration pressure from measurements of mean pulmonary artery and left atrial pressures after lung overinflation at peak inspiratory pressures of around 60 cmH_2O, demonstrated a mild increase in mean transmural microvascular pressure as a result of overinflation when compared to normal conditions [46, 47]. However, any increase in transmural microvascular pressure will have a dramatic effect on edema formation when the microvascular barrier has altered sieving properties [19]. The three basic forces acting on the capillary wall which can eventually result in loss of its functional integrity have been reviewed by West [15] (Figure 2):

1) An increase in the circumferential tension (which is directly proportional to the transcapillary wall pressure and the capillary radius, and inversely proportional to the wall thickness). It may be speculated that ventilation-induced surfactant impairment with alveolar collapse, hypoxic vasoconstriction and redistribution of blood flow to selected capillaries, may increase transcapillary wall pressure and thus circumferential tension in those capillaries. In principal this will result in hydrostatic edema, but it may be speculated that it becomes of the permeability type if transcapillary pressure reaches 40 mmHg or above. Injury at such pressures is not limited to the endothelial cells but also causes breaks in type I cells [48].

2) Surfactant inactivation due to mechanical ventilation results in loss of the supportive 'hoop' function by surfactant on the capillary wall (Figure 2). An increase in surface tension therefore causes a reduction in perivascular pressure, with an increase in distending pressure on the capillary. Nieman et al. showed that the number of alveoli with continuous blood flow (zone III conditions) increases after lung lavage with a detergent solution elevating surface

tension [49].

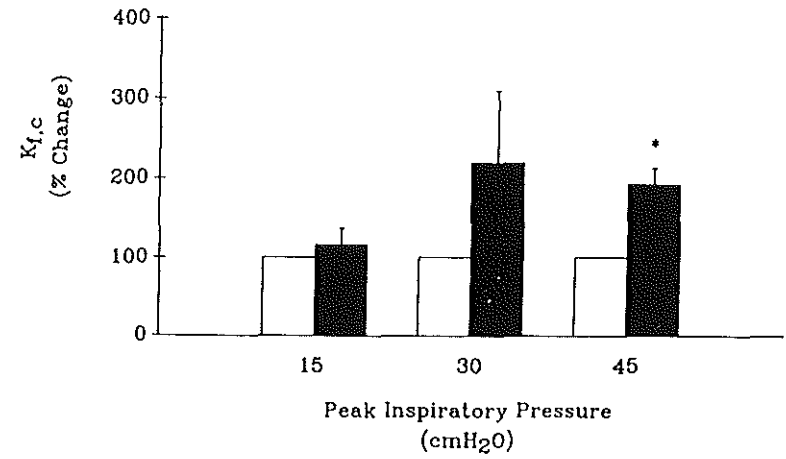
3) Longitudinal tension on the capillary due to lung overinflation. Fu and colleagues have shown that increasing lung volume by increasing transpulmonary pressure from 5 to 20 cmH_2O at a constant capillary pressure of 32 cmH_2O resulted in a significant increase in the number of endothelial and epithelial type I breaks [50]. The increase in the number of endothelial breaks produced by equivalent increases in transpulmonary pressure and capillary transmural pressure were similar. Thus, vascular pressures too low to affect microvascular permeability at low lung volume may increase microvascular permeability when the lung volume is sufficiently increased.

Many studies in open and closed chest animals using different approaches have shown that lung overinflation is associated with changes in microvascular permeability [46, 47 51-53]. The existence of a pressure threshold above which microvascular permeability changes occur has been suggested [54], although others suggested the absence of a well-delimited pressure or volume threshold [55]. Independent of this, it has become clear that microvascular injury secondary to ventilation occurs at much lower airway pressures and volumes in isolated perfused lungs with inactivated surfactant due to dioctyl succinate, as compared to ventilation of healthy lungs [56] (Figure 5). These studies suggest that lungs with an impaired surfactant system are more susceptible to overinflation than healthy lungs and that minor surfactant alterations, such as those produced by spontaneous ventilation during prolonged anesthesia [57, 58] are sufficient to synergistically increase the harmful effects of overinflation on permeability of the endothelial barrier [59].

Similarly, whereas either oleic acid or mechanical ventilation in isolated lungs did not significantly affect capillary permeability, the combination of the two did [60]. Studies in intact animals suggested a synergism rather than additivity between lung injury induced by mechanical ventilation and α-naphthyl-thiourea [59] in inducing endothelial permeability.

c Disturbed fluid balance over the epithelial barrier due to mechanical ventilation

It is known that more than the endothelium or interstitial spaces, the epithelium is rate-limiting for solute and fluid movement between blood and alveolus [61, 62]. Effects of overinflation on epithelial permeability have been studied in fluid-filled in situ lobes, to exclude the effect of surface tension. As the epithelium is progressively stretched during static inflation there is a non-reversible opening of water-filled channels between alveolar cells resulting in free diffusion of small solutes and even albumin across the epithelial barrier [63, 64] (Figure 6). Such changes were shown to occur only at high distending pressures and have been attributed to peak inspiratory epithelial overstretching which occurs due to inflation in the supra-physiological range only [65]. Experimental studies with small solutes like ^{99m}Tc-DTPA have shown that the rate of clearance of this tracer from the alveolar space increases with increases in lung volume, whether caused by large tidal volume ventilation [66] or PEEP [67].



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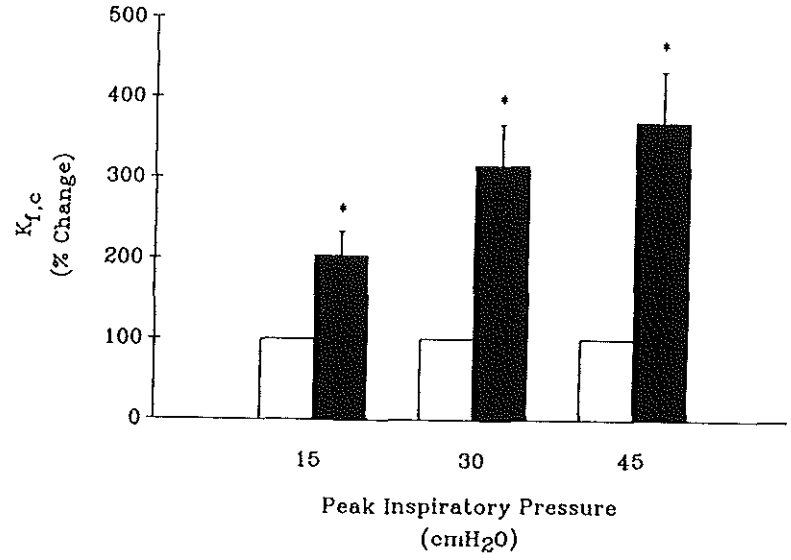

B

Figure 5. Difference in change in capillary filtration coefficient ($K_{f,c}$) from baseline in lungs not pretreated with dioctyl succinate detergent (A) and pretreated with dioctyl succinate detergent (B), which inactivates surfactant, and then ventilated at peak inspiratory pressures of 15, 30 and 45 cmH_2O. The capillary filtration was significantly more increased from baseline at 15, 30 and 45 cmH_2O peak inspiratory pressure in animals pretreated with dioctyl succinate detergent. Open bars = baseline; solid bars = dioctyl succinate detergent plus ventilation. $^*p < 0.05$ compared with baseline values. *(Used with permission from Reference 56)*

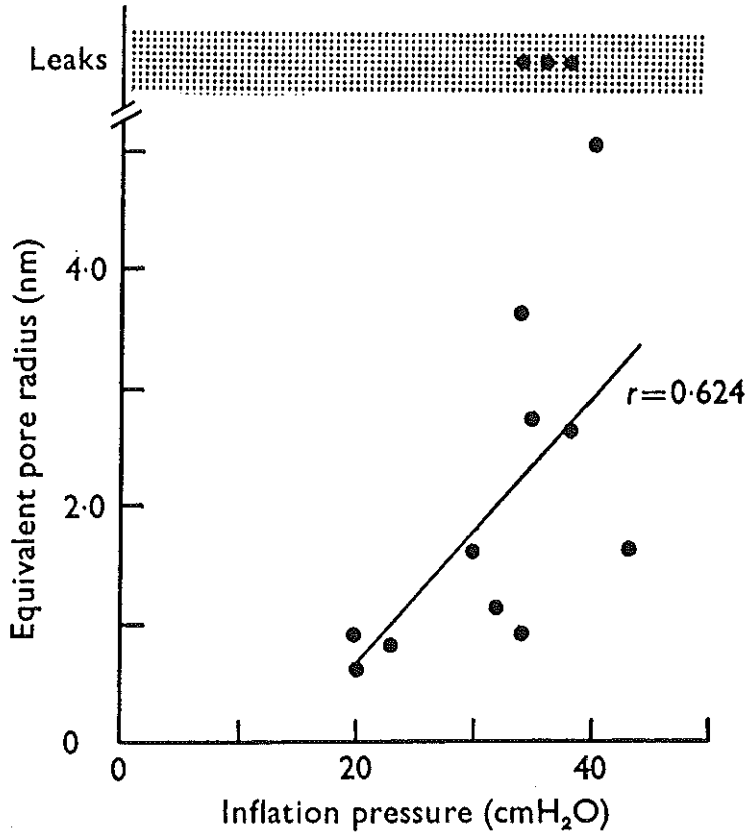

Figure 6. Effect of inflation pressure on the epithelial permeability of fluid-filled in situ sheep lobes. Permeability is characterized by an equivalent pore radius. A linear relationship was observed between inflation pressure and pore radius. At the highest levels of inflation, free diffusion of albumin was sometimes observed, indicating the presence of large leaks. *(Used with permission from Reference 63)*

Due to damage of both the epithelial and endothelial barrier, surfactant components may be lost into the bloodstream [68-71]. More importantly, intra-alveolar protein infiltration will develop which results in dose-dependent inhibition of surfactant [72-75]. As surfactant is rate-limiting for the transfer of proteins over the alveolo-capillary barrier, loss of surfactant function will lead to further protein infiltration. This may result in a self-triggering mechanism of surfactant inactivation.

d Changes in lymph flow

It is clear that mechanical ventilation leads to severe disturbance of both the fluid balance over the endothelial and epithelial barrier. Lymph flow constitutes an important safety factor against the development of pulmonary edema. The pumping action of the lymphatics effectively drains the interstitium under normal conditions and when filtration pressure increases, lymph flow may increase up to 8-fold. If filtration increases more, the capacity of the lymphatics to deal with the volume of liquid presented to them is exceeded and progressive pulmonary edema will develop [76].

e Other effects

Increased filtration during mechanical ventilation also occurs at the extra-alveolar level. Inflating the lung decreases the pressure in the perivascular spaces due to pulmonary interdependence and dilates the extra-alveolar vessels. This has been established in both excised lungs and in in situ lungs in open-chest animals [77, 78].

Role of pressure and volume in ventilation-induced lung injury (VILI)

Studies with high peak inspiratory pressure ventilation where peak inspiratory lung volume was limited by thorax restriction, have suggested that the end-inspiratory lung volume and not end-inspiratory pressure is the main determinant of VILI [51, 79]. However, the alveolar pressure alone as measured in such studies does not provide a measure of alveolar distension. Rather than the absolute airway pressure, the absolute transpulmonary pressure (which is equal to the alveolar pressure minus pleural pressure), is responsible for injury. Therefore, at a given lung-thoracic compliance, absolute transpulmonary pressure and end-inspiratory lung volume are interchangeable and non-discernible with respect to their injurious potential.

Structural damage of the alveolo-capillary barrier due to repeated collapse and reexpansion of alveoli

As discussed above, the basic mechanism for loss of alveolo-capillary barrier function has been considered to be peak inspiratory endothelial and epithelial overstretching with widening of intracellular junctions. However, one idea of ventilation-induced lung injury and epithelial stretching goes back to the pioneering work of Mead who demonstrated that, due to pulmonary interdependence of alveoli (Figure 7), the forces acting on the fragile lung tissue in non-uniformly expanded lungs are not only the applied transpulmonary pressures, but rather the shear forces that are present in the interstitium between open and closed alveoli [80]. An alveolus with surfactant impairment would be predisposed to end-expiratory alveolar collapse and prone to be affected by such 'shear forces'. Shear forces, rather than end-inspiratory overstretching, may well be the major reason for epithelial disrupture and loss of barrier function of the alveolar epithelium and considerable increases in regional microvascular transmural pressure.

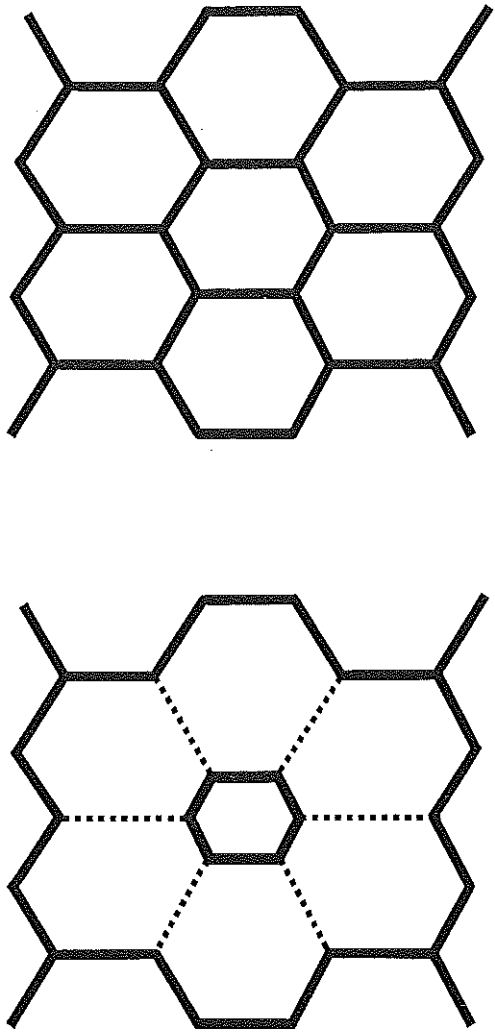

Figure 7. Shear forces are caused between open and closed alveoli due to pulmonary interdependence of alveoli. This figure shows the difference between mechanical ventilation of normal alveoli (upper panel) and mechanical ventilation of the same alveolar unit after surfactant inactivation (lower panel) *(After Reference 80).*

Important evidence for this mechanisms comes from the findings that ventilation at

low lung volumes can also augment lung injury in lungs with an impaired surfactant system [81] a concept first proposed by Robertson et al. [82]. A recent study in a model of subtle surfactant perturbation by dioctyl sodium sulfosuccinate showed that surfactant changes make the lung vulnerable to lung parenchymal injury by mechanical ventilation [83]. These studies confirm earlier work of Nilsson et al. in ventilated premature newborn rabbits with a primary surfactant deficiency. Fetuses treated with surfactant before receiving mechanical ventilation had less bronchiolar epithelial lesions in comparison to non-surfactant treated controls [84].

It has been demonstrated that most endothelial and epithelial permeability changes induced by lung overinflation are reversible [85] with excess pulmonary fluid clearance through the lymphatics [47], although some microvascular permeability alterations [47] and protein in the epithelial lining fluid [85] were shown to persist for longer periods of time.

Effects of increased end-expiratory lung volume by positive end-expiratory pressure (PEEP)

a Effects of PEEP on lung edema

Initial studies have investigated the effect of increasing levels of PEEP at constant tidal volume ventilation, which resulted in higher end-inspiratory pressures and volumes. Such studies found that increasing levels of PEEP reduced shunt [86, 87] and improved oxygenation and lung mechanics [88] which was attributed to reopening of flooded alveoli with redistribution of edema fluid from flooded alveoli into the interstitial spaces [89-91]. Such studies, however, also demonstrated that the use of high PEEP levels did not reduce [86, 88, 92] or even increase edema formation [87, 93]. These findings have been reported in both isolated perfused lungs [86] and in closed-chest healthy animals [88] and closed-chest animals with different forms of lung injury induced by bronchial hydrochloric acid administration [87], alloxan [92], oleic acid [94] or hydrostatic edema due to lobar venous occlusion [93]. Overinflation due to PEEP is probably the explanation for the lack of reduction or even worsening of edema reported with PEEP during such experiments [95].

However, it has now been unequivocally demonstrated in different animal models that ventilation with PEEP at lower tidal volumes results in less edema than ventilation without PEEP and a higher tidal volume for the same peak or mean airway pressure [52, 79, 95, 96] and that, more specifically, PEEP prevents alveolar flooding [33, 52].

b Effects of PEEP on lung parenchymal injury

Studies by Dreyfuss et al. in rats ventilated at peak inspiratory pressure of 45 cmH_2O have shown that damage due to mechanical ventilation begins at the endothelial side after 5 min and rapidly progresses to the epithelium after 20 min [53]. A subsequent study showed a reduction of endothelial injury and the preservation of the structure of the alveolar epithelium by use of 10 cmH_2O of PEEP, which was accompanied by a lack of alveolar flooding [79].

How to explain the beneficial effects of PEEP?

a Reduced microvascular filtration due to capillary compression

Several experiments in closed-chest animals have suggested that PEEP reduces microvascular filtration pressure due to a decrease in cardiac output [86, 92, 97]. It was shown in rats ventilated at peak inspiratory pressure of 45 cmH_2O that the main determinant of lung edema formation is the end-inspiratory lung volume independent of the level of PEEP [98]. Infusion of dopamine to correct the drop in systemic arterial pressure that occurs with PEEP was shown to partially abolish the reduction in pulmonary edema by PEEP (Figure 8) [98]. The effect of PEEP in reducing protein infiltration and permeability of the alveolo-capillary barrier was attributed to a decrease in lung capillary hydrostatic pressure and, therefore, filtration pressure [98]. Such a mechanism occurs at supraphysiological PEEP levels, higher than the level necessary to compensate for the retractive forces of the alveolus, and is attributable to compression of the capillary by adjacent alveoli. However, pulmonary artery pressure or cardiac output were not recorded in that study [98] and loss of the endothelial and epithelial barrier function was not differentiated. Moreover, despite similar arterial pressures, the animals ventilated with PEEP that received dopamine had less edema than animals ventilated without PEEP, suggesting that reduced filtration by capillary compression is not the only reason for the reduction in edema by PEEP and that other mechanisms are acting.

b Reduced filtration due to surfactant preservation

Experiments in the same rat model of overinflation have shown a significant conversion of active into non-active surfactant aggregates compared to non-ventilated controls after lung overinflation; 10 cmH_2O PEEP was shown to prevent a significant conversion of large aggregates into small aggregates compared with non-ventilated controls [33]. This latter study suggests that the beneficial effect of PEEP in reducing protein infiltration after overinflation at peak inspiratory pressure of 45 cmH_2O without PEEP in rats is partially attributable to a reduced filtration by surfactant preservation [33].

Two basic mechanisms have been described in literature which explain the surfactant preserving effect of PEEP during mechanical ventilation. Studies by Wyszogrodski et al. have shown that PEEP prevents a decrease in lung compliance and surface activity of lung extracts, indicating a prevention of loss of alveolar surfactant function during lung overinflation [29]. It was suggested that PEEP prevents alveolar collapse and thus keeps the end-expiratory volume of alveoli at a higher level, thereby preventing excessive loss of surfactant in the small airways by a squeeze-out mechanism during expiration (Figure 3) [99, 100]. Successive studies by Veldhuizen and collegues have shown that the rate of conversion of surfactant large into small aggregates is dependent on tidal volume and on time [31]; changing the respiratory rate [31] or the level of PEEP [32] did not affect surfactant conversion. These studies suggest that the preservation of the surfactant system by PEEP comes from the

reduction in cyclic changes in surface area by PEEP (Figure 4). It should be noted, however, that at a higher functional residual capacity (FRC), comparable changes in tidal volume are accompanied by smaller surface area changes compared to the same volume changes at lower FRC.

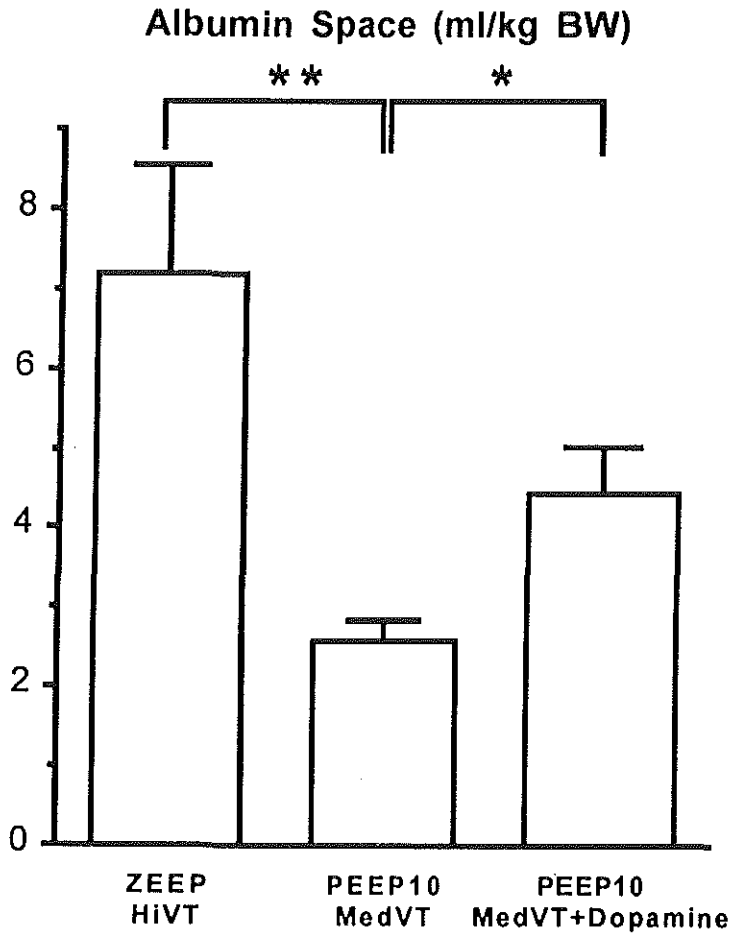

Figure 8 Effect of hemodynamic support with dopamine during 45 cmH_2O peak pressure ventilation with PEEP. The reduction of edema by PEEP was in part abolished when dopamine was administered. **p < 0.001. *(Used with permission from Reference 98).*

To further test the hypothesis that reduced filtration due to surfactant preservation is responsible for the reduction of edema by PEEP, our group conducted a study in which high peak inspiratory pressure ventilation without PEEP was preceded by administration of high amounts of exogenous surfactant [101]. It was shown that an amount of 200 mg/kg bodyweight surfactant preserved oxygenation and lung mechanics after 20 minutes of overinflation at peak inspiratory pressures of 45 cmH_2O without PEEP. Although 400 mg/kg bodyweight surfactant did not reduce the lung tissue content of Evans blue dye it was shown to reduce intra-alveolar accumulation of Evans blue dye [101] (Evans blue dye extravasation has previously been shown to have a good correlation with extravasation of ^{125}I-Albumin [102]). These data provide strong evidence that, besides peak inspiratory overstretching after lung overinflation, surfactant inactivation plays a key role in ventilation-induced intra-alveolar edema formation and that the effect of PEEP in reducing lung permeability to protein is at least partially attributable to its effect on preservation of the surfactant system.

c Splinting open alveolar lung units with an increased collapse tendency by PEEP

The utilization of PEEP to splint open the airways and alveoli at end-expiration in surfactant- deficient lungs may markedly reduce lung injury. Studies in both saline-lavaged isolated perfused rat lungs [81] and saline-lavaged intact animals [103, 104] have shown that ventilation strategies which keep the alveoli open throughout the respiratory cycle by sufficiently high levels of PEEP induce significantly less morphological injury with better preservation of pulmonary compliance than strategies in which alveolar collapse is allowed at end-expiration. Although healthy lungs do not seem to be damaged when terminal units are repeatedly opened or closed for short periods by negative end-expiratory pressure (which nevertheless reduces compliance and alters gas exchange [105]), it does become clear from what is discussed above, that early surfactant changes, which may be induced by mechanical ventilation itself, predispose lungs for ventilation-induced lung injury by repeated opening and closure of alveolar units [83].

The lung as a focus of (systemic) inflammation and infection: role of ventilation-induced lung injury

It is becoming increasingly realized that systemic release of inflammatory mediators [106] and bacterial translocation from the gut into the circulation [107] play a role in MOF. Kolobow et al. suggested that mechanical ventilation may induce local inflammatory reactions in the lung and may possibly, via spread of inflammatory mediators and bacteria, contribute to MOF [108].

a Possible sites of inflammatory mediator release

Experiments with granulocyte-depleted animals have shown that a significant amount of ventilation-induced lung injury may be mediated by activated granulocytes [109] and those ventilation strategies that use high volume [110] and/or cyclic collapse and reexpansion of

alveolar units [111] increase neutrophil influx and activation. The concept of ventilator-induced mediator expression as a result of either damage to the endothelial or epithelial cells or stimulus of stretch receptors present on endothelial cells [112], macrophages [113] and epithelial cells [114] is new (the exact signal transduction pathways have been reviewed elsewhere [115]).

b Ventilation-induced mediator release

Evidence for the release of cytokines during ventilation is limited. High frequency oscillation (HFO) in surfactant-depleted rabbits, which reduces shear stress, has been shown to induce lower levels of the inflammatory chemical mediators platelet-activating factor (PAF), tromboxane (TX) B_2 and 6-keto-prostaglandin (PG) $F_{1\alpha}$ in the lavage fluid compared to conventional mechanical ventilation (CMV) [116]. Interestingly, this could not be demonstrated in healthy unlavaged lungs [116] with an intact surfactant system. Others could not show a difference between thromboxane A_2 and prostacyclin in the perfusate of oleic acid-injured isolated rabbit lungs ventilated with high and low tidal volume (Vt) [117].

Similarly, an increase in TNF-α mRNA in the intra-alveolar cells of surfactant-depleted rabbits after 1 h of CMV was absent when such rabbits were ventilated with HFO. A study by Tremblay and coworkers in isolated lungs investigated the effect of different ventilation strategies on lung inflammatory mediator expression and production of cytokines TNF α, IL-1β, IL-6, IL-10, MIP-2 and γ-IFN in the presence and absence of a preexisting inflammatory stimulus [118]. It was shown that the use of high peak inspiratory lung volumes and not using PEEP during mechanical ventilation have a synergistic effect on the release of pro-inflammatory mediators from the lung tissue into the airways. Ten cmH_2O of PEEP at comparable peak inspiratory lung volumes or lowering peak inspiratory lung volume when ventilating with zero PEEP reduced these cytokine levels [118] (Figure 9). Studies by von Bethmann et al. have shown that prostacyclin, tumor necrosis factor α and interleukin-6 are released into the lung perfusate of isolated perfused and ventilated mouse lungs after artificial ventilation [119]. For TNF release a continuous stimulation by hyperventilation is necessary whereas a short period of 30 minutes of hyperinflation is sufficient to stimulate release of IL-6 for the next 120 minutes [120]. From such studies it may be 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.

Recent animal investigations by Narimanbekov et al. suggest that treatment with antibodies directed against Il-1 reduced albumin infiltration, elastase expression and neutrophil counts in rabbits subjected to pulmonary lavage and 8 hours of hyperoxia and hyperventilation [121]. However, no effect was seen on deterioration of lung compliance and oxygenation which suggests that the different effects of ventilation-induced lung injury may be directed by different molecules.

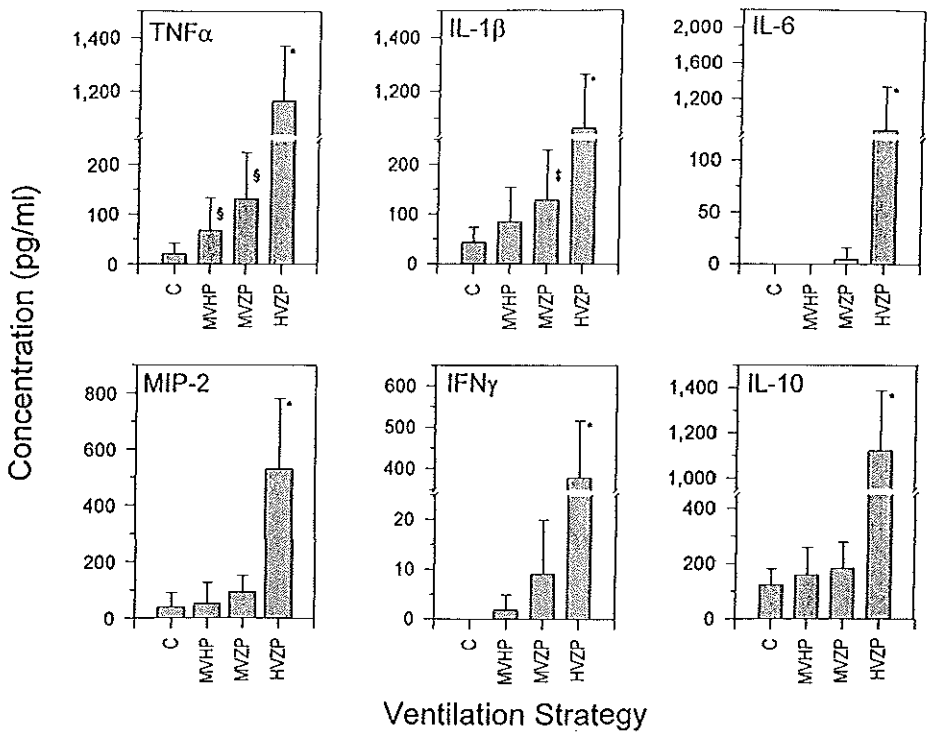

Figure 9. Effect of different ventilation strategies on cytokine concentrations in isolated perfused rat lungs. Four different ventilation strategies were used: controls (C = normal Vt), moderate Vt + high PEEP (MVHP), moderate Vt + zero PEEP (MVZP), high Vt + zero PEEP (HVZP) resulting in the same end-inspiratory distension as MVHP. Major increases in cytokine concentrations were observed with HVZP. *(Used with permission from Reference 118)*

c Ventilation-induced bacterial translocation

Based on the observation that mechanically ventilated ARDS patients often develop pneumonia [122] and septicemia the question may be raised whether damaging mechanical ventilation can promote bacteremia and/or sepsis. It is conceivable that bacteria more readily gain access to the circulation from damaged lung parenchyma than from previously normal lung tissue [123, 124]. It has been established that preserving end-expiratory lung volume with PEEP has a beneficial effect on the course of infection in terms of reducing bacterial counts recovered from the lung tissue after prolonged mechanical ventilation of lungs inoculated with bacteria [125]. Moreover, avoiding high peak transpulmonary pressures and

preserving end-expiratory lung volume with PEEP has been shown to reduce translocation of *Pseudomonas aeruginosa* [125], *Escherichia Coli* [126] and *Klebsiella pneumoniae* [127] from the lung into the bloodstream.

