Strategies to improve oxygenation in experimental acute lung injury

Cover: Scanning electron microscopy photographs of an open alveolus after lung lavage (top), an alveolus after lung lavage followed by a period of mechanical ventilation (middle), and a closed alveolus (bottom).

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STRATEGIES TO IMPROVE OXYGENATION IN EXPERIMENTAL ACUTE LUNG INJURY

STRATEGIEËN TER VERBETERING VAN DE OXYGENATIE IN EXPERIMENTEEL ACUUT LONGFALEN

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Chapter 1

Role of surfactant in the pathophysiology of the acute respiratory distress syndrome: consequences for treatment strategies

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One of the most important clinical syndromes, in which failure of oxygen uptake in the lung leads to severe hypoxia, is the so-called acute respiratory distress syndrome (ARDS). ARDS is a complex of clinical signs and symptoms which occur following diverse pulmonary or systemic insults, including sepsis, shock, pneumonia, trauma, liquid aspiration, hematological disorders, smoke inhalation, and many others [1, 2]. In ARDS, the treatments available are still inadequate and morbidity, mortality, and costs remain unacceptably high [3]. The failure of the lung as a gas exchange organ results in peripheral tissue hypoxia, which appears to be related to the development of multiple organ failure (MOF). Since MOF is the predominant cause of death in ARDS, therapeutic efforts are aimed at improving oxygen delivery to the tissues.

Available treatments include mechanical ventilation with positive end-expiratory pressure (PEEP) and high inspiratory oxygen concentration. However, despite extensive research on new ventilation modes, mortality has not changed much and remains 30-70% [4], as high as when first reported by Ashbaugh et al. in 1967 [5]. It should be realized that mechanical ventilation is only a supportive strategy and that some ventilatory strategies even contribute to lung injury. That is why ARDS may, in part, be a product of our therapy – rather than the progression of the underlying disease [6].

Several strategies for ARDS, such as exogenous surfactant therapy [7], ventilation according to the "Open Lung Concept" [6, 8] and partial liquid ventilation [9] are currently under evaluation. In this chapter, we describe the central role of pulmonary surfactant in the pathophysiology of ARDS, and discuss the impact of these new strategies on surfactant function.

Acute respiratory distress syndrome

ARDS has become a well-recognized condition that is characterized by a heterogenous deterioration of both alveolar ventilation and pulmonary perfusion. ARDS is associated with an increase in alveolar permeability leading to pulmonary edema, hemorrhage and atelectasis. The pathophysiological changes in ARDS include hypoxemia, decrease in functional residual capacity (FRC), decreased total lung capacity (TLC), and decreased lung compliance [10].

As early as 1929, Von Neergaard [11] reported that much larger pressures were required to expand an air-filled lung than a lung filled with fluid (Fig. 1); this important finding suggested that the surface tension at the air-liquid interface of the alveoli influences lung elasticity. The

problem with this discovery was that his work was published in German, and that for 25 years no scientists in the evolving field took serious note of this publication. In 1957, Clements measured surface tension by using a modified surface balance, and demonstrated that lung extracts decreased surface tension [12]. They pointed out that the presence of a material with a very low surface tension prevents atelectasis in the lung.