These data suggest that ventilation-induced changes in the barrier function of the lung epithelium and/or endothelium to bacteria may, to a certain extent, contribute to the development of bacteremia and endotoxemia as it is seen in MOF. Translocation may be due to both an increased translocation of bacteria from the alveolar space directly into the bloodstream or bacterial clearance from the interstitial spaces due to increased lymph flow which promotes drainage of bacteria from the lymphatics into the bloodstream.

Physiological rationale to prevent ventilation-induced lung injury

The experimental data presented demonstrate that ventilation settings that prevent lung injury in both healthy and diseased lungs should recruit all alveoli and prevent their collapse at end-expiration. Moreover, these ventilation strategies should prevent alveolar overdistension or adverse hemodynamic changes due to overinflation.

a Preventing overdistension

Gattinoni et al. showed that patients with early ARDS and collapsed dependent lung regions, have a reduced volume of aerated lung [128]. Volume controlled mechanical ventilation will predominantly ventilate this aerated healthy portion of the lung with overdistension in such regions. If one assumes that 75 % of the lung is consolidated and only 25% is ventilated, then even small tidal volume ventilation with e.g. 7 mL/kg bodyweight, would result in tidal volumes of 28 mL/kg in such lung regions with a danger of overdistension and further lung impairment. Use of pressure-controlled time-cycled modes of mechanical ventilation in which the alveolar pressure can never exceed the peak inspiratory pressure set on the ventilator is then preferable to reduce dangerous alveolar overdistension in these lung areas [129].

Recent clinical investigations have shown that to prevent overdistension in ARDS patients, (which was defined as ventilation below the upper inflection point of the P-V curve of such lungs) tidal volumes have to be decreased to well below those commonly accepted as normal in healthy patients [130]. Other studies suggest that such decreased tidal volumes may increase oxygen delivery due to better hemodynamics [131, 132].

Preliminary reports of reduced tidal volumes by end-inspiratory airway pressure limitation in patients with or at risk of ARDS showed no reduction in mortality rate [133-135]. Such findings may be explained by the hypercapnia, which was accepted as a consequence of tidal volume reduction. Hypercapnia may have several negative effects [136].

b Open up the lung and keep it open at end expiration

Another explanation may be the fact that alveoli first have to be recruited to prevent dangerous shear forces between open and closed alveoli. It has been shown that avoiding

large pressure-volume variations with HFO does not totally prevent lung injury if HFO does not maintain lung volume [137], which can be achieved by a preliminary sustained static inflation to recruit the greatest possible number of lung units before starting HFO [138]. Such recruited alveoli should be kept open during the whole respiratory cycle [139].

Gattinoni et al., using CT scan imaging, have shown that PEEP markedly reduces the fraction of lung that undergoes tidal reopening and closure during mechanical ventilation of patients with ARDS and that the pressures required to open up all alveoli, especially the ones in the dependent lung, are very high [140]. Their studies show that using peak pressures of 50 cmH_2O were insufficient to fully recruit alveoli in the dependent parts of the lung and/or PEEP pressures as high as 20 cmH_2O were insufficient to keep the recruited alveoli fully open [140].

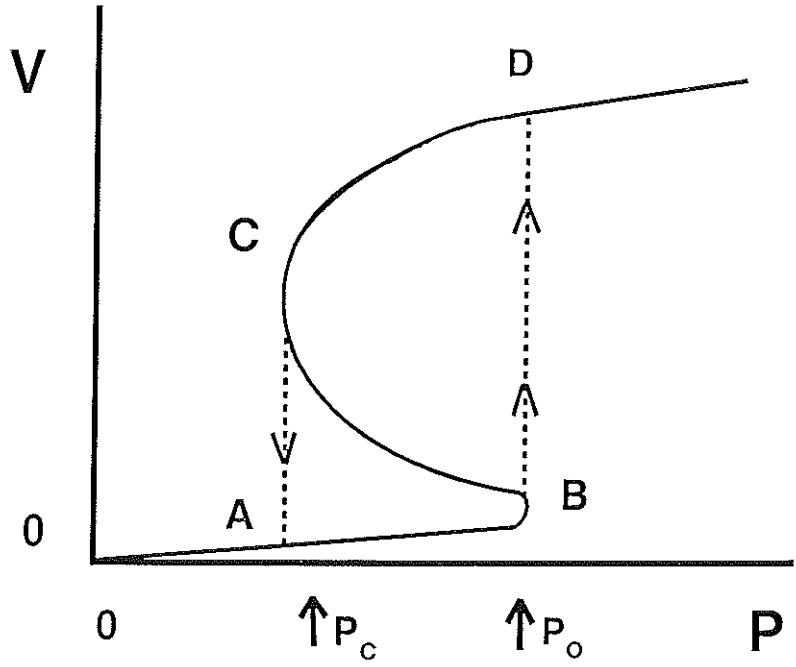

Figure 10 Schematic drawing of a pressure-volume (P-V) curve showing the proposed mechanisms of alveolar recruitment and collapse. With the increase of pressure during the inspiration the corresponding change in volume is given by the pressure-volume curve (solid line). At A the alveolus is still collapsed. It is not before point B that the critical opening pressure, Po, is reached, the increase in alveolar volume is immediate (dashed line) and reaches D. The alveolus is recruited (note that the intra-alveolar pressure is the same at B and D). When the pressure is reduced to the closing pressure (Pc) at point C the change in volume follows the solid line and the alveolus collapses again.

The rationale for high opening pressures to recruit the lung and the need for lower pressures to keep them open can be deduced from the pressure-volume curve of the individual alveolus (Figure 10). It was suggested by Staub and collegues that the behavior of alveoli is quantal in nature [141]. Alveoli are either open or closed. A critical opening pressure has to be reached until previously collapsed alveoli can be opened (Figure 10). Once they are open, they remain open until the pressure drops below a critical level, then immediate collapse occurs. Reopening requires the high recruiting pressure again. Any state between open and closed is unstable and impossible to maintain. If this transition from closed to the open and from the open to the close state occurs within each single breath, the mechanisms described by Mead (Figure 7) postulated that structural damage is caused. Practical directions on how to open up the lung in clinical practice are beyond the scope of this chapter and have been described elsewhere [142, 143]. Earlier data [143] and recent data by our group [144] suggest that when applied early during induction of ALI in rats, this open lung approach prevents a decrease in pulmonary compliance associated with modes of ventilation that do not maintain an open lung during induction of ALI, and suggest that it is necessary to apply this open lung strategy early during the course of ALI/ARDS [143, 144].

A recent clinical study by Amato et al. showed that a ventilation strategy aimed at opening atelectatic lungs and keeping them open at all times in combination with a treatment strategy of permissive hypercapnia and a restriction on the size of tidal volume and limited peak inspiratory pressures, resulted in a higher rate of weaning from mechanical ventilation, lower rate of barotrauma, and improved 28 day survival in ARDS patients compared to conventional ventilation [9, 10].

c Surfactant therapy in acute respiratory distress syndrome

Reestablishing a physiological surface tension at the air-liquid interface by application of exogenous surfactant during mechanical ventilation will prevent end-expiratory collapse and dangerous shear forces between open and closed alveoli (Figure 11) [11]. A recent multicenter, randomized pilot study showed that with high dose exogenous surfactant therapy, mortality of patients with ARDS of different etiology could be decreased from 43.8% to 17.6% with better oxygenation and less ventilatory requirements [8]. Although a recent multicenter trial showed no effect on oxygenation or survival of nebulized surfactant administration in sepsis-induced ARDS patients [145], these results were likely caused by the low quantity of surfactant deposited in the peripheral lung tissue. Higher doses of broncho-scopically administered surfactant have been shown to improve oxygenation in patients with sepsis-induced ARDS [146].

d Liquid ventilation in ARDS

Abolishing the alveolar air-liquid interface by totally filling the lung with perfluorocarbons, liquids which allow gas exchange over the alveolo-capillary barrier to continue due to their

capability to dissolve high amounts of oxygen and carbon dioxide, is another possibility to prevent alveoli from collapse and prevent dangerous shear forces [147]. However, the technical requirements for this technique do not make it easily routinely applicable in clinic. In the technique of partial liquid ventilation (PLV) the lung is partially filled up with perfluorocarbons and tidal volumes of gas are superimposed [147]. Preliminary reports could not shown an improved survival and only minor improvements in oxygenation [148]. As perfluorocarbons have a constant surface tension and no dynamic surface tension behavior like surfactant (Figure 11) [149], they may predispose the lung to relative overinflation at high airway pressures and to alveolar collapse at low airway pressures when gas ventilation is superimposed. Therefore, the technique of PLV is less suitable for therapy in ARDS than surfactant therapy (Figure 11).

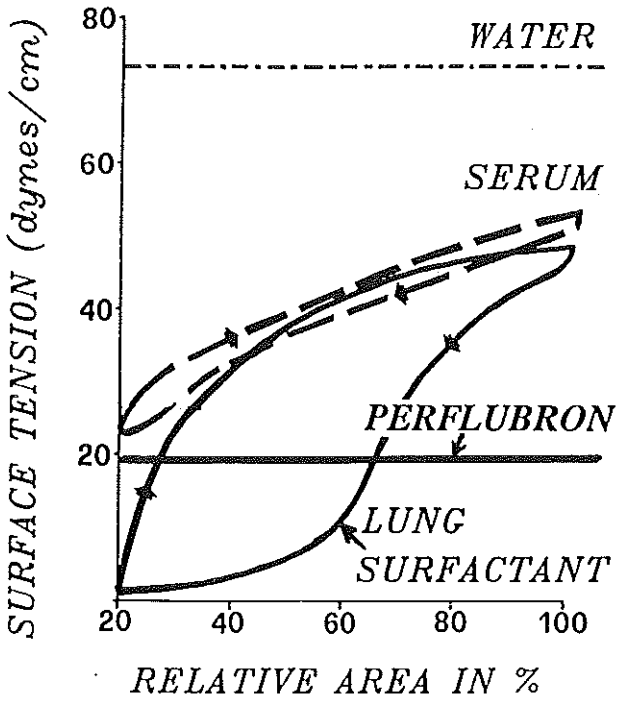

Figure 11. The surface tension behaviour of lung surfactant, serum, perflubron and water. Lung surfactant shows dynamic surface tension behaviour, with low surface tension for lower surface areas and higher surface tension for higher surface areas. In ARDS, the lung surfactant at the air-liquid interface is replaced with serum, which displays much higher surface tensions for each surface area when compared to lung surfactant. Water does not display dynamic surface tension behaviour and neither does perflubron. They both show a constant surface tension for different surface areas, which will predispose the lung alveoli to end-expiratory collapse and peak inspiratory overstretching when perfluorocarbons are present at the alveolar air-liquid interface.

e **Nitric oxide**

Inhalation of exogenous nitric oxide has been shown to be beneficial with respect to reducing pulmonary hypertension and improving arterial oxygenation in neonates with respiratory distress syndrome and in adults with ARDS [150, 151]. The main reason for pulmonary hypertension may well be hypoxic pulmonary vasoconstriction due to alveolar collapse and therefore recruitment of alveoli with abolishment of hypoxic vasoconstriction may well prove to be superior to the use of nitric oxide in reducing pulmonary artery pressure. Moreover, "blood gas cosmetics" by nitric oxide may direct attention away from establishing an optimal lung protective ventilator setting, and given the fact that nitric oxide is highly reactive [152], it may prove to be detrimental in the treatment of patients on ventilatory support.

How to use monitoring techniques to prevent ventilation-induced lung injury

a **Lung Function measurements**

Lung function measurements (Table 1) should provide basic physiological information on 1) gas transport from the air via the lung into the blood, and 2) should - depending on the level of care - provide techniques which make a differentiation of the cause of the disturbance in gas exchange possible.

Table 1. Monitoring requirements during mechanical ventilation

FIRST LEVEL
Arterial oxygen tension (Pulse oximetry)
Acid base balance
Airway pressuree
SECOND LEVEL
Lung-Thorax mechanics (compliance, resistance)
Intra-pulmonary shunt
Cardiac output and hemodynamics
THIRD LEVEL
Oxygen consumption/delivery and carbon dioxide production
Diffusion capacity
Ventilation-Perfusion mismatching
Lung water

Mechanical ventilation should overcome or prevent hypoxia, which is the most

important and life-threatening parameter during mechanical ventilation. The oxygenation index (PaO_2/FiO_2) measured under standard ventilator settings, can be used to define the state of impairment of the lung, although a lower than optimal oxygenation index does not differentiate between 1) ventilation, 2) perfusion, 3) diffusion or 4) Ventilation/Perfusion (V/Q) problems. At present it is the most reliable and routinely available tool to define the state of openness of the lung under standard ventilation conditions [142].

Peak inspiratory pressure at flow-constant ventilation is a poor parameter to measure alveolar overstretching as it is influenced by a number of factors independent of alveolar pressure and does not allow to define the state of overinflation and/or openness of different lung areas [153]. To prevent repeated alveolar collapse and reexpansion, it has been suggested that lungs should be ventilated above the lower inflection point of their P-V curve [115, 153]; to prevent overdistension it has been suggested that peak inspiratory plateau pressures should be reduced below the upper inflection point of the P-V curve, ventilating them in the most compliant part of their P-V curve [115, 153]. Total lung volume and functional residual capacity (FRC) should always be taken into account when interpreting compliance measurements [154]; lung compliance measurements which are not normalized for lung volume have only a limited information. Thus, if FRC measurements are not possible, one should at least normalize lung compliance values for lean body weight. Furthermore, a normal value for FRC does not differentiate a fully open lung from one with collapsed lung areas in which healthy areas are overinflated. Similarly, as the lower inflection point is the resultant of the P-V curves of different lung parts with a different compliance it may be the resultant of overdistended anterior parts, while posterior lung parts remain collapsed (Figure 12) and may represent both alveolar recruitment and increases in volume of alveoli that are already open. Thus, considering the quite complicated nature of lung mechanical measurements and the fact that no standardized way of lung volume measurements is possible (functional residual capacity measurements are at this time not routinely available), oxygenation index is at present the best way to define the disease state of the lung.

b Morphological measurements

The development of a simple tool for determining regional volumes during ventilation would be a major step forward in the search for safer treatment. Although CT scan may be used for this purpose [140], it is not likely to find its way as a routine applicable tool in clinics because of its price, size and possible hazards. However, electric impedance tomography which has been shown to have the same capabilities as CT scan, but is not limited by its disadvantages, may well find its way as a routine evaluation method in the future [155].

c Biological markers

Finally, numerous biological markers have been identified which may be used to evaluate damage to the alveolo-capillary barrier. These have been recently reviewed elsewhere

[156]. For the endothelium, many specific markers are available [156], but at present only increased protein permeability of radio-active tracers from the alveolar lung compartment into the circulation evaluates the epithelial integrity, which is rate-limiting for the transfer of solutes over the alveolo-capillary barrier [156]. A recent preliminary report suggests the use of purines as a specific marker of epithelial injury [157]. The search should continue for highly specific biological markers in the bloodstream, such as are available for other organs, to monitor the metabolic and biological stress inflicted on the lung by our supportive ventilation therapy.

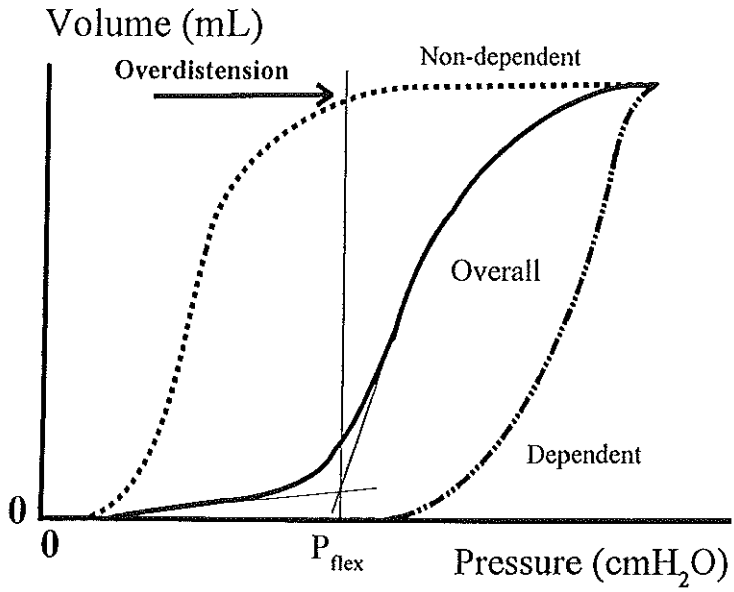

Figure 12. Because the pressure-volume curve of the whole lung in an ARDS lung is the result of parts which have been affected by the disease process to different extents, the lower inflection point of the pressure-volume curve does not have to represent mass alveolar recruitment, but rather overdistension of non-dependent parts while posterior parts remain collapsed.

Monitoring of the lung by means of physiological, morphological and biochemical parameters is a prerequisite for an optimal ventilation strategy in the ICU to prevent or to minimize ventilation-induced lung injury. Monitoring techniques should provide us as much as possible with non-invasive continuous data such as FRC measurements or electric impedance tomography or with minimal invasive data such as on-line blood gases or monitoring of specific biological markers by means of biosensors. Some of these techniques are already available and some are in the experimental, developmental state. If we learn to use them to make the right therapeutic decisions in our patients, than it may be possible to further reduce

the high mortality in the ICU which with we are still confronted.

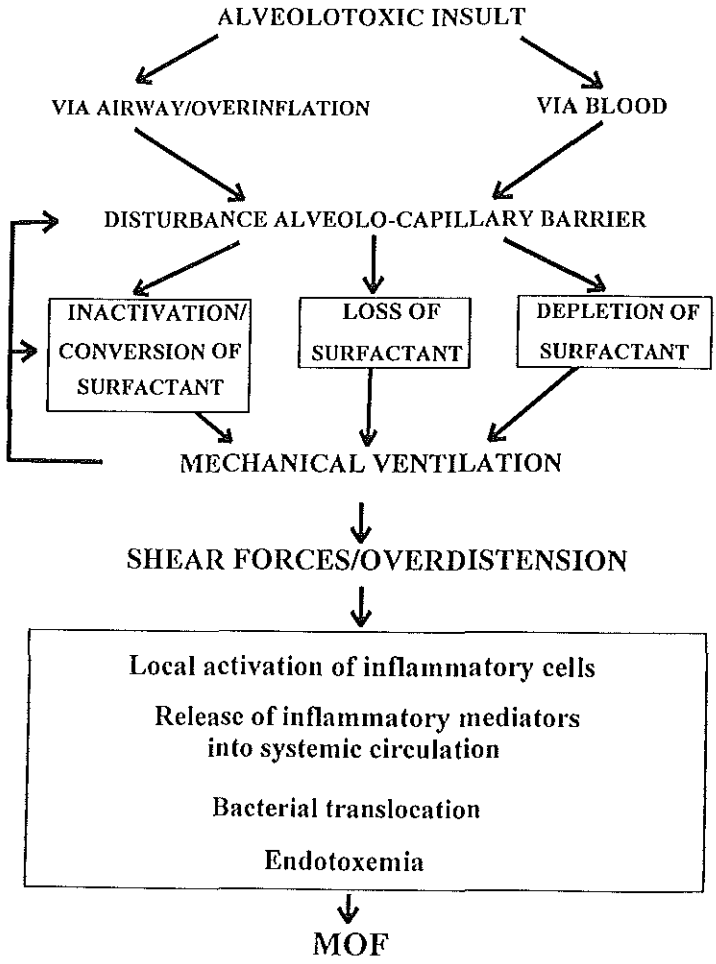

Figure 13. Schematic presentation of the sequence of events that necessitates the use of mechanical ventilation in acute respiratory distress syndrome (ARDS). Mechanical ventilation of a surfactant-deficient lung may lead to the development of shear forces and overinflation of 'healthy' lung parts luxating inflammatory mediator release and bacterial translocation from the lung into the bloodstream, which may finally lead to the development of a generalized inflammation: multiple organ failure (MOF). Important benefits in mortality from MOF may therefore be achieved by lung therapies that are directed at reducing shear forces and/or overdistension.

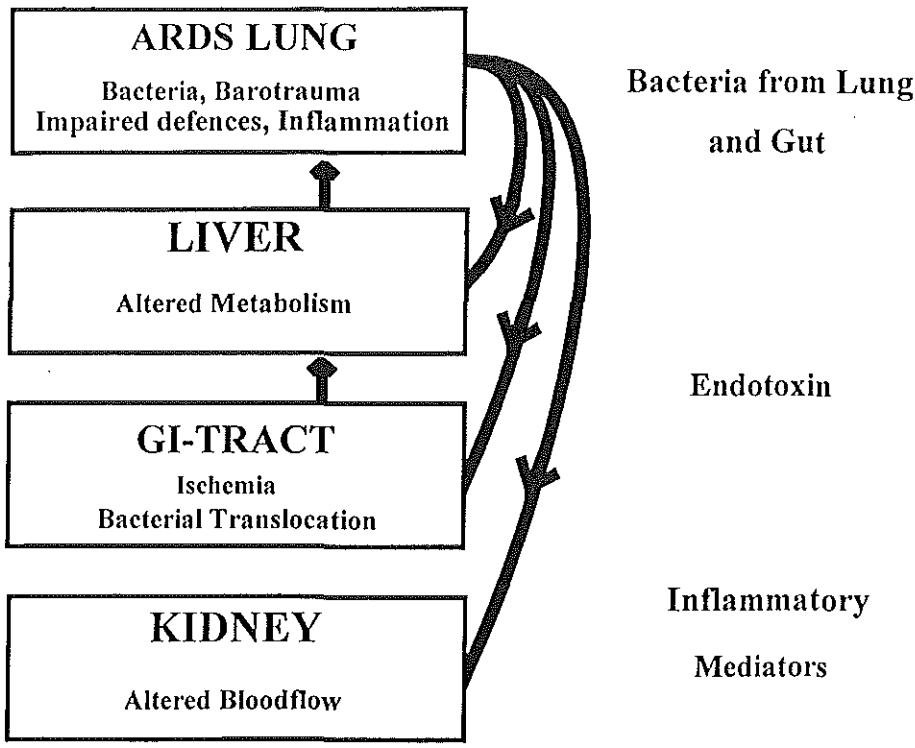

Figure 14. Organ-organ interaction, which can perpetuate the secondary multiple organ failure (MOF). The inflammatory lung as seen with adult respiratory distress syndrome (ARDS) is usually the initiating focus, which may be the source of inflammatory mediators and endotoxemia. GI, gastrointestinal.

FUTURE PROSPECTS

Surfactant changes play a key role in ventilation-induced lung injury. Mechanical ventilation strategies should avoid both continuous alveolar overdistension and repeated collapse and reexpansion of alveoli by keeping all alveoli open at end-expiration. Surfactant therapy is likely to become another important additional therapeutic measure during ventilatory treatment of patients with ARDS, and recent data suggest that it may increase the effectiveness of antibiotic therapy for lung infection [158], which has important implications for patients on mechanical ventilation with ventilator-associated pneumonia [122].

Recent animal studies suggest that such protective lung strategies may prevent the release of inflammatory mediators from the lung and the transfer of bacteria and bacterial endotoxins to the bloodstream. This sequence of events in shown in Figure 13. Such treatments may prevent the development of systemic inflammation with other organs being affected (Figure 14) and may have an important influence on mortality rates of ARDS.

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Chapter 2

Surfactant impairment after mechanical ventilation with large alveolar surface area changes and the effects of positive end-expiratory pressure

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Summary

We have assessed the effects of overinflation on surfactant function and composition in rats undergoing ventilation for 20 minutes with 100% oxygen at a peak inspiratory pressure of 45 cm H_2O, with or without 10 cm H_2O PEEP (group 45/10 and 45/0, respectively). Mean tidal volumes were 48.4 (SEM 0.3) ml · kg^{-1} in group 45/0 and 18.3 (0.1) ml · kg^{-1} in group 45/10. Arterial oxygenation in group 45/0 was reduced after 20 minutes compared with group 45/10 (305 (71) vs 564 (10) mmHg); maximal compliance of the P-V curve was decreased (2.09 (0.13) versus 4.16 (0.35) ml · cm H_2O^{-1} . kg^{-1}); total lung volume at a transpulmonary pressure of 5 cm H_2O was reduced (6.5 (1.0) vs 18.8 (1.4) ml · kg^{-1}) and the Gruenwald index was less (0.22 (0.02) vs 0.40 (0.05)). Broncho-alveolar lavage fluid from the group of animals ventilated without PEEP had a greater protein concentration (2.18 (0.11) vs 0.76 (0.22) mg · ml^{-1}) and a greater minimal surface tension (37.2 (6.3) vs 24.5 (2.8) mN · m^{-1}) than in those who underwent ventilation with PEEP. Group 45/0 had an increase in non-active to active total phosphorus compared with non-ventilated controls (0.90 (0.16) vs 0.30 (0.07)). We conclude that ventilation in healthy rats with peak inspiratory pressures of 45 cm H_2O without PEEP for 20 min caused severe impairment of pulmonary surfactant composition and function which can be prevented by the use of PEEP 10 cm H_2O.

Introduction

Pioneering work by Mead and Collier in 1959 showed that mechanical ventilation in dogs resulted in a progressive decrease in pulmonary compliance which was reversed by periodic forced inflations [1]. Greenfield, Ebert and Benson related these changes in compliance to pulmonary surfactant by demonstrating increased surface tension in lung extracts from dogs whose lungs were ventilated with peak inspiratory pressures of 28-32 cm H_2O for 1-2 h [2].

Intra-alveolar oedema in healthy rats subjected to intermittent positive pressure ventilation (IPPV) at high inflation pressures (HIPPV), without positive end-expiratory pressure (PEEP) was first demonstrated by Webb and Tierney [3] and later confirmed by Dreyfuss and collegues [4]. Dreyfuss and collegues showed that damage of lung tissue resulting in oedema formation began at the capillary endothelium and progressed rapidly to the alveolar epithelium within 20 min [4]. Using PEEP 10 cm H_2O and the same peak inspiratory pressure, the lung was partially preserved from this high permeability oedema [4,5]. A subsequent report showed that the main determinant of lung oedema formation was the end-inspiratory lung volume and attributed the effect of PEEP to a decrease in lung capillary hydrostatic pressure [6].

We postulate a different explanation for the effect of PEEP which may prevent impairment of the pulmonary surfactant system by reducing the large changes in alveolar surface area which occur during mechanical ventilation with large tidal volumes. The balance of hydrostatic forces is altered when surfactant is impaired: pressure within the alveolar fluid lining is reduced, applying more "suction" to the interstitial space. If PEEP reduces surfactant impairment, this prevents suction-induced pulmonary oedema. To assess this hypothesis, we conducted a study to measure changes in surfactant function and composition after mechanical ventilation with high lung volumes or with PEEP when changes in lung volume were less.

Materials and methods

The study protocol was approved by the local Animal Committee, and the care and handling of the animals conformed with European Community guidelines (86/609/EC).

Twenty-four adult male Sprague-Dawley rats weighting 290-350 g, were anaesthetized with 2% halothane and 65 % nitrous oxide in oxygen, tracheotomized and a catheter was inserted into a carotid artery. During the experiment anaesthesia was maintained with pentobarbital sodium 60 mg $\cdot kg^{-1}$ i.p. (Nembutal; Algin BV, Maassluis, the Netherlands); neuromuscular block was produced with pancuronium 2.0 mg kg^{-1} i.m. (Pavulon; Organon Technika, Boxtel, the Netherlands).

After neuromuscular block, the animals were connected to a ventilator (Servo Ventilator 300, Siemens-Elema, Solna, Sweden) in a pressure-controlled mode, at an (FiO_2) of 1.0, frequency 30 bpm and an I/E ratio of 1:2. In order to re-open the atelectatic lung areas induced by the surgical procedure, mechanical ventilation with a peak airway pressure of 26 cm H_2O and a PEEP of 6 cm H_2O was applied for 30 s. The animals were allocated randomly to one of four groups (n = 6 in each group). Animals in group 7/0 underwent ventilation with

a peak pressure of 7 cm H_2O without PEEP; group 45/0 underwent ventilation with a peak pressure of 45 cm H_2O without PEEP; and group 45/10 underwent ventilation with a peak pressure of 45 cm H_2O and a PEEP of 10 cm H_2O. Ventilatory frequency was set at 60 bpm in group 7/0 and 25 bpm in group 45/10 to maintain ($PaCO_2$) within the normal range. Ventilatory frequency in group 45/0 was the same as in group 45/10. The control group was killed immediately after the surgical procedure without undergoing mechanical ventilation. After ventilation for 20 minutes, the other animals were killed by an overdose of pentobarbital via the penile vein, followed by KCl.

Blood samples obtained from the carotid artery were measured (ABL 505, Radiometer, Copenhagen, Denmark) 1, 10 and 20 minutes after 30 s of ventilation with pressures of 26/6. Mean arterial blood pressure was measured using a Statham P23XL transducer (Spectramed, Oxnard, CA, USA) and recorded (Siemens Sirecust 404-1, Danvers, MA, USA).

During mechanical ventilation, animals were placed in a volume-constant body plethysmograph to allow continuous recording of tidal volume. A pressure transducer (Validyne model DP 45-32, Validyne Engineering Co., Northridge, CA, USA) recorded pressure changes which were sampled at 10 Hz using a 12-bit analog-to-digital converter (DAS 1800, Keithley MetraByte, Taunton, MA, USA).

After the animals were killed, a static pressure-volume plot was recorded using conventional techniques [6]. Maximal compliance (C_{max}) was defined as the steepest part of the pressure-volume deflation curve [8], and was determined separately for each animal. The Gruenwald index, defined as $(2V_5 + V_{10})/2V_{max}$, where V_5, V_{10} and V_{max} = lung volumes above functional residual capacity (FRC) at transpulmonary pressures of 5, 10 and 35 cm H_2O, respectively, was calculated [9]. FRC was taken to be total lung volume at a transpulmonary pressure of 5 cm H_2O (V_5) [10]; total lung weight was recorded. The lungs were lavaged with saline-$CaCl_2$ 1.5 mmol $litre^{-1}$ [11]. The active component of surfactant in the broncho-alveolar lavage fluid was separated from the non-active surfactant component by differential centrifugation [11], followed by subsequent phosphorus analysis [12], and the ratio of inactive to active surfactant was calculated. The protein concentration of BAL was determined using the Bradford method (Bio-Rad protein assay, Munich, Germany) [13]. Some of the re-suspension (20 µl) of the active surfactant part was used for biophysical analysis of minimal surface tension after 50 cycles on a pulsating bubble surfactometer (PBS; Electronetics Corporation, Tonowanda, New York, USA), as described by Enhorning [14].

Statistical analysis was performed using the Instat 2.0 biostatistics package (GraphPad software, San Diego, CA, USA). Inter-group comparisons were analysed using ANOVA. Intra-group comparisons were analysed with repeated measures ANOVA. If ANOVA resulted in $P < 0.05$, a Bonferroni post-test was performed. All data are reported as mean (SEM).

Results

Data followed a normal distribution. Tidal volume, (PaO_2), ($PaCO_2$) and mean arterial pressure over time in the three ventilated groups are shown in table 1. During the study

period, tidal volumes differed markedly between groups (Table 1). At t = 1 min, (PaO_2) was comparable in the three ventilated groups and remained stable in groups 7/0 and 45/10; (PaO_2) in group 45/0 decreased significantly after 10 min.

Table 1. Tidal volume (Vt) (ml·kg^{-1}), mean arterial pressure (MAP) (mmHg) and blood-gas tensions (torr) of the groups who underwent ventilation during the study (mean ± SEM). Inter- and intra-group comparisons: ANOVA with Bonferroni post-test if ANOVA P<0.05. Significant difference compared with: #t=0 min;‡t=1 min; §t=1, 10 min; *group 7/0 and †group 45/0.

	Group	7/0	45/10	45/0
	Time (min)			
V_t	0	12.2 ± 1.2	18.2 ± 2.2*,†	46.4 ± 2.7*
	1	12.2 ± 1.2	17.8 ± 2.1*,†	49.5 ± 2.7*
	5	11.5 ± 1.0	17.9 ± 2.7*,†	51.0 ± 2.1*
	10	10.4 ± 1.3$^{\#}$	18.5 ± 2.7*,†	49.6 ± 2.2*
	15	10.2 ± 1.3$^{\#}$	18.9 ± 2.7*,†	46.3 ± 2.5*
	20	10.2 ± 1.2$^{\#}$	18.9 ± 2.7*,†	44.9 ± 2.6*
PaO_2	1	522 ± 18	511 ± 18	492 ± 12
	10	497 ± 16	525 ± 13	524 ± 17
	20	508 ± 24†	564 ± 10†‡	305 ± 71§
$PaCO_2$	1	35.3 ± 3.3†	31.5 ± 1.9	23.4 ± 1.9
	10	37.6 ± 3.8†	42.0 ± 3.1†	21.1 ± 1.8
	20	39.6 ± 4.3†	43.1 ± 3.8†‡	24.8 ± 1.7
Mean blood	0	113.1 ± 5.2	97.0 ± 9.4	103.5 ± 10.9
pressure	1	134.8 ± 5.7	65.2 ± 7.9*	77.3 ± 9.3*
	10	136.5 ± 7.7	85.4 ± 8.6*	92.3 ± 5.8*
	20	137.6 ± 5.8	97.6 ± 15.3	64.7 ± 8.1*

Table 2 shows recovery of BAL fluid and post-mortem data for C_{max}, Gruenwald index, V_S, total lung weight, total phosphorus, protein concentration of BAL and minimal surface tension for the three ventilated groups and the non-ventilated control group. There was no statistical difference between group 7/0 and the control group for any variable. However, there were significant differences, between group 7/0 and group 45/0 for all variables except total phosphorus.

Table 2. Recovery of lavage fluid and post-mortem data for maximal compliance (Cmax), Gruenwald index, V_5, total lung weight, total phosphorus, protein concentration of BAL and surface tension in all four groups after the ventilation period (mean ± SEM). Inter-group comparisons: ANOVA with Bonferroni post-test if ANOVA P<0.05. *Group 45/0 significantly different compared with all oter groups.

	Group			
	Control	**7/0**	**45/10**	**45/0**
Recovery BAL fluid (%)	74.4 ± 1.9	75.6 ± 2.2	75.0 ± 3.2	73.3 ± 1.2
C_{max} *(ml · cm* $H_2O^{-1} \cdot kg^{-1}$*)*	4.11 ±	4.49 ±	4.16 ±	2.09 ±
Gruenwald index	0.41±	0.46±	0.40±	0.22±
V_5 *(ml · kg*$^{-1}$*)*	17.0 ± 1.5	16.0 ± 0.8	18.8 ± 1.4	6.5 ± 1.0*
Total lung weight (gram)	1.9 ± 0.1	2.0 ± 0.1	2.5 ± 0.1	3.9 ± 0.1*
Total phosphorus (μmol)	3.8 ± 0.9	3.6 ± 0.3	4.5 ± 0.2	3.1 ± 0.3
Protein conc. BAL (mg·ml^{-1}*)*	0.44±	0.30±	0.76±	2.18±
Min. surf. tension (mN· m^{-1}*)*	24.3 ± 1.3	26.6 ± 1.8	24.5 ± 2.8	37.2 ± 6.3*

Figure 1 shows that the ratio of non-active to active surfactant was greater in group 45/0 compared with controls. Group 45/10 showed no impairment of any variable compared with the other groups or with controls. In addition, all variables were significantly different between groups 45/10 and 45/0.

Discussion

In this study we used an established rat model of ventilation-induced lung injury first developed by Webb and Tierney [3] and later intensively investigated by Dreyfuss and collegues [4,5]. The role of changes in the pulmonary surfactant, however, has never been investigated in this animal model. The study was designed to better understand the relation between changes in lung morphology and permeability, and changes in the pulmonary surfactant system. We used established techniques to characterize the pulmonary surfactant system. Significant changes in surfactant function and composition occurred after lung overinflation without PEEP for a period as short as 20 min. Surfactant composition, characterized by significant conversion of active into non-active surfactant, was changed after ventilation with HIPPV, without PEEP, compared with controls who did not undergo ventilation. Impairment of surfactant function after lung overinflation was associated with impairment of lung mechanics and an increase in minimal surface tension of lung lavage fluid extracts. Impairment of surfactant composition and function caused by lung overinflation was prevented by the use of PEEP 10 cm H_2O. These findings support the hypothesis that the

beneficial effect of PEEP in this model of ventilation-induced lung injury is mediated by prevention of impairment of surfactant composition and function.

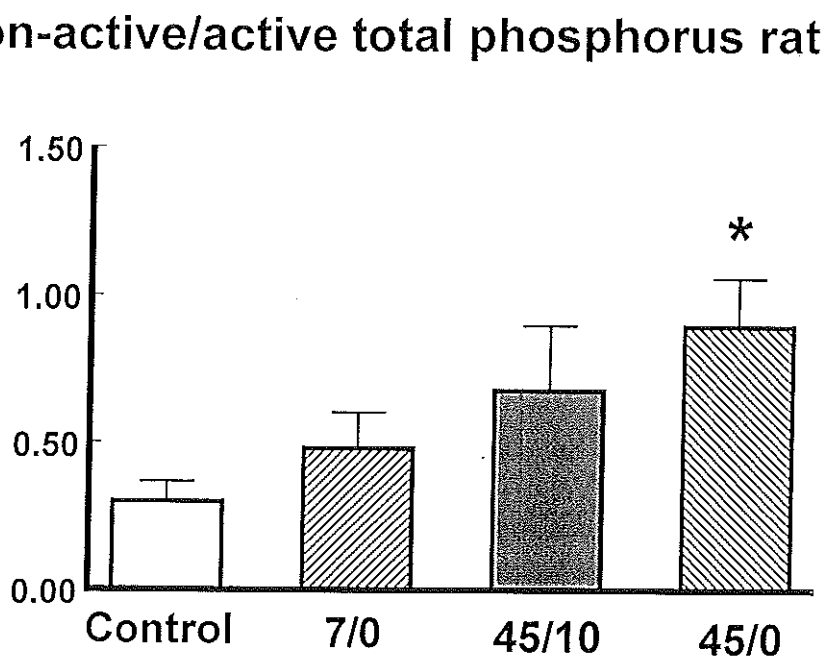

Figure 1. The ratio of non-active to active total phosphorus. Group 45/0 has a significant conversion of active into non-active total phosphorus during the ventilation period compared to non-ventilated controls (Values are mean ± SEM; *p<0.05 group 45/0 versus control).

Protein concentration after mechanical ventilation with high lung volumes was increased in BAL fluid in group 45/0; PEEP 10 cm H_2O prevented protein accumulation. These data are consistent with previous studies in this model which showed less accumulation of lung water, lung protein permeability and absence of intra-alveolar oedema with PEEP 10 cm H_2O during ventilation with high peak inspiratory lung volumes [3,5].

The exact mechanism of ventilation-induced lung injury, and contributory factors, are still disputed. Experiments in rats with high peak inspiratory pressure ventilation of 45 cm H_2O, where peak inspiratory volume was limited by thorax restriction, have shown clearly that high peak inspiratory pressures alone do not induce lung injury [5]. However, a high peak inspiratory lung volume with peak inspiratory overstretching alone can also not explain

ventilation-induced lung injury, as the use of 10 cm H_2O of PEEP at identical peak inspiratory lung volumes almost completely prevented histologically assessed lung injury [5]. Although a role for surfactant in lung injury by lung overinflation was suggested [5], our data are the first to show the association of surfactant changes in lung injury with mechanical ventilation using high lung volumes.

Several mechanisms are involved in the changes in surfactant function during mechanical ventilation. First, mechanical ventilation combined with overinflation enhances the release of surfactant from pneumocytes type II into the alveolus [15-18]. This material may be lost from the alveoli into the airways by compression of the surfactant film if the alveolar surface area becomes smaller than the surface area occupied by the surfactant molecules, so that surface-active material moves into the airways [19,20]. This mechanism of surfactant depletion after mechanical ventilation was first shown by Faridy in isolated rat lungs [20]. Mechanical ventilation increased the surface activity of lavage fluid of the pulmonary airways; this change in activity was dependent on the duration of ventilation and the size of the tidal volume [20]. Studies by Wyszogrodski and colleagues have shown that PEEP prevents a decrease in lung compliance and surface activity of lung extracts, indicating prevention of loss of alveolar surfactant function during lung overinflation [19]. It was suggested that PEEP prevents alveolar collapse and thus maintains the end-expiratory volume of alveoli at a higher level, thereby preventing excessive loss of surfactant in the small airways during expiration [21].

Second, the pulmonary surfactant system can be divided into active and non-active subfractions by differential centrifugation [11]. The active subfractions, which represent tubular-myelin like forms of surfactant, are the metabolic precursors of the non-active components, which represent small vesicular structures [22]. Gross and Narine were the first to show that conversion of active into non-active surfactant subfractions is dependent on cyclic changes in surface area in vitro [23]. To maintain an adequate pool of functional surfactant subfractions in the airspaces in vivo, it is necessary to maintain a balance between secretion, uptake and clearance of the active and non-active surfactant subfractions [22]. Recent in vivo studies by Veldhuizen and colleagues in rabbits attributed surfactant conversion to a change in alveolar surface area associated with mechanical ventilation [24,25]. They found that changing ventilatory frequency [24] or the level of PEEP [25] did not affect the rate of conversion but that conversion of surfactant subfractions is dependent on tidal volume and time [24]. We found a significant conversion of active to non-active surfactant in group 45/0 compared with non-ventilated controls, but no statistically significant conversion in the other two groups who underwent ventilation compared with controls. We suggest that the large tidal volume in group 45/0 was able to induce significant surfactant conversion within 20 min. However, this 20-min period was too short to cause a significant difference in surfactant conversion in the groups ventilated with lower tidal volumes compared with non-

ventilated controls. The exact mechanisms underlying surfactant conversion as a result of changes in surface area are unknown.

An important function of pulmonary surfactant is to aid fluid balance in the lung, and prevent pulmonary oedema from increased suction forces at the alveolo-capillary barrier [26]. Loss of surfactant function with an increase in surface tension at the air-liquid interphase on the alveolar walls will decrease the pressure in the alveolar fluid, altering the pressure gradient across the alveolo-capillary membrane in the alveolar direction [26-28]. In vitro and in vivo studies have shown that pulmonary oedema, and in particular plasma-derived proteins in this oedema, are capable of inactivating surfactant in a dose-dependent manner [29-32]. This further decreases the pressure in alveolar fluid and thus cause further surfactant inactivation. When this vicious circle of surfactant inactivation has started, the resulting protein-rich pulmonary oedema accounts for much of the surfactant alternations seen in group 45/0. The importance of subtle primary changes in the pulmonary surfactant system in increasing the pressure gradient across the alveolo-capillary membrane, initiating a subsequent cascade of protein inactivation, was recently shown in a model of surfactant perturbation by dioctyl sodium sulfosuccinate, which does not cause any other damage of the alveolo-capillary barrier [28]. The study also showed that changes in surfactant make the lung vulnerable to damage by mechanical ventilation [28].

Findings in animals with induced acute respiratory failure and in patients with acute respiratory distress syndrome (ARDS) suggest that changes in the pulmonary surfactant system play a central role in this disease process [33]. Irrespective of the cause, decreased surfactant function increases the forces acting at the air-liquid interface of the alveolus and can lead to consequences such as decreased pulmonary compliance, decreased FRC with end-expiratory alveolar collapse; right-to-left shunt and hypoxemia with anaerobic metabolism [33]. Such changes necessitate the use of mechanical ventilation to maintain adequate oxygen delivery to the tissues. Mechanical ventilation may, however, perpetuate the alternations in the pulmonary surfactant system as found in this study, indicating that mechanical ventilation with high peak inspiratory pressures in patients with ARDS may impair the function of those alveoli that are still intact. The data also suggest that it is important to use PEEP in these patients to preserve normal surfactant function of alveoli that are not yet affected by the disease process.

We conclude that ventilation of the lungs of healthy rats with high peak inspiratory volumes at peak inspiratory pressure of 45 cmH_2O without PEEP caused severe impairment of pulmonary surfactant composition and function. PEEP prevented this impairment of the surfactant system, probably by reducing the amount of change in alveolar surface area, which prevents: surfactant displacement from the alveolar air-liquid interface into the small airways; increased conversion of active into non-active surfactant subfractions; and increased hydrostatic forces over the alveolo-capillary barrier which could lead to a self-propagating

mechanism of surfactant inactivation.

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Chapter 3

Exogenous surfactant preserves lung function and reduces alveolar Evans blue dye influx in a rat model of ventilation induced lung injury

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Abstract

Background: Intermittent positive pressure ventilation with high peak inspiratory lung volumes (HIPPV) has been shown to induce pulmonary edema and surfactant changes. We tested the effect of exogenous surfactant preceding HIPPV on lung function and permeability.

Methods: Five groups of 6 Sprague-Dawley rats received intratracheal administration of saline or 50, 100 or 200 mg/kg surfactant or no intra-tracheal administration prior to 20 min of HIPPV. Gas exchange was measured. A sixth group served as non-treated, non-ventilated controls. Post-mortem static pressure-volume curves and total lung volume at 5 cmH_2O transpulmonary pressure (V_5) were recorded; Gruenwald index and the steepest part of the compliance curve (C_{max}) were calculated. Active and non-active total phosphorus and minimal surface tension (γ_{min}) of broncho-alveolar lavage (BAL) were measured. In another experiment in 5 groups of 6 rats, Evans blue lung permeability was measured. Four groups received 100, 200 or 400 mg/kg surfactant intra-tracheally or no intra-tracheal administration prior to 20 min HIPPV. A fifth group served as non-treated, non-ventilated controls.

Results: Most active phosphorus was recovered in the group that received 200 mg/kg surfactant. This dose preserved V_5, Cmax, Gruenwald index and oxygenation after 20 min HIPPV and reduced γ_{min} of BAL to control value; 200 and 400 mg/kg surfactant reduced Evans blue permeability.

Conclusions: Exogenous surfactant preceding HIPPV prevents impairment of oxygenation, lung mechanics and minimal surface tension of BAL fluid and reduces Evans blue permeability. These data indicate a beneficial effect of surfactant on ventilation-induced lung injury.

Introduction

The development of pulmonary edema and alveolar flooding in healthy rats after lung overinflation with peak inspiratory pressures of 45 cmH_2O without positive end-expiratory pressure (PEEP) were first demonstrated by Webb and Tierney [1] and were later confirmed by Dreyfuss et al. [2]. The main determinant for edema development is the peak inspiratory lung volume [3]. Experiments with thoracic restriction in this rat model have clearly shown that high peak inspiratory pressures themselves that are not accompanied by high peak inspiratory lung volumes, do not induce lung injury [3].

However, peak inspiratory overstretching by lung overinflation can itself not explain ventilation-induced lung injury, since 10 cmH_2O at the same degree of overdistension (e.g. the same peak inspiratory pressure) in this animal model has been shown to reduce permeability edema and almost completely prevent lung parenchymal injury [2,3]. One study has attributed this reduction in permeability edema by PEEP to a decrease in the pulmonary capillary hydrostatic pressure [4], which will reduce edema formation when the pressure balance between (1) plasma colloid oncotic pressure, (2) capillary hydrostatic pressure, (3) interstitial oncotic pressure and (4) alveolar surface tension at the alveolo-capillary barrier is shifted away from the alveolar direction [5]. However, a recent study by our group in the same rat model has shown a reduction in the amount of surface tension reducing surfactant components after 20 min of lung overinflation without PEEP. Impairment of the surfactant system could be prevented by the use of 10 cmH_2O of PEEP [6] which prevented the conversion of surface active tubular myelin-like forms of surfactant (large aggregates) into non-active components which represent small vesicular structures (small aggregates). Gross et al. were the first to show that conversion of active into non-active surfactant subfractions is dependent on cyclic changes in surface area in vitro [7]. Studies by Veldhuizen et al. in vivo have confirmed that conversion is dependent on the change in alveolar surface area associated with mechanical ventilation [8]. These studies suggest that the reduction in alveolar flooding by PEEP is partially caused by its preservation of the surfactant system and suggest that ventilation-induced surfactant changes play a role in the development of alveolar flooding.

To further elucidate the role of surfactant changes in the pathogenesis of ventilation-induced lung injury, we investigated the effect of different doses of exogenous surfactant preceding lung overinflation on oxygenation, lung mechanics and Evans blue dye permeability.

Materials and methods

The study protocol was approved by the local Animal Committee, and the care and handling of the animals conformed with the principles approved by the Council of the American Physiologic Society. A total of 66 adult male Sprague Dawley rats (body weight 290-350 grams) was used.

Studies on the effects of exogenous surfactant

In the first set of experiments, 36 rats were randomly divided into six groups, anesthetized with nitrous oxide/oxygen/enflurane (Ethrane®, Abbott, Amstelveen, The Netherlands) (65/33/2 volume %), tracheotomized and a metal cannula was inserted into the trachea. The operation area was infiltrated with lidocaine 3.0 mg/kg (Xylocaine®, Astra Pharmaceutica BV, Rijswijk, The Netherlands).

Four groups received: 1.5 ml of saline (group saline) or exogenous surfactant (Leo Pharmaceuticals, Copenhagen, Denmark) dissolved in 1.5 ml of saline at a dose of 50 (group S50), 100 (group S100) and 200 (group S200) mg/kg bodyweight administered into the tracheal cannula over a 5 min period. During this period the animals were turned to the supine, prone and both side-positions and were breathing spontaneously. The surfactant used in this study is a natural surfactant isolated from minced pig lungs as previously described, which contains surfactant proteins B and C, but not surfactant protein A [9]. One group of animals did not receive any intra-tracheal administration (group 45/0). All animals were then allowed to recover from anesthesia and those that were given intratracheal administration could resorb saline from the lung during the subsequent period of spontaneous breathing, this to avoid an interaction between mechanical ventilation and saline in damaging the lung [10].

Thirty minutes after tracheotomy, the animals were re-anesthetised under gaseous anesthesia (see above) and a polyethylene catheter (0.8 mm outer diameter) was inserted into a carotic artery. After this surgical procedure, gaseous anesthesia was discontinued and anesthesia was replaced with pentobarbital sodium 60 mg/kg, intraperitoneally (Nembutal®; Algin BV, Maassluis, the Netherlands) during the remainder of the experiment; muscle relaxation was attained with pancuronium bromide 2 mg/kg, intramuscularly (Pavulon®; Organon Technika, Boxtel, the Netherlands). After muscle relaxation, the animals were connected for 20 min to a ventilator (Servo Ventilator 300, Siemens-Elema, Solna, Sweden) set in a pressure-controlled mode at a peak inspiratory pressure of 45 cmH_2O without PEEP, a frequency of 25 breaths/min, an I/E ratio = 1:2 and an FiO_2 = 1.0.

Blood samples taken from the carotid artery were measured 1, 10 and 20 min after starting mechanical ventilation (ABL505, Radiometer, Copenhagen, Denmark).

After 20 min of mechanical ventilation, all animals were sacrificed (overdose pentobarbital sodium through the penile vene). A sixth group of animals was sacrificed immediately after tracheotomy in an identical way. Static pressure-volume diagrams were then recorded using conventional techniques [11]. For these measurements the thorax and diaphragm were opened. The animals were placed into a volume and temperature constant bodybox and the lungs were reexpanded with pure nitrogen up to a pressure of 35 cmH_2O and subsequently deflated again. This procedure was performed to reopen lung areas that became atelectatic after this surgical procedure. The lungs were then immediately reinflated starting from 0 pressure in steps of 1 cmH_2O up to an intra-alveolar pressure of 35 cmH_2O and subsequently deflated in steps of 1 cmH_2O. This was done by changing the PEEP level on the ventilator while in CPAP mode (Servo Ventilator 300, Siemens-Elema, Sola, Sweden). Pressure changes in the bodybox were

recorded (Validyne model DP 45-32, Validyne Engineering Co., Northridge, CA, USA) at a sampling rate of 10 Hz using a (12-bit) analog-to-digital converter (DAS 1800, Keithley MetraByte, Taunton, MA, USA) and stored in a computer. With the rat still in the bodybox, the pressure signals from the bodybox were calibrated for known volume changes immediately after pressure volume-recordings, by injection of known volumes of air into the bodybox, using a precise syringe. The maximal compliance (C_{max}) was defined as the steepest part of the pressure-volume deflation curve, and was determined separately for each animal[9]. The Gruenwald index, defined as $(2V_5 + V_{10})$ /2 V_{max} (where V_5, V_{10} and V_{max} are the lung volumes above functional residual capacity (FRC) at transpulmonary pressures of 5, 10 and 35 cmH_2O) was calculated [12]. FRC was estimated by measuring total lung volume at a transpulmonary pressure of 5 cmH_2O (V_5) as previously described[12]. For this measurement the lungs and the heart were removed from the thorax. After dissection from the heart, the lungs were reexpanded with nitrogen up to a pressure of 35 cmH_2O to reopen lung areas that became atelectatic during excision. The lungs were then left to deflate against a positive pressure of 5 cmH_2O, which was chosen to compensate for the loss of negative intra-thoracic pressure. The total weight of lungs (W) was registered and the lungs were then immersed in saline at a preset depth to measure the upward force (F). According to Archimedes' principle, this force is caused by fluid displacement equal to the volume of the lungs. V_5 was then calculated as 0.99*F -0.94 *W [13].

Thereafter, the lungs were lavaged with saline/1.5 mM $CaCl_2$ (30 ml/kg) five times. The percentage recovered lung lavage fluid was calculated. The obtained lavage fluid was first centrifuged at 400 g (Beckman GPR, Beckman Instruments Inc., Palo Alto, CA, USA) for 10 min at 4°C to remove cells and cellular debris. The supernatant of this 400 g fraction (crude lavage) was then centrifuged at 40,000 g for 15 min at 4°C (Beckman L8-70M) to separate a surface active surfactant pellet (large aggregates) from a non-surface active supernatant fraction (small aggregates) [14]. The large aggregates were resuspended in 2 ml conversion buffer (0.15 M NaCl/10mM Tris/1 mM $CaCl_2$/0.1 mM EDTA, pH 7.4) [12]. Total phosphorus of the small and large aggregates was determined by phospholipid extraction [15] followed by subsequent phosphorus analysis [16]. Twenty µL of crude lavage and the resuspension of the active surfactant part was used for biophysical analysis of minimal surface tension after 50 cycles on a pulsating bubble surfactometer (PBS; Electronetics Corporation, Tonowanda, New York, USA) as described by Enhorning [17]. This apparatus records pressure across the surface of a bubble, expanded in the sample fluid and communicating with ambient air. The bubble pulsated within a sample chamber at a frequency of 20 pulsations per minute between defined radius limits. The sample temperature was set at 37 °C. From the known pressure gradient across the bubble surface and the minimal bubble radius, the minimal surface tension was calculated according to the Law of LaPlace ($P = 2\ \gamma/r$).

Permeability studies

To further elucidate the exact mechanism of the effect of surfactant in HIPPV shown in the

first part of the study, a second set of studies was performed. Thirty rats were randomly divided into 5 groups of 6 rats and tracheotomized as described above. Identical to the way described above, three groups received exogenous surfactant at a dose of 100, 200 and 400 mg/kg bodyweight (groups S100, S200 and S400) and one group did not receive any intratracheal instillation. After the animals were recovered from anesthesia, allowed to breathe spontaneous and reanesthetised, a carotid artery was cannulated and the animals were connected to the ventilator, to receive mechanical ventilation. A fifth group of animals served as non-treated, non-ventilated healthy controls (group control).

Vascular permeability was quantified by the extravasation of Evans blue (EB) dye over 19 min (Sigma, Steinheim, Germany) which correlates well with the extravasation of radiolabeled albumin at high rates of plasma leakage [18]. The dye (30 mg/ml) was filtered using a 0.22 μm Millipore filter (MILLEX-GV, Millipore Products Division, Bedford, MA, USA) before use [19]. One min after starting mechanical ventilation and after tracheotomy in the control group, EB dye (30 mg/kg) was injected through the penile vene. Nineteen min after EB injection, the lungs were lavaged once with warm saline (30 ml/kg). The lavage was centrifuged at 400 g to remove cells and cellular debris. The high amount of surfactant dissolved in the broncho-alveolar lavage (BAL) was shown to disturb photospectrometric measurements of EB dye concentration. Pilot experiments (not reported) measuring the extinction of the chloroform layer at 620 nm at various EB concentrations in saline after Bligh Dyer extraction, demonstrated that EB does not dissolve in chloroform but completely dissolves in a water-methanol phase. Therefore, one ml of BAL was used for phospholipid extraction according to Bligh and Dyer [15] to separate phospholipids in a chloroform layer from EB in the water-methanol phase.

After BAL, the tissue content of EB dye was determined by perfusing the lung circulation via the pulmonary artery with 20 ml of warm saline (37 °C) to remove intravascular dye. For this purpose the aorta was cut at the level of the diaphragm and the left auriculum was removed from the heart before lung vascular perfusion. EB was extracted from the lungs by incubation at room temperature for 3 days in 12 ml formamide (Sigma) in stoppered tubes [19].

The absorbance of water-methanol extracts of EB from BAL and of the formamide tissue extracts of EB were determined against a water-methanol and formamide blank at 620 nm wavelength and by interpolation from a standard curve of EB in the range 0.5-10 μg/ml in water-methanol and formamide, respectively [19]. It could be demonstrated (data not reported) that after Bligh Dyer extraction there are no substances in the BAL of animals with lung edema not given EB that affect the absorbance for water-methanol at 620 nm. The total amount of EB (mg) recovered from the BAL and in the tissue was calculated.

Statistical analysis

Inter-group comparisons were analysed with ordinary ANOVA. Intra-group comparisons were analysed with repeated measures ANOVA. If ANOVA resulted in a $p<0.05$ a Student-

Newman-Keuls post-hoc test was performed. All data are reported as mean ± standard deviation (SD).

Results

Table 1 gives data on PaO_2 and $PaCO_2$ over time in the five ventilated groups in the studies on lung function. After 20 min, oxygenation decreased in the two groups that did not receive exogenous surfactant. Oxygenation was preserved over time in the group that received 200 mg/kg bodyweight surfactant.

Table 1. Data on blood gas tension (mmHg) of the five ventilated groups during the study period in the lung function experiments. Values are mean (SD). Inter- and intra-group comparisons ANOVA with Student-Newman-Keuls post-hoc test if ANOVA $p < 0.05$. Statistical significance compared with *group 45/0; $^{\#}$group S100; ^{o}group S200; $^{\S}t = 10$ min; $^{\blacktriangle}t = 20$ min.

	Group	45/0	saline	S50	S100	S200
	Time (min)					
PaO_2	1	495.8 (28.2)$^{\blacktriangle}$	481.9 (102.8)$^{\#,o,\blacktriangle}$	510.4 (42.0)$^{\#,o}$	578.7 (25.4)$^{*,\blacktriangle}$	587.5 (25.4)*
	10	518.2 (40.7)$^{\blacktriangle}$	412.5 (123.4)$^{\#,o}$	499.3 (142.3)	566.8 (52.1)$^{\blacktriangle}$	632.4 (39.2)
	20	307.5 (186.8)^{o}	322.0 (150.7)^{o}	443.1 (191.0)	457.7 (114.8)	608.4 (37.7)
$PaCO_2$	1	22.6 (4.1)	24.7 (3.0)	24.4 (5.4)	23.7 (4.0)	23.6 (2.7)$^{\blacktriangle,\S}$
	10	20.6 (3.9)	22.7 (4.0)	21.1 (3.3)	18.9 (2.7)	18.5 (1.6)
	20	23.7 (4.4)	21.8 (7.0)	21.5 (3.9)	19.1 (3.7)	18.2 (2.6)

Gruenwald index, C_{max} and V_5 (Table 2) in group S200 were comparable to non-ventilated controls. The amount of active surfactant in the BAL fluid was higher in group S200 than in all other groups. The resuspension of active surfactant in group S200 showed more surface activity than in the other groups, except for group S100. The minimal surface tension of the

crude lavage fluid in group S200 was comparable to group control but was increased in all other ventilated groups.