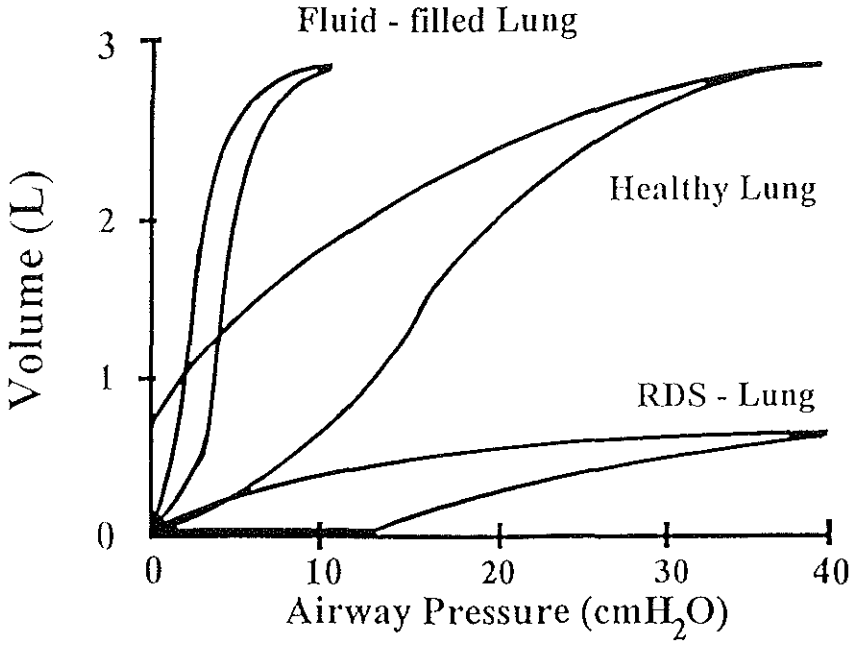

Figure 1. Pressure-volume (P/V) diagram of a healthy air-filled lung, a fluid-filled lung and an ARDS lung. Von Neergaard [11] showed, in 1929, that much larger pressures were required to expand an air-filled lung than a lung filled with fluid. In ARDS, there are even higher pressures required to expand the lung due to the high surface tension at the air-liquid interface in the alveoli, which is caused by surfactant inactivity. ARDS: acute respiratory distress syndrome.

Asbaugh et al., in 1967, demonstrated that the surface tension of broncho-alveolar lavage (BAL) fluid from two adult patients was increased [5]. The clinical and pathological features were very similar to those seen in neonates with respiratory distress syndrome, who have a primary surfactant deficiency that is caused by the immaturity of the lungs. Therefore, they introduced the name adult respiratory distress syndrome (ARDS), and assumed that in the adult patient the pulmonary surfactant became inactivated and was responsible for atelectasis. Nowadays, it is more appropriate to speak about the acute, rather than adult, respiratory distress syndrome, since ARDS is not limited to adults [3].

Since 1967, several studies have analyzed lung surfactant recovered from BAL of patients with ARDS and demonstrated compositional changes in surfactant and/or decreased surfactant content of the lungs [10]. Furthermore, Gregory and colleagues [13] showed that minimal surface tension was increased, and that total phospholipids and surfactant proteins (SP-A and SP-B) were decreased in the BAL fluid obtained from patients at risk to develop ARDS, suggesting that these abnormalities of surfactant occur early in the disease process.

The central role of surfactant deficiency can further be illustrated by studies in animal models of ARDS which demonstrated that exogenous surfactant instillation dramatically improved blood gases and lung mechanics (for reviews see [14-16]). The models of surfactant deficiency in which these improvements could be demonstrated include: acute respiratory failure due to in vivo whole-lung lavage [17-19], neurogenic ARDS [20], respiratory failure as a result of oxygen toxicity [21, 22] or oxidant producing enzymes [23], acute respiratory failure after instillation of hydrochloric acid [24-26], plasma instillation [27] and intoxication with N-nitroso-N-methylurethane (NNNMU) [28] or paraquat [29], and respiratory failure due to pneumonia [30-32].

Thus, there is clinical and experimental evidence of a deficiency of active pulmonary surfactant in patients with ARDS, which would be the rationale for a surfactant replacement therapy.