Table 2. Recovery of BAL fluid and post-mortem data for Cmax, Gruenwald index, V_5, total lung weight, total phosphorus of small aggregates (SA) and large aggregates (LA) and minimal surface (min. surf.) tension of crude lavage and large aggregate resuspension in the lung function experiments in the lung function experiments. Values are mean (SD). Inter-group comparisons ANOVA with Student-Newman-Keuls post-hoc test if ANOVA $p < 0.05$. Statistical significance compared with group *S200; #group control; °group 45/0;†group S100.

Group	Control	45/0	saline	S50	S100	S200
Recovery BAL fluid (%)	74.6 (4.7)	76.6 (4.2)	73.3 $(2.9)^{*}$	71.6 $(1.3)^{*}$	75.6 (4.7)	81.3 (6.6)
C_{max} *(ml/cmH_2O/kg)*	3.9 (0.7)	2.3 (0.5)	2.5 $(0.7)^{\#}$	2.9 $(0.6)^{*}$	3.1 (0.6)	4.2 $(0.9)^{\circ}$
Gruenwald index	0.47 (0.13)	0.25 $(0.08)^{\#,*}$	0.28 $(0.10)^{\#,*}$	0.23. $(0.09)^{\#,*}$	0.37 (0.07)	0.52 (0.21)
V_5 *(ml)*	18.2 (4.1)	6.0 $(2.5)^{\#}$	4.1 $(2.4)^{\#,*,\dagger}$	7.4 (3.2)	9.2 $(2.9)^{\circ}$	15.4 $(3.5)^{\circ}$
Total phosphorus (SA)	0.8 (0.4)	1.3 (0.3)	1.2 $(0.2)^{*,\dagger}$	3.0 $(0.9)^{\#,*,\dagger}$	4.9 $(1.3)^{\#,\circ}$	5.8 $(2.7)^{\#,\circ}$
Total phosphorus (LA)	3.0 (1.6)	2.0 $(0.7)^{*}$	2.6 $(1.0)^{*}$	6.3 $(1.6)^{\circ}$	7.7 $(2.5)^{*}$	17.0 $(6.5)^{\circ,\#}$
Min. surf. tension crude (mN/m)	28.5 (6.5)	40.0 $(1.5)^{\#,*}$	46.1 $(0.7)^{\#,*,\dagger}$	39.5 $(6.8)^{\#,*}$	37.7 $(7.7)^{\#,*}$	29.7 (2.6)
Min. surf. tension LA (mN/m)	24.8 (2.9)	38.4 $(5.9)^{\#}$	45.0 $(3.2)^{\#,*,\dagger,\blacktriangle}$	14.8 $(10.5)^{\#,\circ,\dagger,*}$	6.2 $(7.9)^{\#,\circ}$	1.8 $(1.5)^{\#,\circ}$

In the permeability experiments (Table 3), oxygenation was decreased in group 45/0 after 20 min of HIPPV. Oxygenation after 20 min was preserved and significantly higher in groups S200 and S400 than in groups 45/0 and S100. The amount of EB dye recovered from the tissue was lower in controls than in all ventilated groups; there was no significant difference in the amount of EB dye recovered from the tissue in the ventilated groups. The amount of EB dye recovered from the BAL was significantly higher in group 45/0 compared to group control and significantly lower in groups S200 and S400 than in group 45/0.

Table 3. Data on blood gas tension (mmHg) and permeability indices (mean ± SD) in the five different groups in the permeability studies. Values are mean ± SD. Inter- and intra-group comparisons ANOVA with Student-Newman-Keuls post-hoc test if ANOVA $p < 0.05$. Statistical significance with *group 45/0; #group S100; †group control.

	Group	Control	45/0	S100	S200	S400
	Time (min)					
PaO_2	1		535.9 (24.3)	514.4 (51.4)	538.2 (47.3)	542.9 (22.6)
	10		507.2 (79.4)	561.8 (38.3)	560.0 (39.3)	555.9 (36.6)
	20		280.1 (114.1)	408.4 (154.5)	555.9 (31.1)*,#	585.0 (38.1)*,#
$PaCO_2$	1		26.9 (2.7)	24.0 (1.4)	27.5 (4.1)	27.0 (5.1)
	10		23.4 (3.3)°	19.6 (1.6)°	21.4 (3.9)°	20.0 (1.4)°
	20		21.5 (3.4)°	20.0 (2.7)°	22.3 (5.1)°	19.4 (2.1)°
Evans Blue Tissue (mg)		0.11 (0.05)	0.64 (0.08)†	0.61 (0.25)†	0.58 (0.12)†	0.55 (0.14)†
Evans Blue BAL (mg)		0.06 (0.01)	0.94 (0.36)†	0.53 (0.26)	0.43 (0.40)*	0.28 (0.15)*
Evans Blue Total (mg)		0.17 (0.04)	1.58 (0.43)†	1.14 (0.44)	1.01 (0.45)†,*	0.83 (0.21)†,*

Discussion

This study demonstrates that exogenous surfactant at a dose of 200 mg/kg bodyweight given to rats prior to HIPPV prevents impairment of lung mechanics and oxygenation after 20 min of HIPPV. Moreover, surfactant at a dose of 200 and 400 mg/kg bodyweight significantly reduced the amount of EB dye recovered from the BAL fluid after 20 min of HIPPV. These data show that exogenous surfactant has a beneficial effect on ventilation-induced lung injury.

Changes in permeability of the alveolo-capillary barrier to protein have been attributed to epithelial stretching. Equivalent pore radii indicate that the epithelium, rather than the

endothelium, is primarily responsible for restricting solute transport from the capillaries across the alveolo-capillary membrane into the alveolus [20,21]. As the epithelium is progressively stretched there is an opening of water-filled channels between alveolar cells [22,23].

Important evidence on the role of capillary hydrostatic pressure in inducing edema in the HIPPV rat model comes from the effect of 10 cmH_2O PEEP, which was shown to reduce edema infiltration [1,3]. This effect was attributed to hemodynamic alterations due to PEEP, which will reduce filtration pressure over the alveolo-capillary membrane [4]. Indeed, infusion of dopamine to correct the drop in systemic arterial pressure that occurs with PEEP ventilation was shown by Dreyfuss et al. to partially abolish the reduction in pulmonary edema induced by PEEP [4]; however, this effect was only partial and as pulmonary artery pressure was not recorded in their study it cannot be excluded that the transpulmonary filtration pressure after dopamine infusion, was higher than in the animals ventilated without PEEP [4]. Therefore, it can not be excluded that other factors contribute to intra-alveolar edema development.

Loss of surfactant function with an increase in surface tension at the air-liquid interface on the alveolar walls has been shown to direct the net driving force across the alveolo-capillary membrane to the alveolar side, resulting in intra-alveolar fluid and protein accumulation [5,24,25]. Based on such observations, a recent study by our group postulates a different mechanism for the effect of PEEP on the reduction of lung permeability edema in HIPPV [6]. It describes the mechanisms of surfactant impairment after HIPPV, which include surfactant displacement from the alveolar air-liquid interface into the small airways and increased conversion of active into non-active surfactant subfractions, and shows that PEEP reduces such HIPPV-induced surfactant impairment [6]. Surfactant preservation by PEEP will reduce the contribution of surface tension to fluid and particle transport across the alveolo-capillary barrier, which would be a different explanation for the reduction in permeability edema induced by PEEP [6]. If this mechanism is valid, then exogenous surfactant preceding HIPPV should be able to reduce permeability edema after HIPPV. The present study shows that this is the case and that 200 and 400 mg of exogenous surfactant per kg bodyweight are able to reduce intra-alveolar Evans blue dye influx. This is a substantial amount given the normal total surfactant phospholipid pool size of 10 mg/kg bodyweight in rats [26]. The present data demonstrate that, although peak inspiratory epithelial pore overstretching and capillary hydrostatic pressure are important determinants of permeability edema, surfactant actively stabilizes the fluid balance in the lung and protects the lung from permeability edema at the level of the broncho-alveolar lavage accessible space. Such findings are consistent with recent findings in a model of mild surfactant pertubation by dioctyl sodium sulfosuccinate, which was shown to initiate protein infiltration [27], and previous findings on the rate-limiting effect of supra-physiological amounts of (exogenous) surfactant on solute permeability of normo-ventilated rabbits [28]. The contribution of surface tension to fluid and particle transport across the alveolo-capillary barrier appears to be most prominent on transudation across the alveolo-capillary barrier, as demonstrated by the reduced

EB dye in the broncho-alveolar lavage accessible space, and appears to be less prominent on exudation from the capillary, evidenced by the equal amount of EB recovered from the tissue.

Once protein infiltration has started, plasma-derived proteins dose-dependently inhibit surfactant [29, 30]. This will result in a vicious circle of more fluid and protein influx as a result of increased surface tension with further surfactant inactivation by plasma-derived proteins and more destabilisation of the small airways. In the present study, exogenous surfactant at a dose of 200 mg/kg preceding HIPPV prevented a drop in arterial oxygenation after 20 min of HIPPV and preserved Gruenwald index, C_{max} and V_5 at control values. These findings indicate that exogenous surfactant preceding HIPPV is able to preserve normal end-expiratory lung stability even after 20 min of HIPPV. This end-expiratory alveolar stabilization due to exogenous surfactant is likely caused by a more advantageous protein-phospholipid ratio, which is a critical factor for normal surfactant function [30]. The reason for this more beneficial ratio is two-fold. First, there was a higher amount of surfactant present in the broncho-alveolar lavage accessible space, as evidenced by the higher amount of total phosphorus of both surface active large and non-surface active small aggregates in the animals given exogenous surfactant (Table 2). Second, the reduction in surface tension over the alveolo-capillary barrier towards normal levels by exogenous surfactant will have reduced protein influx. The large aggregate resuspension of the group given 200 mg/kg of exogenous surfactant showed more potential to reduce surface tension than that of non-ventilated controls (Table 2). However, when the influence of surfactant inhibiting proteins in the broncho-alveolar lavage accessible space was included, the net surface tension reducing potential was normalized to the level of controls, evidenced by the normalisation of the minimal surface tension of the crude lavage on the pulsating bubble surfactometer in group S200 (Table 2).

Such disturbance of surfactant function may be the reason for repeated collapse and reexpansion of the lung and, thus, for ventilation-induced lung parenchymal damage [27]. It may thus be suggested, that surfactant changes are (partially) responsible for the lung parenchymal damage previously demonstrated in this animal model[2]. Such a relationship has been previously shown by Nilsson et al. in prematurely delivered rabbits. It was shown that exogenous surfactant preceding mechanical ventilation with both constant tidal volumes (10 ml/kg) and constant peak inspiratory pressures increases lung-thorax compliance and reduces epithelial lesions [31]. Further studies need to be conducted to test such a hypothesis in this HIPPV-induced lung injury model.

The present data show that there is an important interaction between mechanical ventilation and surfactant changes in inducing lung injury. Such changes occur both in a model of both acute lung injury of prematurely delivered animals characterized by an immature surfactant system [31] and, as our data show, in a model of acute lung injury in adult animals, in which surfactant changes are induced by mechanical ventilation itself [6]. It has now been demonstrated that high amounts of exogenous surfactant have a beneficial effect on lung function and, possibly, survival in patients with acute respiratory distress

syndrome [32]. Our data suggest that administration of high amounts of exogenous surfactant may beneficially influence further impairment of lung function due to mechanical ventilation in such patients by protecting the healthy lung areas that are not yet affected by the disease process.

In conclusion, our data show that exogenous surfactant administration preceding 20 min of lung overinflation without PEEP is able to reduce Evans blue accumulation in the broncho-alveolar lavage accessible space and preserve end-expiratory lung stability. These data indicate that exogenous surfactant has a beneficial effect on ventilation-induced lung injury.

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Chapter 4

Pulmonary ^{99m}Tc-human serum albumin clearance and effect of surfactant replacement after lung lavage in rabbits

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Summary

Objective: Pulmonary clearance of technetium-labelled human serum albumin was measured in order to investigate whether the surfactant layer is a rate-limiting factor for the permeability of the alveolar-capillary membrane for ^{99m}Tc-labelled albumin.

Design: Prospective, randomized, controlled trial.

Settings: Research laboratory.

Subjects: Nineteen white New Zealand adult rabbits.

Interventions: Three groups of rabbits were studied: group 1 animals received natural surfactant after lung lavage; group 2 animals underwent lung lavage only and group 3 animals were not lavaged and served as an untreated, healthy control group. All animals were ventilated with high pressures.

Measurements and main results: ^{99m}Tc-labelled albumin was nebulized into the inspiratory line of the breathing circuit with an air jet nebulizer. The clearance measurements were then immediately started. Gamma camera images were obtained in 1-minute frames for 120 minutes and stored in a 64 x 64 image matrix in a computer.

In group 1 animals, surfactant restored bloodgases to near normal, and all animals except one had bi-exponential clearance curves. The half-time of the fast compartment being 35.9 ± 6.4 mins, and the half-life of the slow compartment was 847.5 ± 143.5 mins. All group 2 animals also had bi-exponential clearance curves of the tracer (the half-lives of the fast and slow compartments were 14.6 ± 6.7 and 459.8 ± 167 mins, respectively). The half-lives of both the fast ($p < .01$) and slow ($p < .01$) components were significantly different between groups 1 and 2. Group 3 had a mono-exponential half-life of 580 ± 225 mins.

Conclusions: The use of ^{99m}Tc-human serum albumin as a tracer molecule is possible and feasible. The clearance of this tracer is, in part, determined by the integrity of the pulmonary surfactant system, as it is with ^{99m}Tc-diethylenetriamine pentaacetate.

Introduction

It is well known that the permeability of the pulmonary blood-air barrier increases after surfactant depletion or impairment. Alveolar edema and presence of protein in the alveolar fluid represent major causes of further deterioration of normal lung function [1]. In order to measure and quantitate this change or increase in normal permeability characteristics, some techniques have been developed with the use of radionuclides. The most frequently used tracer is ^{99m}Tc-diethylenetriamine pentaacetate, which is a small, simple-to-obtain and stable molecule. Many reports [2-4] have shown that by using this tracer molecule, the permeability of the alveolo-capillary membrane can accurately be described. The pulmonary surfactant system was shown [3,4] to be rate-limiting for the clearance of ^{99m}Tc-diethylenetriamine pentaacetate from the lung.

However, the use of ^{99m}Tc-diethylenetriamine pentaacetate has some important drawbacks. Thus, for example, a simple increase in lung volume causes a dramatic increase in pulmonary clearance of ^{99m}Tc-diethylenetriamine pentaacetate to levels found in severe pulmonary diseases [5,6]. This sensitivity to volume increases is one of the reasons why some investigators have suggested the use of different, somewhat larger molecules than ^{99m}Tc-diethylenetriamine pentaacetate. One such molecule is ^{99m}Tc-labelled-human serum albumin.

Therefore, in this study, we investigated whether ^{99m}Tc-human serum albumin could be used to quantify the permeability characteristics of the alveolo-capillary barrier instead of using the conventional method with ^{99m}Tc-diethylenetriamine pentaacetate. Moreover, we investigated whether the pulmonary clearance rate of ^{99m}Tc-human serum albumin is decreased, and wheter the clearance characteristics of ^{99m}Tc-human serum albumin are changed in the presence of surfactant, as it is with ^{99m}Tc-diethylenetriamine pentaacetate.

Materials and methods

Animals

Nineteen New Zealand adult rabbits (harlan Laboratory, Zeist, The Netherlands), weighing 3.0 ± 0.5 kg, were used. Approval of the protocol was obtained from the institutional Animal Investigation Committee. Care and handling of the animals were in accordance with the European Community guidelines. The animals were anesthetized with sodium pentobarbital (50 mg/kg body weight/h iv). After tracheotomy, neuromuscular blockage was induced with pancuronium bromide (0.5 mg/kg body weight/h i.m). A carotid artery was cannulated for blood gas measurements and blood pressure monitoring (Figure 1).

All animals underwent pressure controlled ventilation (Servo Ventilator 900 C, Siemens-Elema AB, Solna, Sweden). Initial ventilatory settings were: frequency (*f*) of 30/min, inspiratory/expiratory ratio of 1:2, FiO_2 of 1.0 and a positive end-expiratory pressure (PEEP) of 2 cm H_2O. PEEP was applied to prevent atelectasis formation during preparation. The minute ventilation was set to keep $PaCO_2$ between 30 - 40 torr (4.0-5.3 kPa) by adjusting the peak inspiratory pressure.

Respiratory failure was induced in 13 animals by performing lung lavage [7].

Ventilatory settings were changed as follows: peak inspiratory airway pressure was set at 26 cm H_2O and PEEP at 6 cm H_2O. Lung lavage was performed with 30 mL/kg body weight of isotonic saline at 37°C. Each volume of saline was administered through the tube at a pressure not exceeding 40 cm H_2O. The lungs were lavaged until PaO_2 decreased to < 100 torr (<13.3 kPa) with these ventilatory settings, which were not changed during the observation period. Animals were randomly assigned to group 1 or 2. In group 1 (n=7) natural surfactant was instilled via the tracheal tube after the lavage procedure. Group 2 animals (n=6) received no surfactant. Another group of six animals served as unlavaged, healthy controls (group 3). These animals were ventilated during the 120-min study period according to the settings of groups 1 and 2 after the lavage procedure.

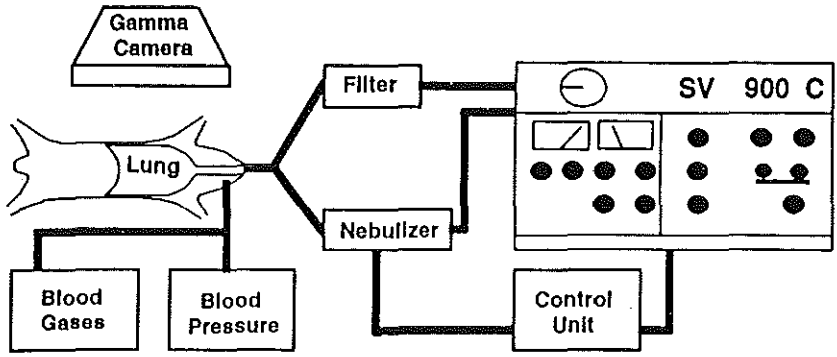

Fig. 1 Schematic representation of the experimental apparatus. *SV 900 C*, Servo ventilator 900 C.

The surfactant used in this experiment is a natural surfactant isolated from pig lungs [8]. Each animal was given 100 mg surfactant phospholipids/kg body weight suspended in 0.9% saline (25 mg phospholipids/mL).

A solution of 1850 Mega Becquerel ^{99m}Tc-human serum albumin was prepared, using a commercial kit (Technescan DRN 4361, Mallinckrodt Diagnostica, Petten, The Netherlands) and placed into an air jet nebulizer (Ultravent, Mallinckrodt Diagnostica, Petten, The Netherlands). Before and after nebulizing, the binding percentage of the ^{99m}Tc-label with the human serum albumin molecule was tested using thin layer chromatography.

The aerosol was then administered via the ventilation circuit [9]. The nebulizer was placed in the inspiratory line of the ventilation circuit. The supply of pressurized air to the nebulizer was controlled by a pneumatic valve which, in turn, was connected to the Servo Ventilator via an electronic circuit. During the period of aerosol administration, the nebulizer was operating during expiration only, filling the tubing in the inspiratory line with aerosol. The particles thus produced were administered with the ensuing insufflation. Aerosol was

administered until a count rate of ~ 300 counts/sec had been reached (after 1 to 2 mins). The clearance measurement was then immediately started. Gamma camera images were obtained in 1-minute frames for 120 minutes and stored in a 64 x 64 image matrix in a computer (PDP 11/34, Digital Equipement Corporation, Maynard, MA).

After 110 min, a small dose of ^{99m}Tc-human serum albumin was injected intravenously in all animals in order to correct for blood background activity [10].

During the study period, blood gas tensions were measured at 30-min intervals during the 2-hr observation period. When the clearance measurement was completed, the animal was killed with an overdose of pentobarbital sodium.

Data analysis

Clearance measurements were analyzed by selecting a region of interest over both lungs and generating a time-activity curve (Fig. 2). Each corrected time-activity curve was analyzed by fitting a mono-exponential equation (Eq. 1) as well as a bi-exponential equation (Eq. 2) to the experimental data:

$$A(t) = A(0)\, e^{-kt} \quad [1]$$

$$A(t) = A_F(0)\, e^{-k(F)\, t} + A_S(0)\, e^{-k(S)\, t} \quad [2]$$

where A(t) is the radioactivity in the lung at any time t, A(0) is the radioactivity in the lung at time zero and k the decay constant. In the bi-exponential analysis, $A_F(0)$ and $A_S(0)$ represent the amount of radioactivity eliminated with the fast (k_F) and slow (k_S) clearance components. Clearance was expressed as the half-life time ($T½ = \ln 2/k$) for the single clearance component in the mono-exponential and for the fast (T½ f) and slow (T½ s) clearance components in the bi-exponential analysis. The relative amount of tracer cleared by the fast clearance component (f_F) was calculated as $A_F(0)/(A_F(0) + A_S(0))$.

The desired quantities (i.e. A(0), k, $A_F(0)$, $A_S(0)$, k_F and k_S) were obtained by minimization of the sum of squares by using the Nelder-Mead simplex method [11,12]. This method for multidimensional minimization which does not rely on the use of gradient information. Different initial values were used to investigate the convergence properties of the method; in this study, similar estimates were obtained for widely different initial values. A lower boundary on the standard error for each parameter estimate was calculated on the basis of the Fisher information matrix. The F test was used for detecting differences between the mono- and bi-exponential fits.

Statistical analysis

Values are expressed as mean ± SD. Differences between the P_aO_2 values of the three groups on t = 0, 30, 60, 90 and 120 mins were evaluated with a non-parametric Kruskal-Wallis analysis of variance test. Subsequent 2 x 2 analysis between the groups was done, using the Mann-Whitney test for unpaired samples. Pre- and post-lavage P_aO_2 data for the surfactant

group (group 1) were evaluated with the Friedman nonparametric test for paired samples.

Differences between the fast and slow components of the half-life of group 1 (surfactant treated) and group 2 (lavaged) and differences between f_f of groups 1 and 2 were evaluated using the Mann-Whitney test for unpaired samples. Differences were considered statistically significant at a $p < 0.05$.

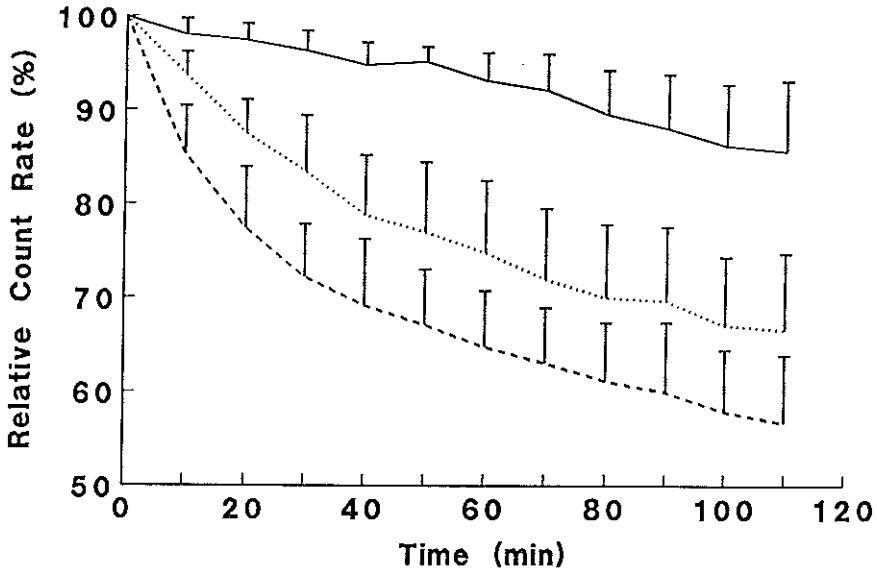

Fig. 2 Time-activity curves of the different groups during the study period. Data are mean ± SD. From top to bottom: solid line control animals (group3); dotted line, surfactant-treated animals (group 1); dashed line lavaged animals (group 2). Group 1 and 2 animals show bi-exponential clearance curves and group 3 animals show a mono-exponential clearance curve.

Results

All animals were kept under stable circulatory conditions throughout the experiment. Testing of the binding percentage of the labelling showed that before nebulization 98 ± 0.4% and after nebulization 99 ± 0.1% of the label was connected to the albumin molecule.

The pulmonary distribution of ^{99m}Tc-human serum albumin was uniform in all animals. There was a distinct difference in clearance of the tracer between the groups.

All clearance curves of ^{99m}Tc-human serum albumin of the lavaged animals (groups 1 and 2) except one, were of a bi-exponential character, with bi-exponential curves fitting

statistically significantly better than mono-exponential curves in all animals. Analysis of the surfactant-treated animals of group 1 resulted in a half-life of the tracer for the fast compartment of 36.0 ± 6.4 mins, and for the slow compartment in a half-life of 847.5 ± 143.5 mins. In one animal of group 1, however, the monophasic equation analysis fitted better than the biphasic equation, and the half-life was 515 mins. The f_F value was 0.27 ± 0.06. In group 2, the half-life of the fast compartment was 14.6 ± 6.6 min, and the half-life of the slow compartment was 460 ± 167 mins. These values, both the fast and slow component, are statistically significantly different from the values of group 1. The relative amount of tracer cleared by the fast compartment (f_F) in the animals of group 2 was 0.32 ± 0.04, which did not significantly differ from the f_F of group 1. The clearance curve of the controls (group 3) was monophasic of character. Group 3 had a half-life of 580 ± 225 mins. Figure 2 shows the time-activity curves of groups 1, 2 and 3 during the study period.

Lung lavage decreased blood gas tensions to < 100 torr (<13.3 kPa) in all lavaged animals (groups 1 and 2). The number of lavages necessary for this decrease was ~ 12 to 14 lavages. In group 1, surfactant restored blood gas tensions almost to normal and there were no statistical differences from the pre-lavage blood gas tensions in this group. There was a statistically significant difference in blood gas tensions between group 1 after lavage and surfactant replacement, and group 3 (controls), except for t = 120 mins. The animals in group 3 were slightly hyperventilated, as shown by the $PaCO_2$ values. The blood gas tensions of group 1 after lavage and surfactant replacement, and group 3 were statistically significantly different from the blood gas tensions in group 2 after lavage. Table 1 shows the blood gas tensions of the three groups during the study period.

Discussion

This study demonstrates that the clearance of ^{99m}Tc-human serum albumin is also, at least in part, determined by the integrity of the pulmonary surfactant system, as it is with ^{99m}Tc-diethylenetriamine pentaacetate. The clearance rates in the surfactant treated animals (group 1) are smaller than in the non-treated group (group 2). The clearance curves of almost all animals in group 1, however, are of a bi-exponential character, in contrast to the control group (group 3) indicating still possible abnormal permeability and transfer characteristics of the tracer through the membrane. The initial fast compartment clearance rate of the surfactant-treated group is, however, significantly slower than that of lavaged animals.

The binding percentage of the ^{99m}Tc-label with the human serum albumin indicates that at least no dissociation has taken place in vitro. In vivo dissociation by infiltrated proteolytic enzymes [13] can not be entirely excluded, although no accumulation of radioactivity in the thyroid and salivary glands was measured during these experiments, which indicates that no large amount of free $^{99m}TcO_4^-$ was formed.

In rabbits, the lung lavage model initially represents a pulmonary surfactant deficiency at the alveolar level [7]. The surfactant layer in the alveoli and airways is washed away by the warmed saline, leading to decreased lung compliance, atelectatic areas and deterioration of blood gas tensions. When mechanical ventilation is applied to these lungs, higher peak

airway and end-expiratory pressures are needed to open up the alveoli and stabilize them during the ventilatory cycle when compared to non-lavaged animals. Exogenous surfactant instillation can restore lung functions and mechanics to almost normal when administered immediately after the last lung lavage [8], as shown in other models of respiratory failure [14].

Table 1. Blood gas tensions of the three groups during the study period (mean ± SD) in torr (kPa). Group 1, lavaged and surfactant-treated animals; Group 2, lavaged animals; Group 3, healthy control animals. A $p < 0.05$ was considered statistically significant (by Mann-Whitney test for unpaired samples): [a]Statistical significance, group 1 compared with group 2; [b]Statistical significance group 2 compared with group 3; [c]Statistical significance, group 1 compared with group 3.

	Group 1		*Group 2*		*Group 3*	
Time (min)	PaO_2	$PaCO_2$	PaO_2	$PaCO_2$	PaO_2	$PaCO_2$
-5	537.5±17.4	32.5±3.0	538.5±16.2	33.8±3.8	-	-
	(17.5±2.3)	(4.3±0.4)	(71.8±2.2)	(4.5±0.5)	-	-
0	78.4±20.3	37.7±4.5	74.2±19.5[b]	35.2±3.8	578.5±33.0[c]	27.8±8.3
	(10.5±2.7)	(5.0±0.6)	(9.9±2.6)	(4.7±0.5)	(77.0±4.4)	(3.7±1.1)
30	460.2±84.4[a]	38.8±4.5	176.7±63.3[b]	34.0±5.3	572.3±36.9[c]	22.2±7.5
	(61.3±10.3)	(5.2±0.6)	(23.5±7.4)	(4.5±0.7)	76.3±4.9	(3.0±1.0)
60	464.9±101.2[a]	40.5±3.9	214.3±116.9[b]	35.8±5.3	579.3±61.3[c]	18.2±6.2
	(62.0±13.5)	(5.4±0.5)	(28.6±14.2)	(4.8±0.7)	(77.2±8.2)	(2.4±0.8)
90	469.7±118.5[a]	40.4±6.8	184.9±112.4[b]	39.5±4.5	587.1±53.9[c]	16.3±6.8
	(62.6±15.8)	(5.4±0.9)	(24.7±15.0)	(5.3±0.6)	(78.3±7.2)	(2.2±0.9)
120	500.1±80.7[a]	38.6±3.8	178.7±94.4[b]	41.3±4.5	553.1±47.5[c]	15.5±7.0
	(66.7±10.8)	(5.2±0.5)	23.8±12.7	5.5±0.6	(73.7±6.3)	(2.1±0.9)

Several workers [15-17] have shown that exogenous surfactant instillation in immature lungs reduces permeability of the alveolar-capillary membrane in the immature. Surfactant thereby restricts formation of pulmonary edema and the influx of water and proteins into the intra-alveolar air spaces. For these permeability measurements, intravenously injected iodine-

labelled albumin was used, and permeability was assessed by measuring the amount of labelled albumin recovered in lung lavage fluid. These studies showed that although the epithelium is of greatest importance in limiting free diffusion of water and solutes into the alveolar air space, without the surfactant layer, high permeability of the membrane exists; this indicates that the phospholipid-protein layer has a critical sealing-off function. It also restores permeability of the alveolar-capillary barrier, as measured by ^{99m}Tc-diethylenetriamine pentaacetate clearance [3, 18-21]. Some workers, however, have queried the sensitivity of this ^{99m}Tc-diethylenetriamine pentaacetate technique based on the fact that, even in healthy lungs, pulmonary clearance of ^{99m}Tc-diethylenetriamine pentaacetate can increase to levels found in severe acute respiratory distress syndrome by physiological factors, such as increase of lung volume, or even by cigarette smoking [5,6,22]. This led to the hypothesis that ^{99m}Tc-diethylenetriamine pentaacetate (492 dalton, 0.6 nm (2)) could be a too small molecule to 1) allow discrimination between intermediate damage and severe damage to the membrane; and 2) allow discrimination between increased permeability resulting from damage to the membrane and increased due to physiological factors. This hypothesis provided the rationale for using radiolabelled solutes which have a greater molecular weight and radius as, for instance, human serum albumin (69,000 dalton, 3.5 nm [23-27]). It has been reported [28] that epithelial permeability measurements could be better performed using larger molecules whose transfer across the membrane is less influenced by physiological factors such as lung volume. Barrowcliffe and colleagues [21] showed that ^{99m}Tc-albumin is a suitable tracer molecule for measuring epithelial permeability.

The finding that exogenous surfactant therapy restores normal permeability [15-17] is, in part, confirmed by this study, as shown by the slower clearance rates in the surfactant treated animals (group 1) than in the nontreated group (group 2). The reason behind the multi-exponential clearance curves in the surfactant treated animals (group 2) in contrast to the control group (group 3), could be that between the first lung lavage and the moment of exogenous surfactant administration, ventilation-induced damage had already developed. Studies by Nilsson and colleagues [29] showed that epithelial disruption takes place after only a few breaths in case of surfactant deficiency combined with mechanical ventilation. This damage could largely be prevented by direct surfactant instillation to the lungs [30]. In the present study, surfactant instillation probably prevented further damage to the epithelium with subsequent, at least partial, restoration of normal integrity of the alveolar-capillary membrane. Inhomogeneity of surfactant distribution in the lungs, probably due to the still imperfect surfactant administration techniques and surfactant preparations, also may have caused differences in clearance rates [31]. Alveoli receiving almost no surfactant have a higher permeability, and alveoli receiving sufficient amounts of surfactant probably had normal or even decreased permeability for the tracer molecule [4]. This feature of the technique might enable the evaluation of different surfactant preparations, in which the more effective ones will spread more homogeneously in the lungs, leading to restored monophasic clearance characteristics.

In the lavaged animals (group 2), a bi-phasic clearance curve exists, probably caused by a combined effect of the induced alveolar surfactant deficiency and the nonoptimal mechanical ventilation mode. Lung lavage could affect individual alveoli and/or lung regions to a different degree, inducing differing degrees of damage and resulting in different half-lives of the tracer. This biphasic clearance curve is also seen in a model of nitrogen dioxide-induced lung injury [32].

Increased alveolo-capillary permeability with increased intra-alveolary albumin can inactivate surfactant [33], which may lead to a vicious circle of protein infiltration and subsequent surfactant inactivation, as seen in the respiratory distress syndrome [8]. Our results show a possible mechanism: surfactant depletion in this model causes increased human serum albumin permeability. We demonstrate that exogeneous surfactant replacement prevents increased permeability of the alveolo-capillary barrier for human serum albumin.

Nilson and Wollmer [34] performed ^{99m}Tc-Albumin and ^{99m}Tc-diethylenetriamine pentaacetate clearance measurements in rabbits after dioctyl sodium sulphosuccinate administration, inducing surfactant dysfunction, followed by oleic acid administration, inducing severe damage of the alveolor-capillary membrane. The authors [34] found increased clearances for both substances in the oleic acid model. With detergent, however, clearance was raised for Tc-diethylenetriamine pentaacetate but not for ^{99m}Tc-Albumin. The authors [34] suggested that the epithelial barrier may be rate-limiting for ^{99m}Tc-Albumin, not the surfactant barrier. The detergent model results in depletion of surfactant at the alveolar surface but does not result in increased pulmonary microvascular membrane permeability to macromolecules [35]. Increased microvascular permeability, however, does occur in our model in combination with ventilation [7], which is an explanation for the faster clearance of ^{99m}Tc-Albumin in our study.

The control group (group 3) in the present study showed a mono-exponential clearance curve for ^{99m}Tc-human serum albumin, which has also been demonstrated by others [21,28]. The rate of clearance is so slow that one cannot exclude mechanisms of transport other than diffusion across the alveolar-capillary barrier with these small quantities of protein. This transport, for instance, could be due to an active vesicle transport system [36].

The data of this study show that for ^{99m}Tc-human serum albumin clearance measurements, the surfactant layer is also a rate-limiting factor. Questions remain concerning in vivo stability and possible dissociation of the ^{99m}Tc-human serum albumin tracer and the exact path of 'diffusion' of the molecule. These problems need to be addressed in further studies.

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Chapter 5

Conventional ventilation modes with small pressure amplitudes and high end-expiratory pressure levels optimize surfactant therapy

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Summary

Objective: High frequency oscillation studies have shown that ventilation at high end-expiratory lung volumes combined with small volume cycles at high rates best preserves exogenous surfactant and gas exchange in lavaged lungs. We investigated whether surfactant composition and gas exchange can also be preserved by conventional modes of mechanical ventilation which combine high levels of positive end-expiratory pressure (PEEP) with small pressure amplitudes.

Design: Prospective, randomized, non-blinded, controlled study

Setting: Research laboratory

Subjects: Thirty male Sprague-Dawley rats

Interventions: Rats were lung-lavaged and treated with exogenous surfactant (100 mg/kg). After 5 minutes, four different ventilator settings (FiO_2 = 1.0) were applied for three hours in 4 groups of rats [peak inspiratory pressure (cm H_2O); static PEEP (cm H_2O); I/E ratio; frequency]: 26/2/1:2/30 (group 26/2); 26/6/1:2/30 (group 26/6); 20/10/1:2/30 (group 20/10-static); 20/6/7:3/130 creating an auto-PEEP of 4 cmH_2O (group 20/10-auto).

Measurements and Main Results: In all groups, PaO_2 increased immediately to pre-lavage values after surfactant therapy. In group 26/2, PaO_2 deteriorated to post-lavage values within 30 min when PEEP was decreased to 2 cm H_2O, whereas PaO_2 remained stable for three hours in the other groups. The $PaCO_2$ increased in groups 26/2 and 20/10-static; $PaCO_2$ could not be reduced by increasing ventilation frequency up to 130 in group 20/10-static. Groups 26/6 and 20/10-auto remained normocapnic. Broncho-alveolar lavage protein concentration was higher in groups 26/2 and 26/6 compared to groups 20/10-static and 20/10-auto. There was significantly more conversion of surface active large aggregates into non-active small aggregates in group 26/2 compared to groups 20/10-static and 20/10-auto.

Conclusions: We conclude that exogenous surfactant composition is preserved by conventional modes of mechanical ventilation which use small pressure amplitudes and adequate oxygenation is maintained by high end-expiratory pressure levels. Effective carbon dioxide removal could be achieved by applying a ventilation mode that creates auto-PEEP and not by a mode which applies the same level of PEEP by static-PEEP only.

Introduction

In neonates with respiratory distress syndrome (RDS), exogenous surfactant immediately reverses hypoxemia and has decreased mortality by 30-40% [1]. There are indications that surfactant therapy may also be beneficial in pediatric patients with acute RDS [2,3].

It is known from experimental studies that ventilation strategy influences the effect of surfactant therapy [4-6]. Studies by Froese *et al.* have shown that high frequency oscillation (HFO) at high lung volumes when combined with surfactant therapy, could improve PaO_2 to a constant level with lower alveolar protein influx and a higher amount of active surfactant at the end of the study period than conventional mechanical ventilation (CMV) where PaO_2 decreased over time [5].

Recent data suggest that differences in conversion of active into non-active surfactant in both healthy animals and animals with acute lung injury receiving surfactant therapy [6-8] are caused by differences in cyclic changes in alveolar surface area. As tidal volumes were ten-fold higher during CMV than during HFO in the study by Froese and colleagues [5], this raises the question whether lower rates of surfactant conversion can also be obtained by modes of CMV that combine small volume cycles at high rates with high PEEP levels. We therefore investigated the effect of different pressure amplitudes and PEEP settings on exogenous surfactant therapy with respect to gas exchange, protein influx and conversion of active into non-active surfactant during a three-hour ventilation period in lung-lavaged rats. Moreover, the effect of auto-PEEP on $PaCO_2$ versus a mode creating the same level of PEEP by static PEEP only was investigated.

Materials and methods

This study was approved by the Institutional Review Board for the care of animal subjects. Care and handling of the animals were in accordance with National Institute of Health guidelines.

The non-blinded studies were performed in male Sprague-Dawley rats (n = 24) with a body weight of 260-330 g (IFFA Credo, The Netherlands). After induction of anesthesia with nitrous oxide, oxygen and ethrane (66/33/3%), a polyethylene catheter (0.8-mm outer diameter) was inserted into the right carotid artery for drawing arterial blood samples. Before tracheotomy, the animals received pentobarbital sodium 60 mg/kg bodyweight i.p. (Nembutal®; Algin BV, Maassluis, The Netherlands) and the inhalation of ethrane was decreased by 50%. After a metal cannula was inserted into the trachea, muscle relaxation was given with pancuronium bromide 2.0 mg/kg, i.m. (Pavulon®; Organon Technika, Boxtel, The Netherlands) and the animals were connected to a ventilator. Anesthesia was maintained with pentobarbital sodium i.p. (Nembutal®; 60 mg/kg/h) and muscle relaxation was attained with pancuronium bromide i.m. (Pavulon®; 2 mg/kg/h). Body temperature was kept within normal range by means of a heating pad.

The animals were mechanically ventilated in parallel (6 animals simultaneously) with a Servo Ventilator 300 (Siemens-Elema AB, Solna, Sweden) at the following ventilator

settings: pressure constant time cycled mode, frequency of 30 breaths/min, peak inspiratory pressure (PIP) of 12 cm H_2O, PEEP of 2 cmH_2O, I/E ratio of 1:2, and an FiO_2 of 1.0. Initially, PIP was increased to 20 cm H_2O for 1 min to open up atelectatic regions in the lungs. After this opening up procedure, the ventilator settings were returned to the previous ones and a 0.15 ml blood sample was taken and replaced by heparinized (10 IE/ml) saline (0.9% NaCl). PaO_2, $PaCO_2$, and pH were measured by conventional methods (ABL 505, Radiometer, Copenhagen, Denmark). Next, respiratory failure was induced by repeated whole-lung lavage as described by Lachmann *et al.* [9]. Each lavage was performed with saline (32 ml/kg) heated to 37 °C. Just before the first lavage, PIP and PEEP were elevated to 26 and 6 cm H_2O, respectively. Lung lavage was repeated 5-8 times with 5 min intervals to achieve a PaO_2<85 torr (11.3 kPa). Five minutes after the last lavage, blood gases were measured and within 10 min each animal received 1.2 ml of a surfactant suspension (25 mg/ml) at a dose of 100 mg/kg. The surfactant used in this experiment is a natural surfactant isolated from pig lungs as previously described [10].

Five minutes after surfactant replacement the animals were randomized in groups of six to be pressure-constant time-cycled ventilated with an FiO_2 = 1.0 at different PIP, PEEP, I/E ratio and frequency settings. One group (n=6) was ventilated with a peak pressure of 26 cmH_2O, a PEEP of 2 cm H_2O, an I/E ratio of 1:2 and a frequency of 30 breaths/min (group 26/2); a second group (n=6) was ventilated with a PIP of 26 cm H_2O, a PEEP of 6 cm H_2O, an I/E ratio of 1:2 and a frequency of 30 breaths/min (group 26/6); a third group (n=6) was ventilated at a PIP of 20 cm H_2O and a PEEP of 10 cm H_2O, an I/E ratio of 1:2 and a frequency of 30 breaths/min (group 20/10-static). A fourth group (n=6) was ventilated at a PIP of 20 cm H_2O, a PEEP of 6 cm H_2O, an I/E ratio of 7:3 and a frequency of 130 breaths/minute necessary to create an auto-PEEP of 4 cm H_2O (group 20/10-auto). The total level of PEEP was recorded with a tip catheter pressure transducer (Raychem EO 2A 121, USA) in combination with a Siemens Sirecust 1280 monitor (Siemens, Danvers, Mass., USA) from a Y-connection piece with the tip located in one lumen of the Y-connection piece proximal to the tracheal tube in each animal. It was verified that end-expiratory flow was zero in the groups ventilated with static PEEP by recording time-flow curves (Servo Screen, Siemens-Elema, Solna, Sweden).

Blood gases were recorded at 5, 30, 60, 120 and 180 min after surfactant replacement in all four groups. At the end of each experiment, all animals were killed with an overdose of pentobarbital sodium injected through the penile vein.

After killing, the lungs were lavaged five times with saline/1.5 mM $CaCl_2$ (32 ml/kg) [11]. The percentage of lung lavage fluid recovered was calculated. The active component of surfactant in the broncho-alveolar lavage was separated from the non-active surfactant component by differential centrifugation [11] followed by subsequent phosphorus analysis [12] and the ratio of inactive to active surfactant was calculated. To get an indication of the concentration of plasma-derived inhibitory proteins in the lavage fluid, protein concentration in the supernatant of the 40,000 g centrifugation was determined [13] using a

photospectrometer (Beckman DU 7400, Fullerton CA, USA) at 595 nm with bovine serum albumin (Sigma St Louis, MO, USA) as a standard.

To investigate the effect of ventilation frequency on carbon dioxide removal in the group ventilated with a PIP of 20 cm H_2O and a static PEEP of 10 cm H_2O, a group of 6 rats was prepared as described above and surfactant depleted and ventilated accordingly. Five minutes after surfactant therapy, the ventilator was set at FiO_2 = 1.0; PIP = 20 cm H_2O; PEEP = 10 cm H_2O; I/E ratio = 1:2. Frequency was set at 30, 60, 90 and 130 breaths per minute in a non-randomized order with 15 min intervals; bloodgases were recorded.

Statistical analysis was performed using the Instat 2.0 biostatistics package (GraphPad software, San Diego, CA, USA). Inter-group comparisons were analysed with ANOVA. Intra-group comparisons were analysed with repeated measures ANOVA. If ANOVA resulted in $p \leq 0.05$ a Bonferroni post-test was performed. Statistical significance was accepted when $p \leq 0.05$. All data are reported as mean ± standard deviation (SD).

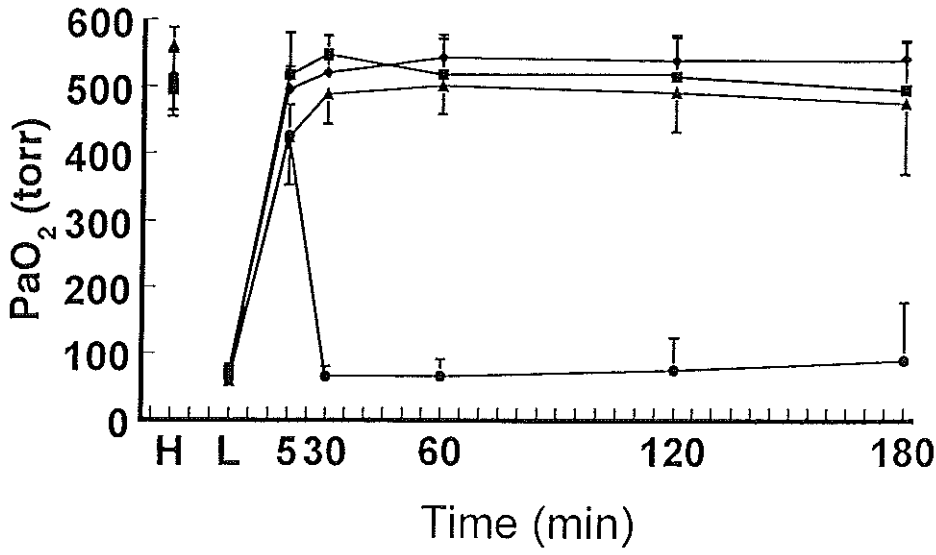

Figure 1. PaO_2 values in torr (mean ± SD) of group 26/2 (●), group 26/6 (▲), group 20/10-static (■) and group 20/10-auto (◆) over time. H = healthy, L = after lavage. Time 5, 30, 60, 120 and 180 min indicate PaO_2 values 5, 30, 60, 120 and 180 min after exogenous surfactant treatment. Statistical significant differences have been indicated in the text.

Results

All animals survived the study period. Data followed a normal distribution.

Arterial PaO_2 and $PaCO_2$ before lavage, after lavage and 5 min after exogenous surfactant therapy were comparable in all four groups (Figures 1 and 2). After switching to 26/2 arterial oxygen tensions decreased to post-lavage values and were significantly lower than in the other three groups. Arterial PO_2 was stable and showed no inter-group differences (Figure 1) in the other three groups. The animals in group 20/10-static showed a marked increase in $PaCO_2$ that was significantly higher at all time points compared to the other groups. Arterial carbon dioxide tensions could not be reduced by increasing ventilation frequency up to 130 breaths per minute (Table 1). Group 26/2 also had increasing $PaCO_2$ levels over the ventilation period. Groups 26/6 and 20/10-auto remained normocapnic during the study period.

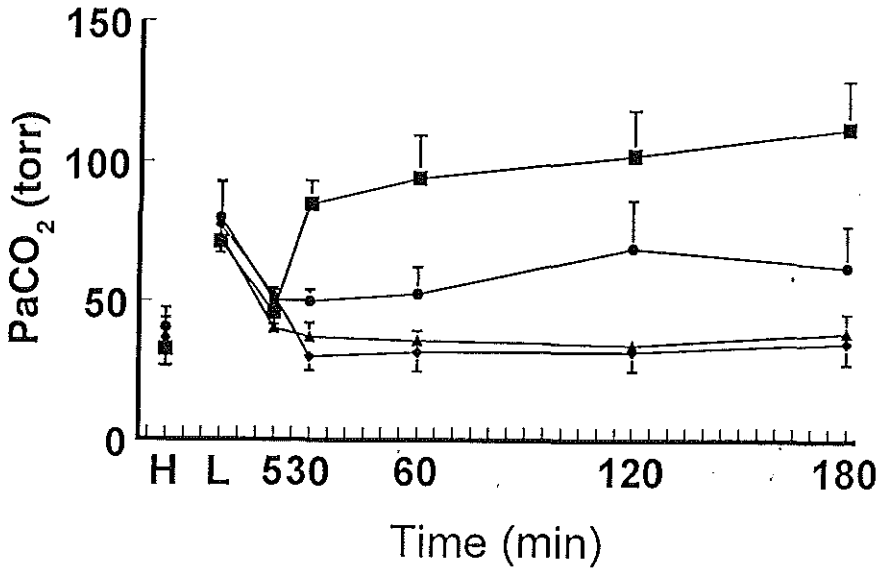

Figure 2. $PaCO_2$ values in torr (mean ± SD) of group 26/2 (●), group 26/6 (▲), group 20/10-static (■) and group 20/10-auto (◆) over time. H = healthy, L = after lavage. Time 5, 30, 60, 90, 120 and 180 min indicate $PaCO_2$ values 5, 30, 60, 120 and 180 min after exogenous surfactant treatment. Statistical significant differences have been indicated in the text.

The recovery of the lavage fluid (mean ± SD) was 87.8 ± 1.3, 92.0 ± 2.3, 85.6 ± 4.7 and 87.3 ± 1.9% in groups 26/2, 26/6, 20/10-static and 20/10-auto, respectively. The recovery was significantly higher in group 26/6 than in groups 20/10-static and 20/10-auto.

Differences in the ratio of small to large surfactant aggregates have been depicted in figure 3 (0.69 ± 0.23, 0.46 ± 0.35, 0.13 ± 0.06 and 0.15 ± 0.06 in groups 26/2, 26/6 20/10 static and 20/10 auto). Protein concentration of broncho-alveolar lavage fluid (mg/mL) between groups has been depicted in figures 4 (1.01 ± 0.20, 0.99 ± 0.41, 0.33 ± 0.11 and 0.41 ± 0.09 in groups 26/2, 26/6 , 20/10 static and 20/10 auto.

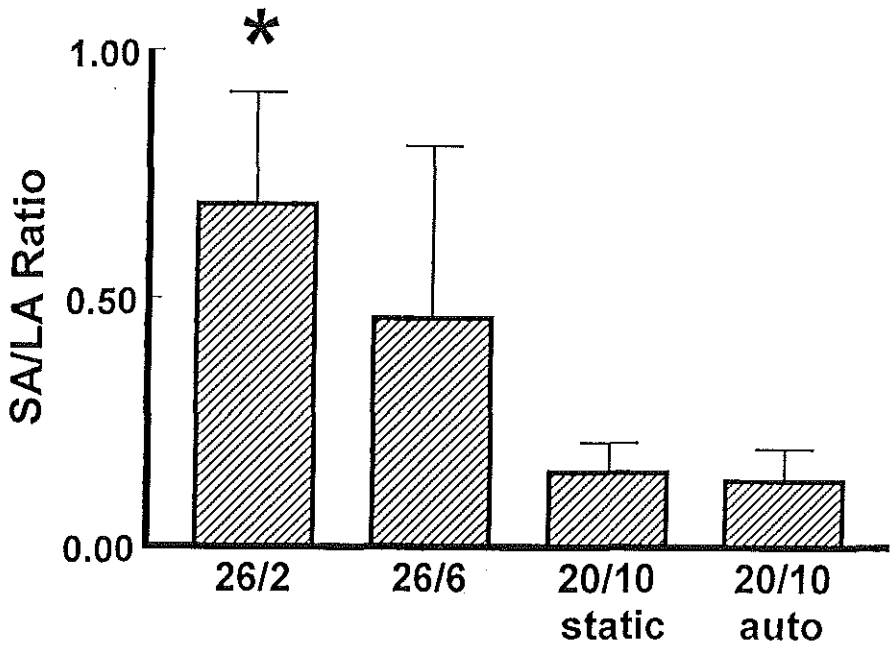

Figure 3. Surfactant small (SA) to surfactant large aggregate (LA) ratio. Group 26/2 showed a significant conversion from surface active large aggregates into non-surface active small aggregates during the ventilation period (values are mean ± SD; * $p \leq 0.05$ versus groups 20/10-static and 20/10-auto).

Discussion

This study shows that during CMV, settings that combine small pressure amplitudes with high

levels of PEEP best preserve the large aggregate, surface-active component of exogenous surfactant. The advantage of applying a mode of ventilation that creates auto-PEEP, compared to a mode that applies the same level of PEEP by static PEEP only, is a more effective CO_2 removal. Moreover, it is shown that PEEP has a major impact on the effect of exogenous surfactant therapy on arterial oxygenation. A ventilator setting with a low PEEP does not preserve oxygenation and blood gases deteriorate immediately, whereas higher levels of PEEP restore oxygenation to pre-lavage values for at least 3 hours.

Table 1. Effect of increasing frequency (bpm) on PaCO2 and PaO_2 in lung lavaged rats given exogenous surfactant (100 mg/kg) and ventilated at PIP of 20 cm H_2O, static-PEEP of 10 cm H_2O and I/E ratio of 1:2. Values are mean ± SD.

	$PaCO_2$ in torr (kPa)	**PaO_2 in torr (kPa)**
Before lavage	27±1 (3.6±0.1)	535±19.3 (71.4±2.6)
After lavage	49±2 (6.5±0.2)	55±4.0 (7.3±0.5)
After surfactant	27±5 (3.6±0.6)	528±37 (70.4±4.9)
Freq. of 30	64±10 (8.5±1.3)	546±71 (72.8±9.4)
Freq. of 60	82±24 (10.9±3.2)	580±67 (77.3±9.0)
Freq. of 90	77±26 (10.3±3.4)	560±53 (74.7±7.1)
Freq. of 130	80±30 (10.7±4.1)	570±62 (76.0±8.3)

Exogenous surfactant therapy is now routinely applied in premature neonates with RDS [1] and several studies have shown a beneficial effect of the use of exogenous surfactant in acute RDS [2,3]. However, in some neonates exogenous surfactant leads to only transient improvements of arterial oxygenation [1]. Although the exact reasons for this are not fully understood, it is becoming increasingly realized that such differences are attributable to: the efficacy of alveolar surfactant delivery of the application technique [14]; the composition of the surfactant preparation [15]; differences in the amount of surfactant inhibitors in the alveolar space [16]; and the mode of mechanical ventilation [4-6].

Data on the influence of mechanical ventilation on exogenous surfactant therapy are limited. Surfactant replacement studies by Kobayashi *et al.* in lung-lavaged rabbits ventilated at a PIP of 20 cm H_2O showed that a PEEP of 4 cm H_2O improved survival and normalized blood gases and compliance as opposed to surfactant-treated rabbits ventilated without PEEP [4]. Our data confirm the importance of a sufficiently high level of PEEP to maintain adequate oxygenation (group 26/6 versus group 26/2).

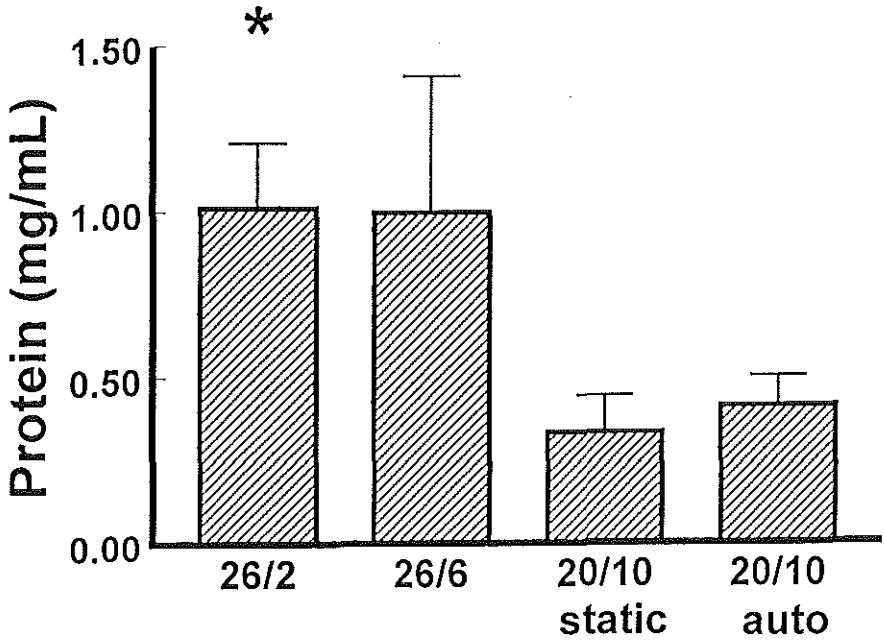

Figure 4. Protein concentration (mg/ml) of the 40,000 g supernatant fraction. Groups 26/2 and 26/6 had a significant increase in protein concentration compared to the other three groups (values are mean ± SD; * $p \leq 0.05$ compared to groups 20/10-static and 20/10-auto).

More recent studies by Froese and colleagues in surfactant treated (80 mg/kg) lung-lavaged rabbits have clearly demonstrated that HFO with small volume cycles at high rates and high end-expiratory lung volumes is most beneficial in exogenous surfactant treatment and leads to a constant improvement of PaO_2 with a low alveolar protein influx and a high amount of active surfactant at the end of a 4 hour study period [5]. However, HFO may not be routinely available in most neonatal intensive care units. The improvements in oxygenation after exogenous surfactant therapy with conventional volume-constant ventilation in the same study showed a decline over time; analysis of surfactant composition at the end of the study period demonstrated a greater conversion of active into non-active surfactant with CMV than with HFO [5]. Such differences in conversion were explained by differences in volume cycles, which were ten-fold higher during CMV than during HFO [5]. Gross *et al.* were the first to show that conversion of active into non-active surfactant subfractions is dependent on cyclic changes in surface area in vitro [17]. To maintain an adequate pool of functional surfactant

subfraction in the air spaces in vivo, it is necessary to maintain a balance between secretion, uptake and clearance of the active and non-active surfactant subfractions [18]. Recent in vivo studies by Veldhuizen *et al.* in rabbits attribute the surfactant conversion to a change in alveolar surface area associated with mechanical ventilation [6, 8]. They found that changing the respiratory rate did not affect the rate of conversion but that conversion of surfactant subfractions is dependent on tidal volume and time [8].

It may be reasoned that also conventional modes of mechanical ventilation that combine small pressure amplitudes with high end-expiratory lung volumes have a beneficial effect on exogenous surfactant composition and function. The low surfactant conversion rate and the adequate oxygenation in group 20/10-static show that this is indeed the case. However, the $PaCO_2$ level indicates that carbon dioxide could not be effectively removed with these settings of the ventilator. Increasing the ventilation frequency in the static-PEEP group to the same level as in the auto-PEEP group had no influence on $PaCO_2$ (Table 1), which indicates that alveolar ventilation could not be increased by increasing ventilation frequency. The more effective carbon dioxide removal is therefore not explained by the ventilatory frequency, but rather by the differences in driving pressures, which is 14 cmH_2O (20 cmH_2O PIP - 6 cmH_2O static PEEP) at the start of expiration in the auto-PEEP group, whereas it is only 10 cmH_2O in the static-PEEP group. High levels of $PaCO_2$ may lead to pathophysiological changes in the cardiovascular system and central nervous system [19]. Therefore, although CMV with small pressure amplitudes and high end-expiratory pressure levels preserves the active exogenous surfactant subfraction, the resulting high levels of $PaCO_2$ associated with such ventilator settings (at any of the set ventilatory frequencies) may not be desirable in certain categories of patients [19].

If at pressure-constant ventilation one either increases the I/E ratio at a constant frequency, or increases the frequency at a constant I/E ratio (or both) to establish an expiratory time which will be too short to allow emptying of the lung to the ambient pressure, an auto-PEEP will be created [20-24]. This mode of mechanical ventilation can only be applied during pressure-constant time-cycled mechanical ventilation, and not during volume-constant ventilation, where there is the risk of dangerous lung overinflation. Our data show that when applying the same level of total-PEEP with such a ventilation mode (by 4 cm H_2O of auto-PEEP and 6 cm H_2O static-PEEP), effective oxygenation and carbon dioxide elimination can be achieved with the same level of preservation of the active surfactant subfraction as with static PEEP only. Our findings on carbon dioxide elimination confirm previous results with this type of mechanical ventilation in patients with acute RDS [23].

It has been established that (plasma-derived) proteins inhibit surfactant dose-dependently [16]. Therefore, to establish an optimal function of exogenous surfactant, it is necessary to maintain an optimal ratio between surfactant phospholipids and such inhibitory proteins. Next to higher levels of active surfactant, CMV with small pressure amplitudes and high end-expiratory lung volumes, both with static and auto PEEP, also resulted in a lower intra-alveolar protein influx than in animals ventilated with higher pressure amplitudes and

low PEEP levels; such findings will also have influenced PaO_2 levels.

We conclude that pressure-constant time-cycled ventilation with high PEEP levels in a mode creating auto-PEEP may be a useful ventilation mode after exogenous surfactant therapy. In our study it resulted in steadily improved blood gases and effectively preserved surfactant. It may thus reduce the necessary amount of exogenous surfactant and treatment costs in clinical practice. Moreover, our data confirm that even changing ventilator settings during conventional mechanical ventilation has a major impact on exogenous surfactant therapy. Future studies are necessary to confirm such findings under clinical conditions and to compare this type of mechanical ventilation to flow-constant, pressure-limited ventilation and HFO.

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Chapter 6

Purine in broncho-alveolar lavage fluid as marker of ventilation-induced lung injury

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Summary

Objective: To investigate in a rat model of ventilation-induced lung injury (VILI) whether metabolic changes in the lung are reflected by an increased purine concentration [adenosine, inosine, hypoxanthine, xanthine and urate; an index of adenosine-triphosphate breakdown] of the broncho-alveolar lavage fluid (BALF) and whether purine can thus indirectly serve as a marker of VILI.

Design: Prospective, randomized, controlled trial

Setting: Research laboratory

Subjects: Forty-two male Sprague-Dawley rats

Interventions: Five groups of Sprague-Dawley rats were subjected to 6 minutes of mechanical ventilation One group was ventilated at a peak inspiratory pressure (PIP) of 7 cmH_2O and a positive end-expiratory pressure (PEEP) of 0 cmH_2O. A second group was ventilated at a PIP of 45 cmH_2O and a PEEP of 10 cmH_2O. Three groups of Sprague-Dawley were ventilated at a PIP of 45 cmH_2O without PEEP; prior to mechanical ventilation two of these groups received intra-tracheal administration of saline or exogenous surfactant at a dose of 100 mg/kg and one group received no intra-tracheal administration. A sixth group served as non-ventilated controls.

Measurements and Main Results: BALF was collected in which both purine concentration (μM, mean ± SD, ANOVA $p < 0.0001$) and protein concentration (mg/mL, ANOVA $p < 0.0001$) were determined. Statistical differences were analysed by a one-way ANOVA with a Student-Newman-Keuls post-hoc test. Purine and protein concentrations were different between groups (ANOVA p-value for purine and protein < 0.0001). Both purine and protein concentration in BALF were increased in 45/0 (3.2 ± 1.9 and 4.2 ± 1.6, respectively) compared to 7/0 (0.4 ± 0.1, $p < 0.05$ and 0.4 ± 0.2, $p < 0.001$) and controls (0.2 ± 0.2, $p < 0.01$ and 0.2 ± 0.1, $p < 0.001$) and in group 45/Na (5.8 ± 2.5 and 4.2 ± 0.5) compared with 7/0 (purine and protein $p < 0.001$) and controls (purine and protein $p < 0.001$). PEEP prevented an increase in purine and protein concentration in BALF (0.4 ± 0.3 and 0.4 ± 0.2, respectively) compared to 45/0 (purine $p < 0.01$; protein $p < 0.001$) and 45/Na (purine and protein $p < 0.001$). Surfactant instillation preceding lung overinflation reduced purine and protein concentration in BALF (2.1 ± 1.6 and 2.7 ± 1.0) compared to 45/Na (purine $p < 0.001$; protein $p < 0.01$); Surfactant instillation reduced protein concentration compared to group 45/0 ($p < 0.01$).

Conclusions: This study shows that metabolic changes in the lung as a result of VILI are reflected by an increased level of purine in BALF and that purine may thus serve as an early marker for VILI. Moreover, the study shows that both exogenous surfactant and PEEP reduce protein infiltration and that PEEP decreases the purine level in BALF after lung overinflation.

Introduction

Ventilation-induced lung epithelial permeability changes can be described by increased protein concentration of the broncho-alveolar lavage fluid (BALF) [1] and increased lung clearance of radio-active tracer molecules [2]. Changes in the pulmonary surfactant system, which have been shown to be rate-limiting for the clearance of ^{99m}Tc-human serum albumin from the lung, may be (partially) responsible for such changes [2, 3]. These permeability changes, however, are no direct reflection of ventilation-induced morphological changes or beginning injury of the lung parenchyma [1].

From studies in animals and patients it is known that disturbance of the delicate equilibrium between adenosine-triphosphate (ATP) supply and demand in ischemic heart tissue, leads to excretion of myocardial ATP-catabolites [adenosine, inosine, hypoxanthine, xanthine and urate] into the systemic circulation [4]. These purines can thus serve as biochemical markers for ischemic heart disease [4]. Studies in isolated perfused rat lungs have shown that ventilation with hypoxic gas mixtures results in increased urate concentrations of lung perfusate and lung tissue homogenates [5]. These changes have been attributed to hypoxia-induced pulmonary edema resulting in pertubation of adenine turnover and, more likely, to direct metabolic alternations due to hypoxia [5]. We hypothesized that metabolic changes in the lung tissue induced by ventilation-induced edema or (beginning) lung parenchymal injury are reflected by purine production of the lung tissue and that purines may thus indirectly serve as markers of ventilation-induced lung injury (VILI). To test this hypothesis we used a rat model of lung injury induced by intermittent positive pressure ventilation at high inflation pressures [6-9]. The effect of exogenous surfactant instillation and positive end-expiratory pressure (PEEP) on ventilation-induced metabolic changes in lung tissue reflected by purine release and their effect on protein permeability in this animal model were investigated.

Materials and methods

The study protocol was approved by the Institutional Review Board for the care of animal subjects. Care and handling of the animals were in accordance with the National Institute of Health guidelines. The animals had free access to commercial chow (Hope farms, Woerden, The Netherlands).

A pilot experiment was designed to get an indication of the applicability of purine in both the blood and BALF as marker of metabolic changes in the lung tissue as a result of VILI. Four adult male Sprague-Dawley rats (body weight 290-340) were anesthetized with nitrous oxide/oxygen/halothane (65/33/2 volume %), a metal tube was placed in the trachea and a catheter was inserted into a carotic artery. Two animals were connected to a ventilator (Servo Ventilator 300, Siemens-Elema, Solna, Sweden) for 6.5 min set in a pressure-controlled mode, at an FiO_2 of 1.0, a frequency of 30 breaths/min and an I/E ratio of 1:2. During mechanical ventilation, anesthesia was replaced with pentobarbital sodium (Nembutal; Algin BV, Maassluis, The Netherlands) 60 mg/kg, intraperitoneally; muscle relaxation was

attained with pancuronium bromide (Pavulon; Organon Technika, Boxtel, The Netherlands) 2.0 mg/kg, intramuscularly. In order to re-open atelectatic lung areas induced by the surgical procedure, the airway pressure was increased to a peak inspiratory pressure of 20 cmH_2O and a PEEP of 3 cmH_2O for 0.5 min. Two rats served as non-treated, non-ventilated controls and were killed immediately after preparation (overdose pentobarbital through the penile vene).

In the main experiment, in 38 animals a metal tube was put in the trachea as described above. Thirty-two of these animals were randomized to one of the following five experimental groups to be mechanically ventilated for 6.5 minutes. After an opening procedure identical to the one described in the pilot experiment, one group of animals was ventilated with a peak inspiratory pressure of 7 cmH_2O without PEEP (group 7/0, n=6). A second group was ventilated with a peak inspiratory pressure of 45 cmH_2O with 10 cmH_2O PEEP (group 45/10, n=6). Three other groups were ventilated with a peak inspiratory pressure of 45 cmH_2O without PEEP; immediately prior to mechanical ventilation one of these groups received 100 mg/kg body weight surfactant dissolved in 1 mL of saline (group 45/S, n=7); a second group received 1 mL of saline only (group 45/Na, n=6); a third group did not receive any intra-tracheal administration (group 45/0, n=7). After the ventilation period, the animals were killed by an overdose of pentobarbital through the penile vein. A sixth group of animals (n=6) was randomized to serve as non-treated, non-ventilated controls (control group) and was killed (overdose pentobarbital through the penile vene) immediately after a metal tube was placed in the trachea.

All chemicals were purchased from Sigma (St. Louis, MO) unless stated otherwise. In the pilot experiment, immediately after the ventilation period, 3 mL of heparinized blood were drawn from the arterial line. In all animals, the lungs were immediately lavaged with warm saline (37°C, 30 mL/kg). The blood and the BALF were centrifuged (Beckman GPR, Palo Alto, CA) at 4°C at 400 x g for 10 min to remove cells and cellular debris. The plasma and part of the BALF were both immediately mixed 1:1 with a cold aqueous solution of dipyridamole 20 µM and EHNA 10 µM. These mixtures were deproteinized with equal amounts of cold perchloric acid (8%, w/v), and then centrifuged at 3000 x g for 10 min at 4°C. The pH of the supernatant fluid was adjusted to 5-7 with 6 M KOH/2 M K_2CO_3. The solution was then centrifuged at 11,000 g. The supernatant was stored at -80°C until further analysis.

Protein concentration in the BALF was determined with the Bradford method (Bio-Rad protein assay, Munich, Germany) using a photospectrometer (Beckman DU 7400, Fullerton, CA) at 595 nm with bovine serum albumin as a standard [10].

Purine was determined by reversed phase high-performance liquid chromatography (HPLC) according to Smolenski *et al.* [11]. In brief, a C_{18} column (Hypersil ODS 3 µm, 150 x 4.6 mm; Alltech, Deerfield, IL) was employed combined with a C_{18} guard column (Hypersil ODS 5 µm, 7.5 x 4.6 mm). The system configuration consisted of a Waters 510 pump, a cooled Waters 712 WISP autosampler, a Spectra Focus forward optical scanning detector (Spectra-Physics, San Jose, CA) and a Waters Millenium 2010 data system (Waters, Milford,

MA). Peaks were detected at 254 nm (hypoxanthine, xanthine, inosine, adenosine) and at 280 nm (uric acid). BALF purines were identified based on their retention times, their co-elution with standards and their 254/280 ratios.

Statistical analysis:
Intra-group comparisons were analyzed with a one-way ANOVA. If ANOVA resulted in a $p < 0.05$ a Student-Newman-Keuls post-hoc test was performed. All data are reported as mean ± standard deviation (SD).

Results
ANOVA for both purine and protein resulted in a p-value < 0.0001.
In the pilot experiment, total purine levels in the blood were 12.4 and 6.6 µM in the 2 control animals and 9.0 and 5.6 µM in the 2 animals in group 45/0. Total purine levels in BALF were 0.4 and 0.6 µM in the 2 control animals and 2.6 and 1.7 µM in the two ventilated animals.

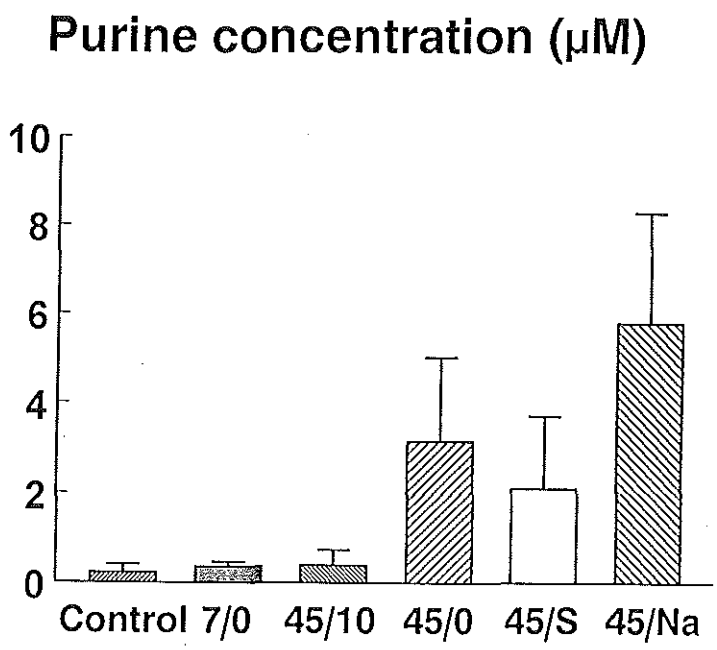

Figure 1. Purine concentration in broncho-alveolar lavage fluid (µM, mean ± SD) in all experimental groups. See text for statistical differences.

Both purine concentration (Fig. 1) and protein concentration (Fig. 2) in BALF were significantly increased in groups 45/0 compared to non-ventilated (group C, purine $p < 0.01$;

protein $p < 0.001$) and ventilated controls (group 7/0, purine $p < 0.05$; protein $p < 0.001$) and in group 45/Na compared to non-ventilated (purine and protein $p < 0.001$) and ventilated controls (purine and protein $p < 0.001$); in group 45/S only protein concentration was significantly increased versus ventilated and non-ventilated controls (both $p < 0.001$).

The group ventilated with 10 cmH_2O PEEP at the same peak inspiratory pressure (45/10) showed no increase in purine concentration (Fig. 1) or protein concentration (Fig. 2) in BALF compared to ventilated and non-ventilated controls. Both total purine and protein concentrations were significantly lower in the group ventilated with 10 cmH_2O than in the animals ventilated at the same peak inspiratory pressure without PEEP (purine $p < 0.01$; protein $p < 0.001$ and group 45/Na (purine and protein $p < 0.001$).

Surfactant instillation preceding lung overinflation without PEEP prevented a significant increase in purine concentration after lung overinflation compared to controls, significantly lowered purine and protein concentrations in BALF compared to saline-treated animals (purine $p < 0.001$; protein $p < 0.001$) and lowered protein concentrations compared to non-treated (45/0) animals ($p < 0.01$).

Protein concentration was significantly lower in group 45/10 than in 45/S ($p < 0.001$). Purine concentration was significantly lower in group 45/0 than in 45/Na ($p < 0.001$).

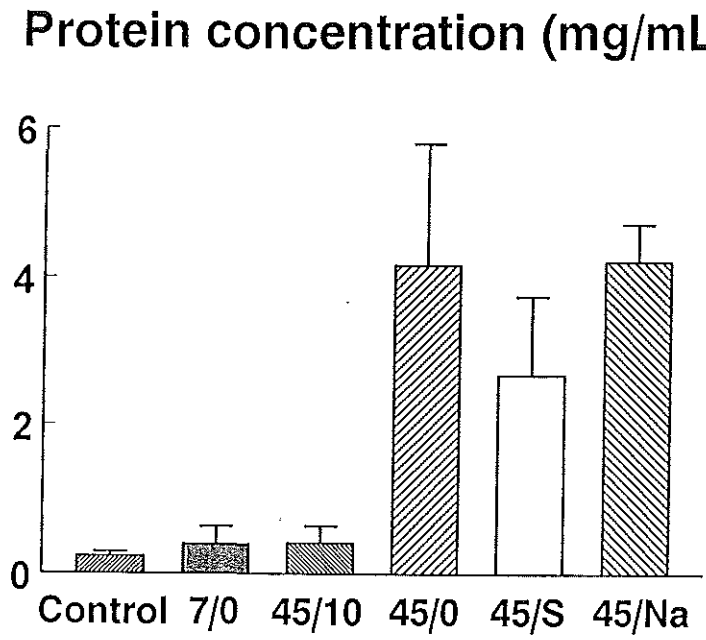

Figure 2. Protein concentration in broncho-alveolar lavage fluid (mg/mL, mean ± SD) in all experimental groups. See text for statistical differences.

Discussion

This study demonstrates that early metabolic changes in rat lung tissue as a result of mechanical ventilation at peak pressures of 45 cmH_2O without PEEP for 6 min are reflected by a significant increase in purine concentrations in BALF compared to ventilated and non-ventilated controls. At the same peak pressure PEEP decreases the purine level in BALF. Both exogenous surfactant and PEEP reduce protein infiltration, which is increased after lung overinflation.

Increasing data supports the role of mechanical ventilation in the pathogenesis of acute lung injury (ALI), and the clinical relevance of ventilation-induced lung injury is becoming increasingly realized [12]. The exact mechanisms of VILI and contributing factors are still disputed [13]. Experiments in rats with high peak inspiratory pressure ventilation of 45 cmH_2O, where peak inspiratory volume was limited by thorax restriction, have clearly shown that rather than peak inspiratory pressure, the peak inspiratory lung volume is injurious [8]. More recently, mechanical ventilation at low lung volumes has become recognized as an important contributing factor to ventilation-induced lung injury if end-expiratory alveolar collapse is allowed [14].

Experiments in rats [6, 7] showed that mechanical ventilation with high peak inspiratory pressures of 45 cmH_2O without PEEP results in permeability and structural changes of the alveolo-capillary barrier comparable to those seen in acute lung injury. The use of 10 cmH_2O of PEEP at such peak pressures prevents ventilation-induced permeability and lung parenchymal changes. This PEEP effect is attributable to both decreased lung capillary hydrostatic pressure [8] and preservation of the surfactant system [3], which decrease filtration pressure over the alveolo-capillary barrier. Our data on protein concentration of BALF in groups 45/0 and 45/10 are consistent with such findings.

In the present study, we evaluated whether purines are formed in VILI and may be used as early markers to show metabolic changes in lung tissue. Moreover, to further elucidate the mechanisms of VILI, we investigated the effect of PEEP and surfactant on ventilation-induced purine and protein release in the epithelial lining fluid. We chose to ventilate for only 6 min because early signs of increased capillary permeability (which included an increase in ^{22}Na distribution space, dry lung weight and fractional albumin uptake [7]) are already present after 6 min of mechanical ventilation in this animal model.

The pilot experiment showed no increase in serum purine concentration in rats subjected to lung overinflation compared to controls. This in contrast to experiments in isolated perfused rat lungs after hypoxic stress which showed increased urate concentrations in a Krebs-Ringer bicarbonate perfusion buffer [5]. We suggest that a possible increase in plasma purine concentration as a result of ventilation-induced lung parenchymal metabolic changes or injury, is obscured by the high basal total purine concentration of plasma [4]. Therefore, we did not further investigate the use of total purine levels in the plasma as a marker of (metabolic changes in) VILI.

In group 45/0 there was an increase in total purine concentration of BALF compared

to PEEP-treated and ventilated and non-ventilated control animals. It has been shown in this animal model, that the combination of a high peak inspiratory lung volume and a lack of PEEP is the main stimulus for lung injury [3, 6-9]. A previous publication by our group [3] has shown that the tidal volume is 48.4 mL/kg in group 45/0, 18.2 mL/kg in group 45/10, and 12.2 mL/kg in group 7/0. The tidal volume of spontaneously breathing, healthy Sprague-Dawley rats has been reported as 7.2 mL/kg [15]. These differences in tidal volume and PEEP are likely the most important reason for the observed differences in protein infiltration and recovery of purine in BALF.

In contrast to ATP, purine has no polarity restrictions in crossing cell membranes. Therefore, first, purine release in these experiments on VILI may indicate that cells with an intact cell membrane suffered from stress by a stimulus, that resulted in increased conversion of ATP. Such metabolic stress would be reversible. Second, the purine may have also been released from cells with a ruptured cell membrane, which would indicate irreversible cell damage. With the present data, we are not able to make a distinction between the two. However, it is evident that the most injurious type of mechanical ventilation (the combination of high end inspiratory lung volumes without PEEP) resulted in the highest levels of purine in BAL.

In the rat model of VILI used in the present study, both the lung endothelium and epithelium are possible sites of purine release [8]. Another cause for the increased purine concentration in BALF may be purine from the blood, reaching the alveolar space by increased permeability of the alveolo-capillary barrier.

In the rat, the average cell number (10^8)/ cm^3 in the lung is: 1.38, 2.65 and 7.86 for type I, type II and endothelial cells, respectively [16] and the volume percentage of those cells in the lung tissue is 12.6, 9.7 and 26.4%, respectively [16]. The ATP concentration of cultured type II cells has been reported as 5.0 nmol/10^6 cells [17], which would equal 1325 nmol/g type II tissue; that of the cultured myocardial vascular endothelium is 14.2 nmol/g wet tissue [18]. We do not know of any publication that defines the ATP content of pneumocytes type I. However, we could exclude the type I cell as a possible site of purine release and consider the type II cell as the only site in the lung epithelium from which purines were released. It is likely that the type II cell can produce purine because metabolically it is highly active and contains sufficient amounts of ATP-ase [16]. Even when we assume that the type I cell does not contribute to purine production, only on the basis of the ATP content of the type II cell the potential for purine production in the lung epithelium would be a factor 34 greater than that of the endothelium.

During mechanical ventilation at a peak inspiratory pressure of 45 cmH_2O without PEEP in rats, the pulmonary infiltrate consists of pure plasma [7]. The plasma protein concentration in the rat has been reported as 80 mg/mL [20]. By multiplying the protein concentration of the BALF (4.2 ± 1.6 mg/mL) with the amount of BALF (8.8 ± 0.7 mL), it is calculated that the amount of protein that infiltrated the alveolar space in the rats ventilated at a peak inspiratory pressure of 45 cmH_2O without PEEP was 37 mg. Therefore, the amount

of plasma that infiltrated the alveolar space was about 0.5 mL. The average plasma purine concentration in the rat in our pilot experiments was 8.4 μM. Therefore, the total amount of purine in the alveolar space as a result of plasma infiltration would be 4.2 nmol. If this would be totally recovered in the BALF, the maximal concentration of purine in BALF attributable to plasma infiltration would be 0.4 μM, which is 14% of that actually found. These calculations and the fact that BALF rinses the lung epithelium, give strong support to the idea that the increased purine concentration in BALF in our study reflects early epithelial changes and not endothelial changes or serum purine infiltration.

The most likely candidate for ventilation-induced hypoxic metabolic changes and/or mechanical stress injury is the surfactant producing type II pneumocyte, whose surfactant pool becomes impaired as a result of lung overinflation [21-23]. Recent investigations in this rat model of lung injury induced by lung overinflation have shown that mechanical ventilation without PEEP induces impairment of the surfactant system, which is prevented by the use of PEEP [3]. Our data on the PEEP-treated group 45/10 suggest that surfactant preservation may have resulted in decreased purine concentration in BALF after PEEP, which may reflect prevention of hypoxic metabolic changes and/or mechanical stress of the pneumocyte type II. Decreased protein permeability in the surfactant-treated group 45/S is then attributable to prevention of a decrease in pressure of the alveolar fluid, altering the pressure gradient across the alveolo-capillary barrier into the alveolar direction [3, 24], which is in accordance with previous results on exogenous surfactant therapy in surfactant-depleted rabbits [2].

Concluding, the data from this study show that purines in BALF reflect ventilation-induced metabolic changes to the lung tissue and may thus indirectly serve as markers of VILI. The most likely origin of purine is the epithelial type II cell. Moreover, our data provide strong evidence that in this animal model of VILI, early changes of injury are not confined to the endothelium [7] and that the epithelium also suffers from early changes of injury. These findings are especially interesting since, at present, no specific marker for lung epithelial injury in ALI is available [1]. The study also shows that both PEEP and surfactant are capable of reducing protein infiltration in VILI; PEEP also decreased purine concentration of BALF.

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Chapter 7

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

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Summary

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 x 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 hours after inoculation. Arterial blood samples were obtained and cultured before and 180 min after mechanical ventilation and immediately before sacrifice in 2 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 hours 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.

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 development of bacteremia with *K. 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 x 10^5 colony forming units (CFU)/ml *Klebsiella pneumonia* (ATCC 43816; serotype 2) was prepared as follows: stationary-phase cultures were prepared by incubation for 16 hours at 37 °C in Mueller-Hinton broth (MHB; Difco Laboratories, Detroit, Michigan, USA). After proper dilution and reincubation for 90 minutes 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 gram) 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 per minute; 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 seconds. 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 having 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.

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 mean ± standard deviation (SD). Differences in the number of positive blood cultures for *Klebsiella pneumoniae* at t = 22 h and t = 25 h in all groups were determined with an exact χ^2-test on a 2 x 5 contingency table. If p was less than 0.05, Fisher's exact post-tests on 2 x 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 100µl of saline containing 3.5-5.0 x 10^5 CFU *K. pneumoniae*/ml.

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

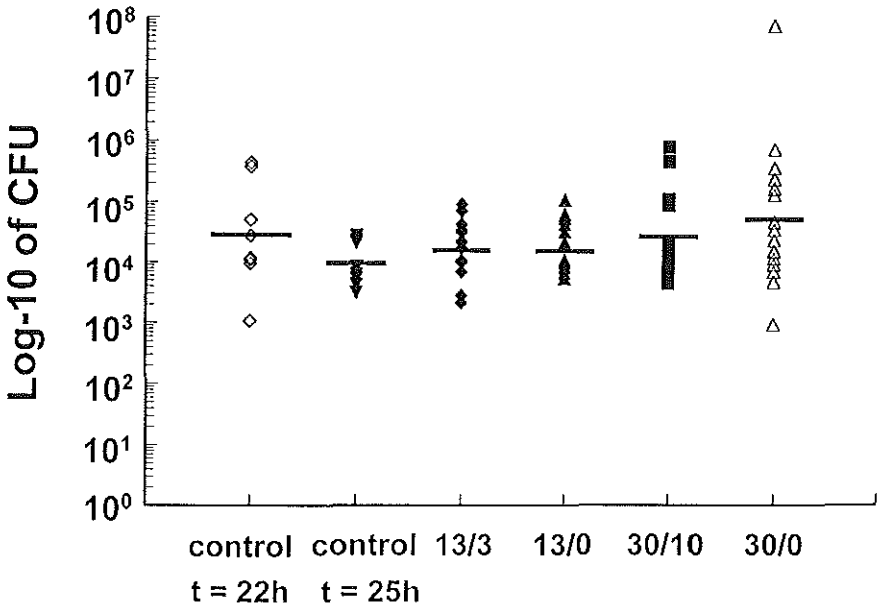

Figure 1. Quantitative lung bacterial count in four experimental and two control groups. There were no significant differences in the number of bacteria recovered from the lung tissue.

Five animals were excluded from analysis because the animals 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 groups were bacteremic.

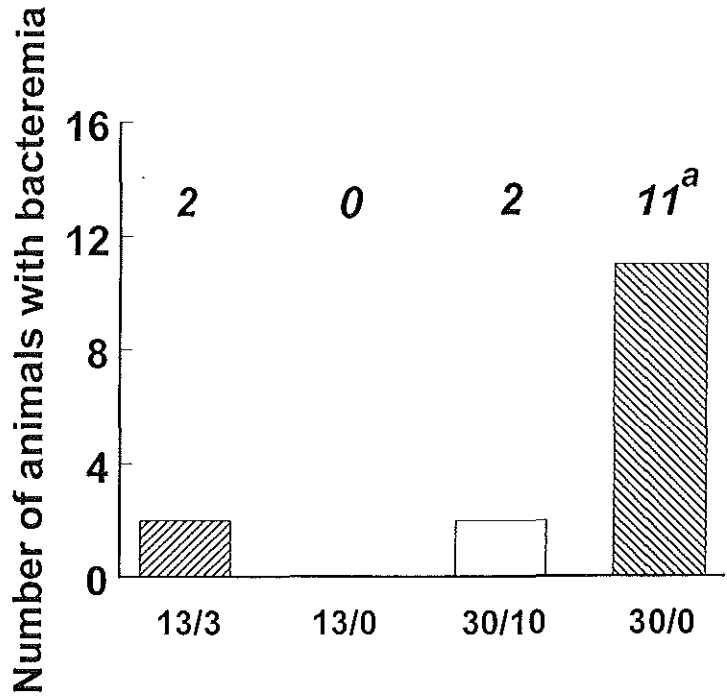

Figure 2. Number of animals with positive blood cultures for *K. Pneumoniae* in 2 ml of blood at t = 180 min, that were not bacteremic at t = 0 min (n=16 in each group). [a]p < 0.05 versus all other groups.

Arterial oxygenation over time is given in figure 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.

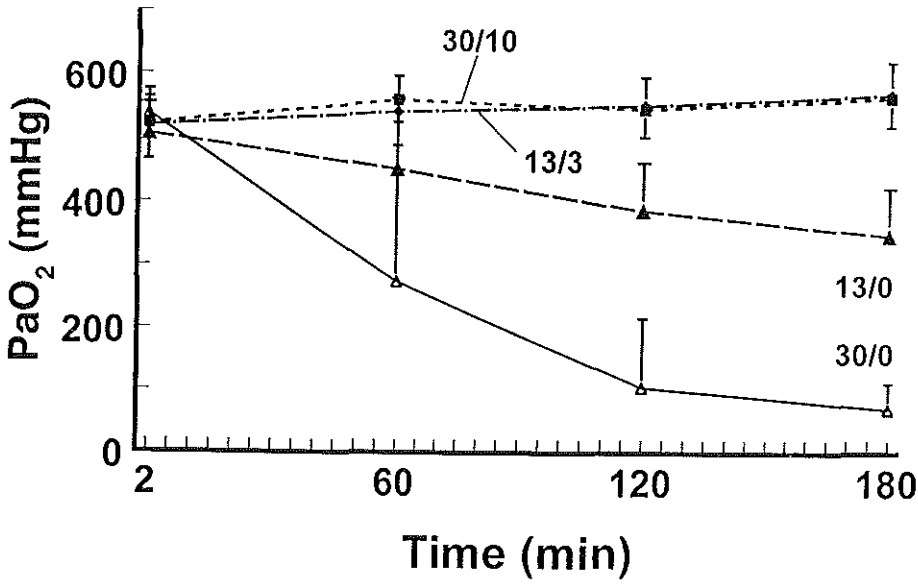

Figure 3. PaO_2 values (mean ± SD) of the different ventilated groups. Legends indicate peak inspiratory pressure/positive end-expiratory pressure.

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 hours after inoculation. Inoculation just before 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 intratracheal inoculation of healthy rats with 8 x 10^4 CFUs *K. pneumoniae*, bacteremia does not develop in the first 24 hours after inoculation [7]. Our pilot experiments showed that inoculation with a higher count of *K. pneumoniae* (3.5 x 10^5 CFUs) in healthy non-ventilated animals induced bacteremia in 5 out of 8 animals after 22 hours. Inoculation with a lower count of *K. pneumoniae* (3.5 x 10^3 CFUs) did not induce bacteremia 22 hours 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 hours after inoculation in the group inoculated with 3.5 x 10^3 CFUs induced bacteremia in 3 out of 7 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 partially prevent permeability edema 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 (Figure 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 mmHg in group 30/0 is well above those currently regarded as safe (50 mmHg) 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 development of bacteremia, they are not likely to be the main cause for 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 hours

(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.

Table 1. Data on arterial carbon dioxide tension and pH over time (mean ± SD) in the ventilated groups. [a,b,c]Intra-group comparisons over time; $p < 0.05$ vs [a]t = 2 min; [b]t=120min; [c]t=180 min (Repeated measures ANOVA with Bonferroni post test if $p < 0.05$). [d,e,f]Intergroup comparisons; $p < 0.05$ [d]group 30/0; [e]group 13/0; [f]group 30/10 (Ordinary ANOVA with Bonferroni post test if $p < 0.05$).

		Group			
Time		***30/0***	***30/10***	***13/3***	***13/0***
2'	**pH**	7.41 ± 0.07	7.35 ± 0.08	7.35 ± 0.07[d]	7.41 ± 0.05
	pCO_2	32.4 ± 6.1	37.9 ± 5.9[d]	37.8 ± 4.8[a,d]	35.8 ± 5.0
60'	**pH**	7.36 ± 0.13[b,c]	7.38 ± 0.04	7.40 ± 0.07	7.34 ± 0.06[a,b,c]
	pCO_2	43.4 ± 11.2[a,c]	43.8 ± 4.4[a,b,c]	42.8 ± 7.3[a,e]	50.7 ± 8.4[a,b,c]
120'	**pH**	7.18 ± 0.17[a]	7.39 ± 0.06[d,e]	7.40 ± 0.05[d,e]	7.25 ± 0.07[a]
	pCO_2	54.1 ± 10.9[a,c]	39.3 ± 5.2[d,e]	44.8 ± 6.1[a,e]	66.1 ± 16.1[a,c,d]
180'	**pH**	7.08 ± 0.12[a]	7.42 ± 0.07[a,d,e]	7.40 ± 0.06[d,e]	7.18 ± 0.08[a,b,f]
	pCO_2	77.0 ± 17.5[a]	37.8 ± 6.1[d,e]	45.1 ± 4.3[a,d,e]	81.0 ± 14.3[a]

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-induced 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 intra-pulmonary 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] which in group 30/0 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 metastasis, 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 liver [24], which would increase the likelihood of 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 development of bacteremia is mediated by a direct effect of 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 bacteremia 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 likely attributable to ventilation-induced damage of the alveolo-capillary barrier, which results in lowering of the threshold for bacterial translocation. When translated 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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Chapter 8

Different ventilation strategies affect lung function but do not increase TNF-α and PGI_2 production in lavaged rat lungs *in vivo*

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Summary

Background: This study was designed to investigate the role of alveolar end-expiratory collapse in inducing ventilation-induced inflammatory mediator release from the lung parenchyma *in vivo*.

Methods: In adult rats, mechanically ventilated with 100% oxygen, acute lung injury was induced by repeated lung lavage to obtain a PaO_2 < 85 mmHg (peak pressure/PEEP: 26/6 cmH_2O). Then, animals were randomly divided to receive either exogenous surfactant therapy, partial liquid ventilation, ventilation with high PEEP (16 cmH_2O, open lung concept (OLC)), ventilation with low PEEP (8 cmH_2O), or ventilation with an increase in peak inspiratory pressure (to 32 cmH_2O). Two groups of healthy non-lavaged rats were ventilated at peak pressure/PEEP of 32/6 and 32/0 cmH_2O, respectively. Blood gases were measured. PGI_2 and TNF-α concentrations in serum and broncho-alveolar lavage fluid (BALF) as well as protein concentration in BALF were determined after 90 and 240 minutes and compared to ventilated and non-ventilated controls.

Results: Ventilation strategies directed at prevention of alveolar collapse improved oxygenation and reduced BALF protein levels. The lavage procedure appeared to favor TNF-α release into BALF in a subgroup of animals. Ventilation with high PEEP at high mean airway pressure levels (open lung concept) increased BALF PGI_2 levels, whereas BALF TNF-α levels showed no difference between groups. Serum PGI_2 and TNF-α levels did not increase compared to controls as a result of mechanical ventilation.

Conclusions: Although ventilation strategies markedly differed with respect to their injurious potential in acute lung injury, there were no indications for ventilation-induced systemic or pulmonary PGI_2 and TNF-α release. Mechanical ventilation at high mean airway pressure levels increased PGI_2 levels in the broncho-alveolar lavage accessible space.

Introduction

Recent studies in rodents have shown that mechanical ventilation itself can be sufficient to elicit production and release of pro-inflammatory mediators [1-3]. In isolated non-perfused rat lungs Tremblay et al. have shown that mechanical ventilation at tidal volumes of 40 ml/kg body weight without positive end-expiratory pressure (PEEP) induces inflammatory mediator expression after 2 h in the lung tissue and results in inflammatory mediator release into the broncho-alveolar lavage accessible space [2]. In the same study, the use of 10 cmH_2O PEEP was shown to reduce inflammatory mediator expression and release at the same degree of end-inspiratory overstretching [2]. These responses occurred in both healthy rat lungs and lungs of rats exposed for 50 minutes to lipopolysaccharide (LPS) [2]. While these studies provided evidence that injurious ventilation strategies may result in pulmonary mediator release, the studies from von Bethmann et al. indicated that ventilation may result even in systemic mediator release [1, 3]. In isolated and perfused mouse lungs from healthy donors a peak inspiratory pressure (PIP) of 25 cmH_2O with 2 cmH_2O of PEEP induced inflammatory mediator release into the perfusate, whereas a PEEP of 10 cmH_2O at the same level of PIP did not [1, 3]. The study supports the idea proposed by Kolobow et al. that detrimental modes of mechanical ventilation may not only induce local inflammatory reactions in the lung but, via the spread of inflammatory mediators, also contribute to systemic multiple organ failure [4].

The mechanism of the ventilation-induced mediator release is unknown at present, but may result from: 1) stimulus of stretch receptors present on endothelial cells [5], macrophages [6] or epithelial cells [7] and/or 2) intrapulmonary neutrophil accumulation and activation [8]. It is becoming increasingly realized that next to peak inspiratory overstretching of the lung parenchyma [9, 10], impairment of the surfactant system (as a result of mechanical ventilation) [11, 12] with subsequent repeated end-expiratory alveolar collapse and re-expansion [13], contributes to lung parenchymal stretch [13], neutrophil accumulation [8] and ventilation-induced lung injury [13].

The present study was designed to elucidate a possible role of repeated alveolar collapse and re-expansion during mechanical ventilation in inducing inflammatory mediator release from the lung in an *in vivo* rat model of acute lung injury. We compared the effect of ventilation strategies directed at prevention of alveolar end-expiratory collapse with strategies that do not prevent end-expiratory collapse.

Materials and methods

Preparation of animals

The study was approved by the local Animal Committee of the Erasmus University Rotterdam. Care and handling of the animals were in accord with the European Community guidelines (86/609/EC).

The studies were performed in male Sprague-Dawley rats (body weight 250-330 g,

Harlan CPB, Zeist, The Netherlands). After induction of anesthesia with 2% enflurane in 65% nitrous oxide in oxygen, a sterile polyethylene catheter (0.8 mm outer diameter) was inserted into a carotid artery for drawing arterial blood samples. Before tracheotomy, the animals received 60 mg/kg pentobarbital sodium, i.p. (Nembutal®; Algin, Maassluis, The Netherlands) and the ethrane concentration was decreased to 0.5-1.0%. Thereafter, a sterile metal cannula was inserted into the trachea.

Non-ventilated animals

One group of animals served as non-treated, non-ventilated controls (group Control, n = 10). Six animals served as non-ventilated, tumor necrosis factor-α (TNF-α) positive controls. They were injected 15 mg/kg lipopolysaccharide intraperitoneally (LPS, 5 ml Salmonella abortus equi S. form, Metalon GmbH, Wusterhausen, Germany) and exposed for 90 (n = 3) and 240 (n = 3) minutes (group LPS). All these animals were killed immediately after induction of anesthesia.

Mechanical ventilation

After cannulation of the trachea, in all other animals muscle relaxation was induced by pancuronium bromide 2 mg/kg, i.m. (Pavulon®; Organon Teknika, Boxtel, The Netherlands) followed by immediate connection to a ventilator. Body temperature was kept within normal range by means of a heating pad. The animals were mechanically ventilated in parallel, with a Servo Ventilator 300 (Siemens Elema, Solna, Sweden) in a pressure constant time cycled mode, with the following settings: frequency of 30 breaths per min (bpm), peak inspiratory pressure (PIP) of 13 cmH_2O, positive end-expiratory pressure (PEEP) of 3 cmH_2O, inspiratory/expiratory ratio of 1:2, and 100% oxygen. Initially, PIP was increased to 20 cmH_2O for half a minute to recruit atelectatic areas (open-up procedure). Thereafter, the ventilator settings were returned to the previous ones and blood gases were recorded (Instrumentation Laboratory, Synthesis 25, Milan, Italy). Anesthesia was maintained with hourly injections of pentobarbital sodium (60 mg/kg/h, i.p.) and muscle relaxation was maintained with hourly injections of pancuronium bromide (2 mg/kg/h, i.m.).

Healthy control animals

One group of non-lavaged (=Healthy (H)) ventilated animals served as controls and ventilator settings were not changed. Ventilation was continued for 35+90 (n = 10) and 35+240 (n = 10) min (group 13/3H). The 35-min period was included to compensate for a 35-min period in which the animals in the other ventilated groups were being lavaged (see below).

Lavaged animals

In 100 animals which received mechanical ventilation, acute lung injury was induced after the open-up procedure by repeated broncho-alveolar lavage (= lavage (L)), according to Lachmann and colleagues [14]. Each lavage was performed with saline (32 ml/kg) heated to

37°C. Just before the first lavage PIP and PEEP were elevated to 26 and 6 cmH_2O, respectively. Lung lavage was performed over a 35-min period and repeated 4-5 times to achieve a $PaO_2 \leq 85$ mmHg. Immediately after lavage, these animals were randomized to be treated with:

- partial liquid ventilation (PLV) at a dose of 15 ml perfluorocarbon per kg bodyweight (Perflubron®; Alliance Corp., San Diego, USA) for 90 (n = 10) and 240 (n = 10) min (group PLV).
- exogenous surfactant at a dose of 120 mg/kg for 90 (n = 10) and 240 (n = 10) min (group Surf). The surfactant used was isolated from minced pig lungs, that were processed as previously described [15]. The freeze-dried material was suspended in warm saline to a concentration of 40 mg/ml, and administered intratracheally, for which the animals were disconnected from the ventilator. The surfactant suspension was administered as a bolus followed by a bolus of air (12 ml/kg), directly into the endotracheal tube via a syringe, and was immediately followed by reconnection to the ventilator.
- an increase in PEEP of 2 cmH_2O for 90 (n = 10) and 240 (n = 10) min resulting in a PIP of 28 cmH_2O and a PEEP of 8 cmH_2O (group 28/8)
- an increase in PIP of 6 cmH_2O for 90 (n = 10) and 240 (n = 10) min resulting in a PIP of 32 cmH_2O and a PEEP of 6 cmH_2O (group 32/6)
- an open lung concept for 90 (n = 10) and 240 (n = 10) min (group OLC). In these groups the lung were opened by increasing PIP to 40 cmH_2O, PEEP to 20 cmH_2O, I/E ratio to 1:1 and frequency was set at 100 bpm after lung lavage. After 2 to 3 min, PIP was decreased to 32 cmH_2O and PEEP was set at 16 cmH_2O. These settings were shown not to result in auto-PEEP because end-expiratory flow on a ServoScreen (Siemens) connected to the ventilator was zero.

Other ventilator settings were not changed. In all ventilated animals, blood gases were further recorded at 5, 30, 60, 90 min (in the animals ventilated for 90 min) and at 5, 30, 60, 120, 180 and 240 min (in the animals ventilated for 240 min) after starting the experimental mode of mechanical ventilation.

Chemical analysis

At the end of the study period, a broncho-alveolar lavage was performed in all animals with saline (32 ml/kg heated to 37 °C) and 4 ml of heparinized blood was taken from the arterial line. The animals were then killed by an overdose of pentobarbital sodium through the penile vena. The blood and the broncho-alveolar lavage fluid (BALF) were centrifuged at 4 °C at 400 x g for 10 min to remove cells and cellular debris. Supernatant of both blood and BALF were taken and snap-frozen on liquid nitrogen and stored at -80 °C until further analysis.

The protein concentration of the BAL supernatant was determined with a photospectrometer (Beckman DU 7400, Fullerton CA, USA) at 595 nm using the Bradford method (Bio-Rad protein assay, Munich, Germany) with bovine serum albumin (Sigma St Louis, MO, USA) as a standard [16].

Prostacyclin was assessed as the stable metabolite 6-keto-PGF_{1a} and was measured by EIA (Cayman, Ann Arbor, MI, USA). Rat TNF-α was assessed by rat specific ELISA obtained from Genzyme (Cambridge, MA, USA).

Non-lavaged ventilated animals

To investigate whether washout of alveolar macrophages might have affected TNF-α release, 40 healthy animals were not lavaged (=Healthy (H)) but underwent mechanical ventilation only. After the open-up procedure, they were ventilated for 35 min at control settings (PIP 13 cmH_2O; PEEP 3 cmH_2O; I/E ratio = 1:2; frequency 30 breaths/min; FiO_2 = 1.0) to compensate for the 35-min lavage period in which the animals in the other experimental groups were being lavaged and ventilated. After this period the animals were exposed to the following ventilator settings (other ventilator settings were not changed):

- a PIP of 32 cmH_2O with 6 cmH_2O of PEEP for 90 (n = 10) and 240 (n = 10) min (group 32/6 H).
- a PIP of 32 cmH_2O without PEEP for 90 (n = 10) and 240 (n = 10) min (group 32/0 H). In this group dead space was increased to keep $PaCO_2$ > 20 mmHg.

A group of non-ventilated control animals (n = 6) and 2 groups of ventilated control animals (13/3 H) ventilated for 90 (n = 3) and 240 (n = 3) min were included.

Blood gas analysis was performed as described above. Blood was taken and a broncho-alveolar lavage was performed. The samples were treated and protein and TNF-α concentrations were determined as described above.

Statistical analysis

Intra-group comparisons for PaO_2 and $PaCO_2$ were analysed with a repeated measures ANOVA. Inter-group comparisons for protein, mediator levels and PaO_2 and $PaCO_2$ were analysed with ANOVA. If ANOVA resulted in a $p \leq 0.05$, a Tukey-Kramer post-test was performed. Statistical significance was accepted when $p \leq 0.05$. All data are reported as mean ± SD.

Results

Arterial oxygenation and carbon dioxide levels over time with statistically significant differences have been indicated in Table 1 and Table 2, respectively. In groups 28/8 and 32/6 oxygenation levels did not significantly recover from post-lavage values. In groups OLC, PLV and Surf oxygenation levels were restored to pre-lavage values after 60 min, although in group PLV they gradually decreased over the next 3 hours. In group 32/0 H, oxygenation levels decreased over time as a result of mechanical ventilation only, whereas they remained stable in group 32/6 H.

The protein concentration was increased in the BALF of all lavaged animals after 90 and 240 min (Figure 1). However, treatment with OLC, surfactant or PLV partially reduced the BALF protein concentrations.

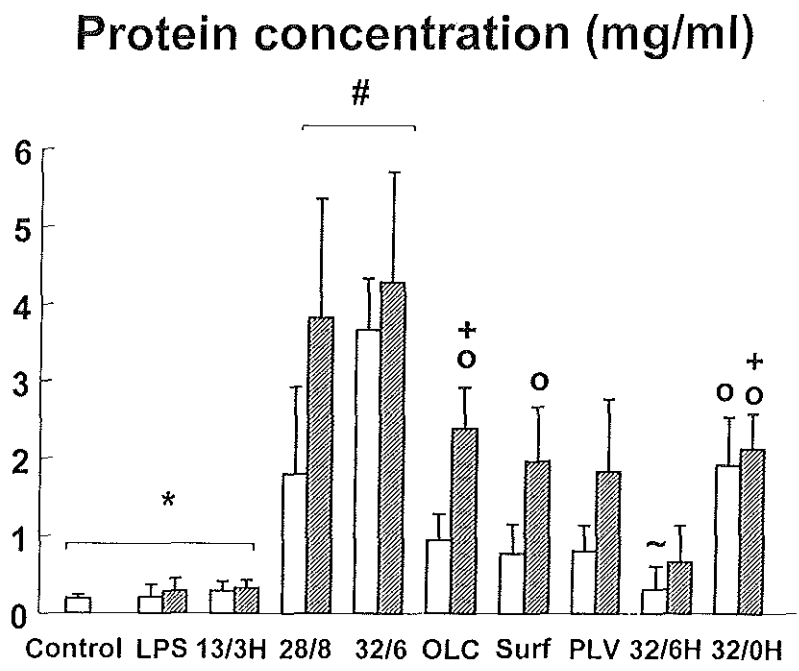

Figure 1 Protein concentration in the BALF in the different experimental groups after 90 (open) and 240 (dashed) min of mechanical ventilation. Data are mean±SD from groups of 10 animals. Statistically significant ($p<0.05$) * vs group 28/8 90 and 240, 32/6 90 and 240, OLC 240, PLV 240, Surf 240, 32/0H 90 and 240; # vs group 28/8 90, OLC 90 and 240, PLV 90 and 240, Surf 90 and 240, 32/0H 90 and 240, 32/6H 90 and 240; + vs groups OLC 90, PLV 90 and Surf 90; ° vs groups 32/6 90 and 240; ~ vs groups PLV 240 and 28/8 90.

Since previous studies had shown that ventilation alone may be sufficient to cause release of the eicosanoid prostacyclin [3] as well as the important pro-inflammatory cytokine TNF [2, 3] from isolated lungs *in vitro*, in the present study we have focused on these two mediators. Figures 2 and 3 depict data on BAL and serum concentration of TNF-α and PGI_2, respectively. There was no significant increase in serum concentrations of these mediators caused by the ventilation procedures. Increased TNF levels were found only in the serum of the LPS-treated animals. TNF levels in the BAL were not significantly increased, although the data suggest that there might be responsive and unresponsive animals: after 90 min 14 out of 50 animals that were lavaged and ventilated exceeded a threshold of 200 ng/ml TNF in the BALF, compared with only 2 out of 33 in the non-lavaged animals ($p < 0.05$ in Fisher's exact test). Thus, although not all animals responded, the number of animals that released increased amounts of TNF was significantly higher among lavaged animals. This division in responders and non-responders may also explain the high standard deviations. However, the increased TNF levels in the BALF of the responsive animals did not correspond to increased

prostacyclin levels in the same animals. With respect to prostacyclin in BALF, significantly increased values were observed in OLC group after 240 min only.

Discussion

This study demonstrates that ventilation strategies that prevent end-expiratory collapse, as indicated by levels of arterial oxygenation, reduce protein accumulation in the broncho-alveolar lavage accessible spaces in lung lavaged rats as compared to ventilation strategies which do not prevent alveolar collapse. TNF-α concentrations of BALF showed no differences between groups, although surfactant deficiency might have favored TNF production in a subgroup of animals. Thus, our *in vivo* findings did not confirm previous studies in isolated lungs subjected to different modes of hyperinflation [1-3]. Of note, the large increase in serum TNF induced by LPS, shows that under our conditions changes in serum TNF would have been noticed. Furthermore, in our study high PEEP at high levels of mean airway pressure (OLC group) resulted in a significant release of PGI_2 in the broncho-alveolar lavage accessible space, which is somewhat in contrast to the increases in lavage mediator levels of isolated lungs as a result of mechanical ventilation at high PIPs without PEEP [2].

In vivo studies in intact, healthy rats at tidal volumes comparable to the ones used by Tremblay et al. [2] in their *ex vivo* isolated and non-perfused rat lungs (40 ml/kg and above), have shown that the permeability changes as well as the changes in lung mechanics and oxygenation associated with this type of mechanical ventilation [9-12], can (at least partially) be prevented by application of PEEP or administration of exogenous surfactant preceding mechanical ventilation. Both of these measures are supposed to reduce repeated alveolar collapse and reexpansion [13], which induces stretch on the lung parenchyma [13] and may result in injury even at low airway pressures [17]. This suggests that ventilation-induced mediator expression is to an extent attributable to primary ventilation-induced surfactant changes with repeated alveolar collapse and reexpansion [11]. The reduction of the level of inflammatory mediators by PEEP application in isolated lungs [2] supports this hypothesis. The application of ventilator settings resulting in tidal volumes of 40 ml/kg and above, however, is lethal in intact rats within one hour, likely due to respiratory failure [18]. This period, however, is within the time frame of first assessment of inflammatory mediators in the *ex vivo* studies reported above [1-3].

The use of *ex vivo* lung preparations has other limitations. First, unlike *in situ*, isolated lungs are prone to overstretching by the loss of thoracic restriction at end inspiration [19]. This may become particularly pertinent during ventilation with high volumes and may be one of the major reasons for the lack of agreement between our data and those of isolated lungs which released inflammatory mediators at pressures as low as 25 cmH_2O [2, 3]. Second, not intrapulmonary pressures but absolute transmural pressures determine ventilation-induced lung injury and will be higher in isolated lungs than in *in situ* lungs [9, 20] unless special

precautions are taken [3]. Third, priming of ischemia on lung expression of various mediators *ex vivo* cannot always be ruled out. Other limitations of *ex vivo* preparations include the absence of innervation and perfusion [2] as well as perfusion by an artificial buffer instead of blood [1, 3]. Taken together, tissue responses to stress in isolated lung preparations appear not be identical to those seen *in vivo*.

For the reasons presented above, the role of repeated alveolar collapse and reexpansion in ventilation-induced inflammatory mediator expression *in vivo* was investigated in a rat lung lavage model at more clinically relevant moderately high peak airway pressures. Broncho-alveolar lavage increases surface tension of the alveolar lining fluid and decreases lung-thorax compliance and functional residual capacity by 35% [21, 22]. Treatment procedures to prevent repeated alveolar collapse and reexpansion are aimed at: 1) counterbalancing the increased retractive forces by applying pressure-controlled ventilation that recruits collapsed lung areas by applying an inspiratory pressure that overcomes the opening pressure of collapsed but recruitable lung units. After recruitment, ventilation pressures are reduced and PEEP is set just above the critical closing pressure of these lung units to prevent end-expiratory collapse [23], 2) decreasing alveolar surface tension by application of surface active material (exogenous surfactant therapy) [24], and 3) partial liquid ventilation, in which ventilation is superimposed on lungs that are filled with perfluorocarbons thus preventing expiratory collapse [25]. Our data demonstrate that all these strategies recruited collapsed alveoli, as indicated by the improved oxygenation (PaO_2/FiO_2 > 500 mmHg) compared to after broncho-alveolar lavage. Moreover, the protein levels were lower than in groups 28/8 and 32/6, even at comparable PIPs in group OLC. These findings can be explained by the reduction in lung parenchymal shear stress by prevention of repeated alveolar collapse and reexpansion of recruitment strategies, and epithelial stretch in particular [26]. This will increase permeability of the alveolo-capillary barrier to protein [26] and, due to alveolar collapse, increase suction to the interstitial spaces [12]. However, despite the differences in lung function and shear stress with these different ventilatory strategies no differences in serum TNF-α or PGI_2 concentrations were found.

Mechanical ventilation with high PEEP levels at high levels of mean airway pressure resulted in an increase in the PGI_2 level of the BALF after 240 min of mechanical ventilation (group OLC). It may be speculated that PGI_2 release as a result of mechanical ventilation forms a self-regulating physiological adaptation mechanism of vasodilatation of the lung microvasculature. This will protect the lung from reduced capillary perfusion as a result of compression associated with mechanical ventilation at high mean airway pressures. As the barrier function of the alveolo-capillary membrane in lung lavage lungs is lost even to large molecules [27], PGI_2 may freely diffuse over the alveolo-capillary barrier, which makes its origin unclear. Stretching of both cultured rat lung cultures [28] and cultured endothelial cells [29] has been shown to result in PGI_2 production.

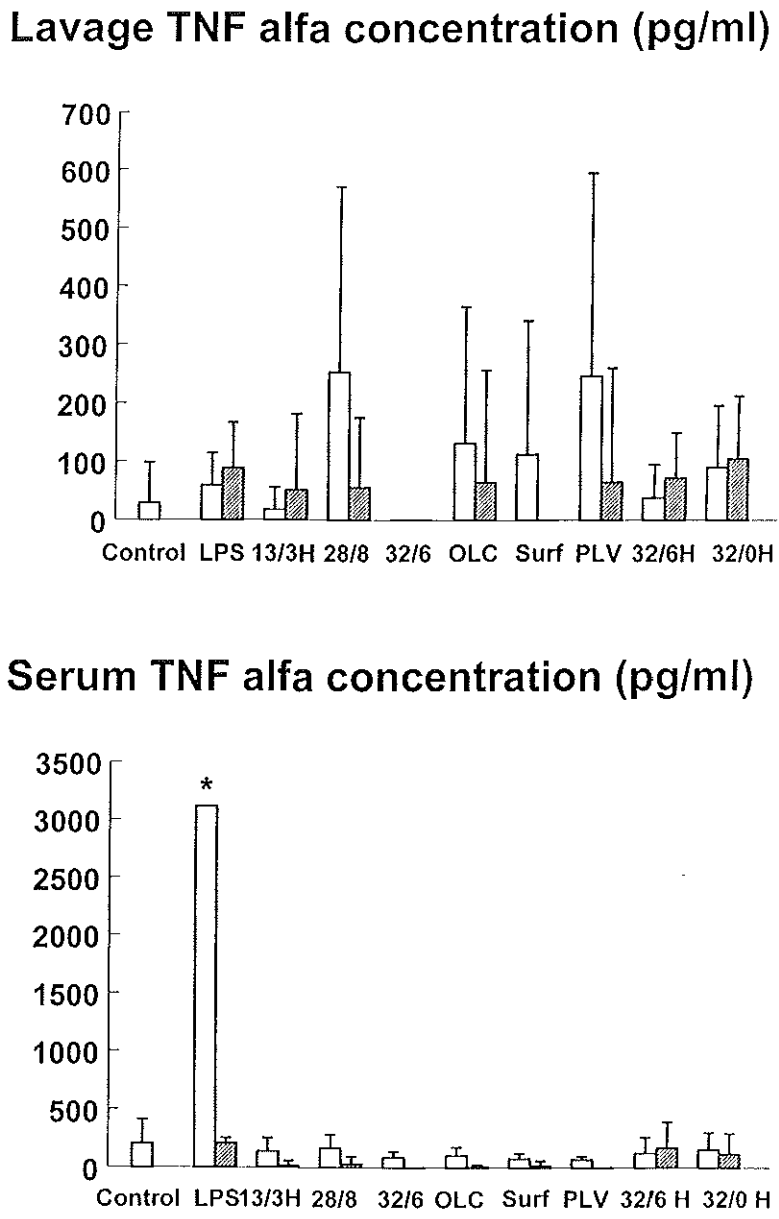

Figure 2. BALF (A) and serum (B) concentrations of TNF-α in the different experimental groups after 90 (open) and 240 (dashed) min of mechanical ventilation. Data are mean±SD from groups of 10 animals (except control n = 16 and 13/3H n = 13). Fig. 2A: There were no statistical differences between groups in BAL. Fig 2B: Statistically significant ($p<0.05$) * vs all other groups

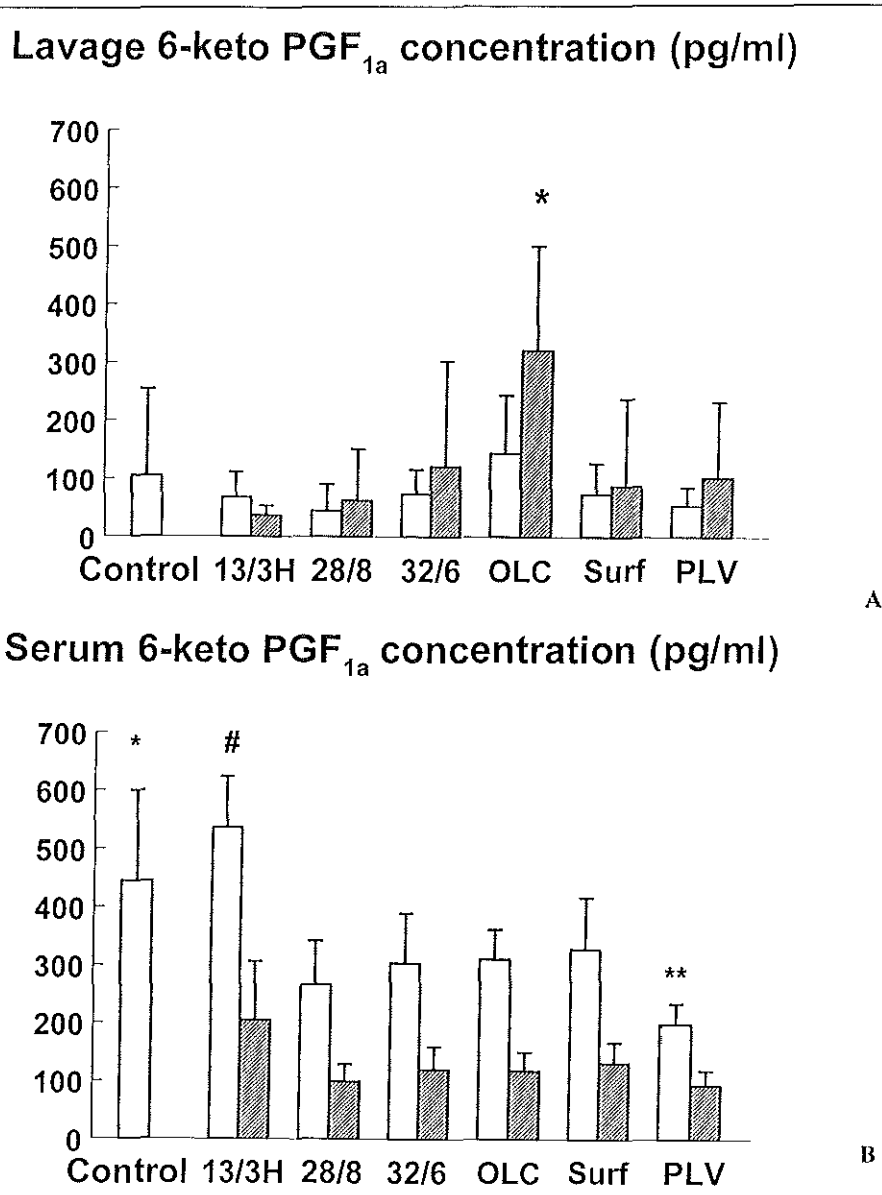

Figure 3. BALF (A) and serum (B) concentrations of 6-keto-PGF_1 in the different experimental groups after 90 (open) and 240 (dashed) min of mechanical ventilation. Data are mean±SD from groups of 10 animals. Fig. 3A: statistically significant ($p<0.05$) vs *all other groups. Fig. 3B: In all ventilated groups the values at t = 90 min within one group were higher than the values at t = 240 min. Within the lavaged animals, all values at t = 240 were lower than the values at t = 90 min in all groups except for PLV 90 versus 32/6 240 and OLC 240. Statistically significant ($p<0.05$) * vs all other groups except Surf 90 and 13/3 90; # vs all other groups except control; ** vs. 32/6 90, OLC 90 and Surf 90.

Table 1. Arterial oxygenation (PaO_2) in the different ventilated groups over time (mean (SD)). Statistical differences have been indicated.Intra-group differences: Significant differences vs [1]all time points; [2]30, 60 and 90 min; [3]5, 30, 60 and 90 min; [4]30, 60, 90, 120, 180 and 240 min; [5]60, 90, 120, 180 and 240 min. Inter-group differences: Significant differences [6]28/8 and 32/6 vs all others; [7]non-lavaged vs lavaged; [8] vs OLC; [9]vs OLC, PLV, Surf, 32/6 H; [10]28/8, 32/6 and 32/0 H vs all others.

	Group							
Time	**13/3 H**	**28/8**	**32/6**	**OLC**	**PLV**	**Surf**	**32/6 H**	**32/0 H**
Before	548.5	538.8[1]	530.6[1]	552.7	539.4	543.2	520.6	532.8[4]
	(44.7)	(44.9)	(29.4)	(39.9)	(44.1)	(44.6)	(57.8)	(53.6)
After[7]	564.9	68.8	67.4	66.9[1]	57.1[1]	66.9[1]	510.6	520.2[4]
	(43.2)	(12.6)	(13.9)	(12.8)	(14.2)	(14.6)	(90.6)	(72.5)
5'[6]	549.0	98.9	105.6	556.3	507.4	534.1	530.3	543.9[4]
	(39.9)	(29.4)	(45.0)	(58.3)	(55.6)	(43.8)	(71.5)	(50.2)
30'[6]	563.3	107.5	111.3	572.2	547.2	543.0	518.9	340.0[5,9]
	(53.8)	(54.1)	(64.6)	(49.7)	(51.1)	(41.2)	(63.6)	(190.2)
60'[10]	563.5	110.5	117.7	574.2	556.2	552.1	527.7	127.8
	(46.6)	(67.7)	(73.4)	(45.2)	(44.2)	(48.5	(60.2)	(138.5)
90'[10]	593.0	89.8	92.1	578.6	553.0	564.7	517.2	74.2
	(45.9)	(24.1)	(57.5)	(68.2)	(45.7)	(51.5)	(48.1)	(24.1)
120'[10]	541.4	138.5	130.7	570.2	534.4	522.5	547.2	66.8
	(48.1)	(115.3)	(105.5)	(38.5)	(69.3)	(44.5)	(47.7)	(24.6)
180'[10]	546.6	133.3	125.2	579.0	429.7[2,8]	508.6	542.8	97.0
	(56.5)	(120.2)	(110.7)	(44.7)	(142.5)	(60.7)	(65.8)	(77.6)
240'[10]	554.0	128.0	124.4	597.7	350.6[3,8]	494.0	516.2	67.3
	(60.4)	(124.3)	(82.2)	(47.4)	(188.5)	(81.3)	(113.3)	(14.4)

Table 2. Arterial carbon dioxide tension ($PaCO_2$) in the different ventilated groups over time (mean (SD)). Statistical differences have been indicated. Intra-group differences: Significant differences vs [1]all other time points; [2]A and 5 min; [3]60, 90, 120, 180, 240 min; [4] vs b, 60, 90, 120. Inter-group differences: Significant differences [5]28/8 vs all others; [6]13/3, 32/6 H and 32/0 H vs all others; [7]vs 32/6; [8]32/0 H; [9]vs PLV; [10]vs 28/8

	Group							
Time	**13/3 H**	**28/8**	**32/6**	**OLC**	**PLV**	**Surf**	**32/6 H**	**32/0 H**
Before	33.6 (5.0)	32.9[1] (6.4)	33.3[2] (6.3)	33.5 (4.7)	35.1[3] (5.7)	32.9[3] (6.5)	33.1[3] (5.1)	35.6 (6.5)
After[6]	32.5 (6.3)	55.6 (7.8)	52.6[1] (10.1)	53.3[1] (8.2)	55.3[1] (10.0)	47.8[1] (14.6)	31.6 (5.5)	30.4 (5.6)
5'[5]	30.2[7] (4.1)	53.7 (12.6)	40.8 (10.8)	36.6[8] (7.6)	35.2[3,8] (4.7)	34.5[3] (4.2)	31.9[7,8] (4.6)	26.5[4,7] (2.6)
30'[5]	34.4 (5.7)	50.5 (11.6)	35.6 (8.8)	33.7 (7.9)	29.0 (5.1)	30.3 (3.5)	30.8 (5.5)	30.6 (6.6)
60'[5]	31.1 (6.2)	50.6 (12.2)	33.9[9] (8.0)	34.2[9] (8.3)	25.2 (3.8)	28.6 (3.5)	30.2 (5.4)	35.8[9] (7.0)
90'[5]	28.8 (5.4)	48.6 (10.9)	37.5 (8.1)	30.4 (3.6)	23.1[7] (3.9)	27.4[7] (4.0)	30.1 (3.9)	35.3[9] (6.0)
120'	35.5[2,9] (8.1)	50.9 (12.1)	35.4[9] (11.0)	32.4 (10.7)	23.2[10] (3.5)	28.9[10] (4.3)	27.0[10] (7.0)	35.3[9,10] (7.5)
180'[5]	29.0 (3.2)	49.8 (14.3)	35.4 (9.4)	33.3 (6.0)	23.9[7] (4.3)	28.8 (5.8)	27.0 (6.4)	33.5 (10.7)
240'	33.0[9,10] (4.8)	50.4 (15.7)	38.0 (11.5)	33.9[10] (7.7)	26.3[10] (4.5	30.8[10] (9.3)	29.6[10] (3.0)	35.3 (11.5)

We do not have an explanation for the consistent finding of a decrease in serum PGI_2 concentration over time. It may be the result of decreased flow speeds in the lung vasculature [30], or depletion of a PGI_2 pool, altered PGI_2 metabolism, or a naturally occurring physiological vascular adaptation to changes in lung perfusion as a result of mechanical ventilation.

The BALF control levels of TNF-α were in the same order of magnitude as in isolated rat lungs [2]. Although our data showed no statistically significant effect of lavage or ventilation on BALF TNF levels *in vivo*, our data suggested that lavage may favor TNF release in a subgroup of responsive animals. Alveolar macrophages are primary candidates for mediator release [31] and TNF-α in particular [32], which may be induced by mechanical stretch [6]. The failure to see ventilation-dependent TNF release *in vivo*, could in theory have been due to the washout of alveolar macrophages from the alveolar spaces. To exclude this possibility, we included two groups of healthy, non-lavaged rats exposed with comparable PIPs but different PEEP levels. Again, ventilation strategy profoundly affected the levels of arterial oxygenation and protein infiltration. Interestingly, although serum levels of TNF-α showed a tendency to decrease over time in lavaged animals (90 min vs 240 min), this did not occur in the healthy, non-lavaged lungs. Data by von Bethmann et al. have shown that prolonged stretch is required for TNF-α release [1]. Stretch may have decreased in the lavaged groups due to changes in compliance over time, whereas such changes did not or only insufficiently occur in healthy animals to result in a decrease in TNF-α concentration. Independent of this, end-expiratory collapse did also occur in group 32/0 H as evidenced by the levels of arterial oxygenation. However, both serum and lavage TNF-α levels did not increase in healthy non-lavaged animals as a result of mechanical ventilation compared to non-ventilated controls and were in the same order of magnitude as in the ventilated and lavaged animals. Therefore, it is unlikely that broncho-alveolar washout of macrophages affected broncho-alveolar TNF-α levels.

In conclusion, different ventilation strategies had a profound effect on lung permeability and protein levels in lungs from lavaged rats *in vivo*. However, except for an increase in the level of PGI_2 in the broncho-alveolar lavage fluid by mechanical ventilation with high levels of PEEP (OLC), we could not demonstrate any increase in TNF-α or PGI_2 levels in serum or broncho-alveolar lavage fluid as a result of these different ventilatory strategies in lavaged rats *in vivo*. This is in contrast to previous findings in isolated perfused lungs [1-3]. It is therefore stressed that caution should be exercised to extrapolate the data on ventilation-induced inflammatory mediator expression in isolated lung preparations with high levels of lung parenchymal stretch to *in vivo* preparations and the clinical situation. Moreover, the data demonstrate that acute lung injury and possible ventilation-induced mediator expression are different modalities which do not necessarily coincide with each other. Future studies should investigate the possible interaction between ventilation-induced bacterial translocation [33] and

ventilation-induced inflammatory mediator release *in vivo*, particularly given the high frequency of ventilator associated pneumonia in ventilated patients [34].

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Summary and conclusions

As outlined in *Chapter 1*, the consequences of detrimental forms of mechanical ventilation on the fluid balance over the alveolo-capillary barrier have been extensively described in literature. Moreover, possible mechanisms by which such ventilation strategies exert systemic effects and effects on other organs are becoming increasingly realized. It is now generally accepted that the most detrimental forms of mechanical ventilation combine high peak inspiratory lung volumes with low levels of end-expiratory lung volumes. Ventilation-induced changes of the fluid balance over the alveolo-capillary barrier include permeability and structural changes to both the epithelial and endothelial layers. However, these changes can not be explained by end-inspiratory overstretching alone, since the use of positive end-expiratory pressure (PEEP) has been shown to reduce/prevent permeability and structural changes at the same level of end-inspiratory pressure. The effect of PEEP on lung permeability can be partially explained by the decrease in capillary filtration pressure resulting from it. However, this can not explain how PEEP leads to a reduction in structural damage caused by lung overinflation. As described in *Chapter 1*, changes to the surfactant system as a result of mechanical ventilation may provide a common denominator to explain both permeability and structural changes to the alveolo-capillary membrane as a result of mechanical ventilation, as well as a basis for the effect of PEEP on ventilation-induced lung injury (VILI). Insight in the exact mechanisms of VILI may indicate the ventilation strategies which prevent or minimize VILI and surfactant changes in both healthy and sick lungs: they should, on the one hand, prevent end-inspiratory alveolar overdistension and, on the other, repeated collapse and re-expansion of alveolar units. Future monitoring techniques during mechanical ventilation should provide us with minimal or non-invasive on-line information to enable these therapeutic directives to be achieved, and thus prevent lung parenchymal injury or stress.

Chapter 2 describes the mechanisms of ventilation-induced surfactant changes in a rat model of VILI with high peak inspiratory lung volumes at peak inspiratory pressures of 45 cmH_2O without PEEP. This animal model was first described by Webb and Tierney and later used in studies by Dreyfuss. The mechanisms of ventilation-induced surfactant impairment include: 1) surfactant displacement from the alveolar air-liquid interface into the small airways, 2) increased conversion of active into non-active surfactant subfractions, and 3) inactivation by intra-alveolar plasma proteins which infiltrate the alveolar space as a result of mechanical ventilation. In our study, use of PEEP was shown to prevent the increased conversion of active into non-active surfactant subfractions which was related to preservation of lung mechanics. It was postulated that surfactant changes with an increase in surface tension at the air-liquid interface play a key role in the mechanism of VILI by increasing suction forces at the air-liquid interface of the alveolar walls; surfactant changes will alter the pressure gradient across the alveolo-capillary barrier in the alveolar direction. This will result in a vicious circle of increased surfactant inactivation by protein-rich edema. Studies by

Taskar et al. have shown that such surfactant changes will make the lung more vulnerable to damage by mechanical ventilation. Our data demonstrate that mechanical ventilation may perpetuate alterations in the pulmonary surfactant system, indicating that mechanical ventilation with high peak inspiratory pressure levels without sufficiently high enough levels of PEEP in patients with ARDS, may impair the function of those alveoli that are still intact. Our data support the use of PEEP in patients with ARDS on ventilatory support to preserve normal surfactant function of alveoli that are not yet affected by the disease process.

Although such studies demonstrate that surfactant changes occur in VILI, they do not undisputably prove that surfactant changes play a central role in its mechanisms. Therefore, in *Chapter 3*, we describe a study on the effect of exogenous surfactant therapy, preceding lung overinflation without PEEP, on lung function and lung permeability. It demonstrates that high doses of exogenous surfactant, preceding lung overinflation, preserve lung mechanics and reduce infiltration of Evans blue dye in the alveolar spaces. It has now been demonstrated that large amounts of exogenous surfactant have a beneficial effect on lung function, and, possibly survival in patients with ARDS. Our data suggest that administration of large amounts of exogenous surfactant may beneficially influence further impairment of lung function due to mechanical ventilation in ARDS patients by protecting the lung areas not yet affected by the disease process.

The study presented in *Chapter 4* confirms that surfactant is rate-limiting for the transfer of proteins across the alveolo-capillary barrier, by demonstrating in lung lavaged rabbits, a reduced clearance of radio-labelled ^{99m}Tc-human serum albumin from the lung after exogenous surfactant therapy. The data suggest that fitting a mono-exponential or bi-exponential clearance characteristic to the clearance curve of this tracer molecule, might enable the use of the technique for the evaluation of the efficiency of different surfactant preparations.

In *Chapter 5*, it is demonstrated that a pressure-controlled ventilatory mode that uses small pressure amplitudes and high levels of end-expiratory pressure better preserves the active subfraction of surfactant after exogenous surfactant therapy than ventilation strategies that use higher pressure differences at lower levels of PEEP. Moreover, this ventilation strategy was shown to better preserve oxygenation and reduce alveolar protein infiltration. The advantage of modes of mechanical ventilation that create PEEP by inversed ratio ventilation, with an early interruption of the expiratory flow, over modes of mechanical ventilation that create the same level of PEEP by static PEEP only, is a more effective carbon dioxide elimination. These data show that pressure-controlled inversed ratio ventilation may reduce the required amount of exogenous surfactant and, thus, treatment costs in clinical practice during exogenous surfactant therapy.

Although permeability changes of the alveolo-capillary barrier provide strong indication for structural changes of the alveolo-capillary barrier, they are not a direct reflection of ventilation-induced morphological changes or beginning injury of the lung parenchyma. In *Chapter 6* it is demonstrated that the breakdown products of adenosine-

triphosphate (ATP), the purines, in the broncho-alveolar lavage fluid indicate early metabolic and, possibly, structural changes to the alveolo-capillary barrier as a result of mechanical ventilation. The most likely origin of these ATP breakdown products is the alveolar type II cell. The use of PEEP was shown to prevent ventilation-induced increases in these ATP breakdown products, and exogenous surfactant was shown to prevent a significant increase in these products compared to non-ventilated controls.

As is discussed in *Chapter 1*, there are strong indications from experimental studies that mechanical ventilation induces inflammatory processes that do not remain localized to the lung, but may give rise to a more generalized systemic inflammatory reaction. Such an inflammatory reaction could promote the failure of other organs, leading to the development of MOF, which is still the leading cause of death in ARDS.

Given the fact that the majority of intubated patients develop a ventilation-associated pneumonia, *Chapter 7* discusses one possible mechanism by which mechanical ventilation may influence the development of MOF. It was shown that 3 hours of mechanical ventilation in rats inoculated with *Klebsiella pneumoniae* with a peak inspiratory pressure of 30 cmH_2O without PEEP promotes bacteremia with *Klebsiella pneumoniae*. The use of 10 cmH_2O of PEEP was shown to reduce ventilation-induced bacteremia. Three hours of mechanical ventilation at low peak inspiratory pressures with or without the use of PEEP, did not increase bacterial translocation from the lung into the bloodstream, when compared to non-ventilated controls. When translated to the clinical setting, our data advocate the use of a sufficiently high level of PEEP to prevent bacteremia in intubated patients receiving mechanical ventilation at high levels of peak inspiratory pressure.

The work in *Chapter 8* demonstrates in lung lavaged rats that the ventilation strategy strongly affects the level of lung injury as measured by intra-alveolar protein infiltration and lung function. However, we could not demonstrate differences in the serum levels of TNF-α and PGI_2 with different ventilation strategies. These results were in contrast to findings reported by others which showed differences in the levels of inflammatory mediators in the perfusate of isolated perfused *ex vivo* lung preparations as a result of different ventilation strategies. It is concluded that: 1) lung injury and inflammatory mediator release from the lung tissue are two different entities that do not necessarily coincide with each other *in vivo*, and that 2) inflammatory mediator release from the lung tissue as a reaction to mechanical ventilation may be the result of the limitations of *ex vivo* lung preparations and that caution is required in extrapolating the data from such studies to the clinical situation.

In summary, the studies in this thesis contribute to the knowledge on VILI, providing evidence for the role of surfactant changes and for the possibility of development of systemic inflammatory reactions in the mechanism of VILI.

Samenvatting en conclusies

Zoals uiteengezet in *Hoofdstuk 1*, zijn de consequenties van schadelijke vormen van kunstmatige beademing op de vloeistofbalans van de alveolus-capillair barrière uitgebreid in de literatuur beschreven. Ook groeit het inzicht in de mogelijke mechanismen waardoor deze beademingsstrategieën systemische effecten en effecten op andere organen uitoefenen. Het is nu algemeen geaccepteerd, dat kunstmatige beademing met een combinatie van hoge piek inspiratoire longvolumes met lage eind-expiratoire longvolumes de meest schadelijke is. Ventilatie-geïnduceerde veranderingen van de vloeistofbalans over de alveolus-capillair barrière omvatten zowel permeabiliteitsveranderingen als structurele veranderingen in het longepitheel en endotheel. Echter, eind-inspiratoire uitrekking van het long parenchym kan op zichzelf deze veranderingen niet verklaren, omdat, bij een gelijke mate van eind-inspiratoire druk, het gebruik van positief eind-expiratoire druk (PEEP) permeabiliteits en structurele veranderingen vermindert/voorkomt. Het effect van PEEP op de longpermeabiliteit kan gedeeltelijk verklaard worden door een vermindering van de capillaire filtratiedruk t.g.v PEEP. Echter, dit mechanisme kan de vermindering in structurele schade niet verklaren. Zoals in *Hoofstuk 1* beschreven wordt, vormen veranderingen in het surfactant systeem een gemeenschappelijke verklaring voor zowel permeabiliteits als structurele veranderingen t.g.v. kunstmatige beademing, als ook een verklaring voor het effect van PEEP op beademings-geïnduceerde longschade. De exacte mechanismen van beademings-geïnduceerde longschade geven inzicht in die beademingsstrategieën die zowel beademings-geïnduceerde longschade als surfactant veranderingen in gezonde en zieke longen voorkomen of minimaliseren: Ze moeten enerzijds een te grote eind-inspiratoire uitrekking en anderzijds het herhaaldelijk samenvallen en openen van alveoli voorkomen. Voor de toekomst moet gezocht worden naar minimaal of non-invasieve bewakingstechnieken, waardoor men kan vaststellen of deze therapeutische doelstellingen bereikt worden en dus schade en uitrekking van het longparenchym wordt voorkomen.

Hoofdstuk 2 beschrijft de mechanismen van beademings-geïnduceerde surfactant veranderingen in een rat model van beademings-geïnduceerde longschade t.g.v het gebruik van hoge piek-inspiratoire long volumes bij piek-inspiratoire drukken van 45 cmH_2O zonder PEEP. Dit diermodel werd voor het eerst beschreven door Webb and Tierney (1974) en later ook in studies van Dreyfuss (1985). De mechanismen van beademings-geïnduceerde surfactant schade omvatten 1) surfactant dislocatie van het alveolaire vloeistof-gas scheidingsvlak naar de kleine luchtwegen, 2) een verhoging van de omzetting van actieve in niet-actieve surfactant componenten en 3) inactivatie van surfactant door intra-alveolaire plasmaeiwitten die de alveolair ruimte infiltreren t.g.v kunstmatige beademing. In deze studie werd aangetoond dat PEEP de verhoogde omzetting van actieve in niet-actieve surfactant componenten voorkomt, wat gerelateerd was aan het behoud van de longmechaniek. Verondersteld werd dat surfactant veranderingen met een verhoging van de oppervlaktespanning op het gas-vloeistof scheidingsvlak van de alveolus een centrale rol spelen in het mechanisme van beademings-

geïnduceerde longschade door de zuigkrachten op het gas-vloeistof scheidingsvlak te verhogen. Dit zal leiden tot een verschuiving van de drukgradient over de alveolus-capillair barrière in de richting van de alveolus, wat vervolgens resulteert in een vicieuze cirkel van meer surfactant inactivatie door eiwit-rijk oedeem. Studies door Taskar hebben aangetoond dat surfactant veranderingen de long kwetsbaarder maken voor schade door kunstmatige beademing. Onze data tonen aan dat kunstmatige beademing veranderingen in het surfactant systeem van de long kan induceren, hetgeen weer aantoont dat kunstmatige beademing met hoge piek-inspiratoire drukniveaus zonder voldoende hoge PEEP niveaus in patiënten met ARDS, de functie van gezonde alveoli kan verstoren. Onze data ondersteunen het gebruik van PEEP in patiënten met ARDS, om de normale surfactant functie te beschermen van die alveoli die nog niet zijn aangedaan door het ziekteproces.

Alhoewel deze studies aantonen dat surfactant veranderingen plaats vinden tijdens beademings-geïnduceerde longschade, bewijzen ze niet ontegenzeggelijk dat surfactant veranderingen een centrale rol spelen in het mechanisme van beademings-geïnduceerde longschade. Daarom wordt in *Hoofdstuk 3* een studie beschreven naar het effect van vooraf toegediend exogeen surfactant op de verstoring van de longfunctie en toename van de longpermeabiliteit door ventilatie met hoge piek-inspiratoire longvolumes zonder PEEP. De studie toont aan dat exogeen surfactant toediening in hoge dosering voorafgaand aan ventilatie met hoge piek-inspiratoire longvolumes, leidt tot een behoud van de longmechaniek en een vermindering van de infiltratie van Evans blue naar de alveolaire ruimte. Recent is aangetoond dat surfactant in hoge dosering een positief effect heeft op de long functie en, mogelijk, op de overleving van ARDS patiënten. De data van deze studie suggereren dat toediening van exogeen surfactant in hoge dosering een gunstig effect heeft op de verdere verslechtering van de longfunctie ten gevolge van kunstmatige beademing in ARDS patiënten, door die longdelen te beschermen die nog niet zijn aangedaan door het ziekteproces.

De studie uit *Hoofstuk 4* bevestigt dat surfactant een beperkende factor is voor de filtratie van eiwitten over de alveolus-capillair barrière. In konijnen waarvan de longen gelaveerd zijn, vermindert door exogeen surfactant de klaring van humaan serum albumine, gemerkt met radioactief ^{99m}Tc, vanuit de long naar de bloedbaan. Bovendien veronderstellen de data dat door het correleren van een mono-exponentiële of bi-exponentiële curve op de klaringscurve van dit molecuul de effectiviteit van verschillende surfactant preparaten geëvalueerd kan worden.

In *Hoofdstuk 5* wordt aangetoond, dat een vorm van drukgestuurde kunstmatige beademing die kleine drukamplituden combineert met hoge niveaus van eind-expiratoire druk leidt tot een beter behoud van de actieve component van exogeen surfactant, dan beademings-strategieën die grotere drukamplitudes combineren met lage PEEP niveaus. Bovendien leidt zo'n beademingsstrategie tot een beter behoud van de arteriële zuurstofspanning en vermindert het eiwit infiltratie. Het voordeel van het gebruik van auto-PEEP gecreeërd door een voortijdige onderbreking van de expiratoire flow door het gebruik van inversed-ratio beademing boven het gebruik van statische PEEP, is een effectievere koolstofdioxide uitwas.

Vanuit een klinisch perspectief betekent dit dat drukgestuurde inversed-ratio beademing de hoeveelheid exogeen surfactant, en dus de behandelingskosten tijdens het gebruik van exogeen surfactant kan verminderen.

Permeabiliteitsveranderingen zijn een sterke aanwijzing voor structurele veranderingen van de alveolus-capillair membraam, echter, ze zijn geen directe weerspiegeling van beademing-geïnduceerde morfologische veranderingen of beginnende schade van het long parenchym. In *Hoofdstuk 6* wordt aangetoond dat de afbraakproducten van adenosine-trifosfaat (ATP), de purines, in de broncho-alveolaire lavage als markers van vroege metabole en, mogelijk, structurele veranderingen van de alveolus-capillair membraan t.g.v. kunstmatige beademing kunnen functioneren. De meest waarschijnlijke oorsprong van deze ATP-afbraakproducten is de alveolaire type II cel. Het gebruik van PEEP voorkomt een beademings-geïnduceerde stijging in deze ATP-afbraakproducten en exogeen surfactant voorkwam een significante stijging in deze ATP-afbraaktproducten i.v.m. niet-beademde controle dieren.

Zoals bediscussieërd in *Hoofdstuk 1*, zijn er sterke aanwijzingen uit experimentele studies dat kunstmatige beademing ontstekingsprocessen in de long induceert, die niet gelocaliseerd blijven in de long, maar die aanleiding geven tot een meer gegeneraliseerde systemische ontstekingreactie. Zo'n ontstekingsreactie zou aanleiding kunnen geven tot het falen van andere organen, wat uiteindelijk kan leiden tot het ontstaan van multiorgaan falen (MOF), wat nog steeds de belangrijkste doodsoorzaak is van ARDS.

Gebaseerd op het feit dat de meerderheid van de geïntubeerde patiënten een beademings-geassocieerde pneumonie ontwikkelt, wordt in *Hoofdstuk 7* een mogelijk mechanisme beschreven waardoor kunstmatige beademing het ontstaan van MOF kan beïnvloeden. Er werd aangetoond dat 3 uur kunstmatige beademing met piek inspiratoire drukken van 30 cmH_2O zonder PEEP in ratten geïnoculeerd met *Klebsiella pneumoniae*, bacteremie met dit organisme induceert. Het gebruik van PEEP verminderde deze beademings-geïnduceerde bacteremie met *Klebsiella pneumoniae*. Drie uur kunstmatige beademing met lage piek inspiratoire drukken met of zonder het gebruik van PEEP leidde i.v.m. niet-beademde controle dieren niet tot een stijging van de translocatie van bacteriën vanuit de long naar de bloedbaan. Wanneer dit wordt vertaald naar de klinische setting, ondersteunen onze data het gebruik van een voldoende hoog PEEP-niveau om bacteremie te voorkomen in patiënten die kunstmatig beademd worden met hoge piek-inspiratoire drukniveaus.

De studie uit *Hoofdstuk 8* demonstreert dat verschillende beademingstrategieën in long-gelaveerde ratten de longfunctie en de mate van longschade weerspiegeld door intra-alveolaire eiwit infiltratie, sterk beïnvloeden. Echter, een verschil in de serum spiegels van TNF-α en PGI_2 als gevolg van verschillende beademingsstrategieën, kon niet worden waargenomen. Deze bevindingen zijn niet in overeenstemming met de bevindingen van een andere onderzoeksgroep, die aantoonde dat in geïsoleerde, geperfundeerde *ex vivo* long-preparaten van knaagdieren, de hoogte van de spiegels van ontstekingsmediatoren van de

perfusievloeistof afhankelijk is van de beademingsstrategie. Geconcludeerd kan worden dat 1) beademings-geïnduceerde longschade en beademings-geïnduceerde afgifte van ontstekingsmediatoren twee verschillende fenomenen zijn die, *in vivo,* niet noodzakelijkerwijs samenvallen met elkaar en dat 2) de afgifte van ontstekingsmediatoren vanuit het longweefsel t.g.v kunstmatige beademing in *ex vivo* long preparaten een gevolg kan zijn van de beperkingen van dit soort preparaten en dat voorzichtigheid geboden is met het extrapoleren van de resultaten van deze onderzoeken naar de klinische situatie.

Samengevat dragen de studies bij aan de kennis over beademings-geïnduceerde longschade, door bewijs aan te voeren voor de rol van surfactant veranderingen en de mogelijkheid tot de ontwikkeling van een systemische ontstekingsreactie in het mechanisme van beademings-geïnduceerde longschade.

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Curriculum vitae

De auteur werd geboren op 30 juli 1971 te Hilvarenbeek. In 1990 behaalde hij het VWO-diploma aan het "Onze Lieve Vrouwe" Lyceum te Breda waarna hij aanving met de studie geneeskunde. In mei 1993 begon hij een student-assistentschap op de afdeling Experimentele Anesthesiologie aan de Erasmus Universiteit. Het doctoraal examen geneeskunde behaalde hij in mei 1996 aan de Erasmus Universiteit te Rotterdam. Na een onderzoeksstage van 6 maanden op de afdeling longpathologie van de "University of Michigan" in Ann Arbor in de Verenigde Staten, begon hij in mei 1996 met full-time onderzoek naar beademings-geïnduceerde longschade onder begeleiding van Professor Lachmann, wat uiteindelijk resulteerde in dit proefschrift. In januari 1999 zal hij beginnen met zijn co-assistentschappen welke aan het eind van het jaar 2000 afgerond zullen zijn.

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