Pulmonary Surfactant

Pulmonary surfactant is a complex of phospholipids (80-90%), neutral lipids (5-10%) and at least four specific surfactant-proteins (5-10%) (SP-A, SP-B, SP-C and SP-D), synthesized and secreted

from the alveolar type II cells, lying as a monolayer at the air-liquid interface to reduce the surface tension in the lung [33]. Surfactant is essential for normal breathing at physiological transpulmonary pressures and prevents end-expiratory collapse of the alveoli and small airways; an intact surfactant system is also essential to maintain the fluid balance in the lung [34]. The functional integrity of surfactant depends on the specific proteins [35]. The exact mechanisms involved are not yet clear, but there are indications that SP-A regulates surfactant secretion and re-uptake of surfactant by the type II cells, whilst SP-B and SP-C are thought essential for rapid adsorption of the phospholipid molecules into the monolayer. It has been demonstrated that surfactant, in particular SP-A and D, plays a role in the lung's defence against infection [36].

Pulmonary surfactant and ARDS

Despite diverse etiologies in ARDS, the final pathway results in damage of the alveolar epithelium and endothelium, which leads to high-permeability pulmonary edema. The exact mechanisms responsible for injury to the alveolar-capillary membrane are complex and are still under discussion [37]. The formation of edema leads to wash-out or dilution of the surfactant [38] and/or inactivation of the surfactant by plasma components, such as fibrin(ogen), albumin, globulin and transferin, hemoglobin and cell membrane lipids; these components are known to inhibit pulmonary surfactant in a dose-dependent way [39]. Furthermore, surfactant may also be disturbed by the following mechanisms: breakdown of surfactant by lipases and proteases; phospholipid peroxidation by free radicals; loss of surfactant from the airways due to mechanical ventilation with large tidal volumes; disturbed synthesis, storage, or release of surfactant secondary to direct injury of type II cells [39-41].

Diminished surfactant has far-reaching consequences for lung function. Independent of the cause, decreased surfactant function will directly or indirectly lead to [34]:

- decreased pulmonary compliance
- decreased FRC
- atelectasis and enlargement of the functional right-to-left shunt
- decreased gas exchange and respiratory acidosis
- hypoxemia with anaerobic metabolism and metabolic acidosis

- pulmonary edema with further inactivation of surfactant by plasma constituents.

This phase of acute respiratory distress can persist for days to weeks, but recovery without persistent impairment of lung function is possible. However, when this condition persists for a longer period of time, it is likely that new inflammatory events occur, which can trigger fibroblast proliferation that results in progressive lung fibrosis. In this phase of ARDS lung damage becomes irreversible, and this condition is associated with a very poor prognosis [42].

The pulmonary surfactant system and mechanical ventilation

The deterioration of gas exchange in ARDS that results from the collapse of surfactant-deficient alveoli necessitates the implementation of mechanical ventilation. However, it has been shown that mechanical ventilation itself, in particular when large tidal volumes and high peak inspiratory pressures are used in combination with low end-expiratory pressures, affect the pulmonary surfactant system [43]. The exact mechanisms involved are not yet entirely clear, but it has been shown that the surfactant in the alveolar lining is actively removed from the alveolus towards the larger airways; this can lead to a shortage of surfactant at the alveolar level causing changes in surface tension characteristics in the lung, as seen during or after prolonged periods of mechanical ventilation [43].

During end-expiration the surfactant molecules covering the alveolar epithelium are compressed on the small alveolar areas leading to low surface tension thus preventing the alveoli from collapse. If the surface of the alveolus is smaller than the surface occupied by the surfactant molecules, the molecules are squeezed out of the surface of the alveolus and forced toward the airways. These surfactant molecules are then 'lost' for the alveoli and are eventually cleared from the lungs. During the following inflation of the alveoli, the surface is replenished with surfactant molecules coming from the underlying hypophase where surfactant molecules are 'stored' for later use. During the next expiration, the mechanism repeats itself and again surfactant molecules are forced out of the alveolus and subsequently replenished from the hypophase; this is a continuing cycle (Fig. 2) [43].

The amount of surfactant that must be produced and subsequently secreted by the alveolar type II cells is proportional to the loss of surface active molecules during the breathing cycle.

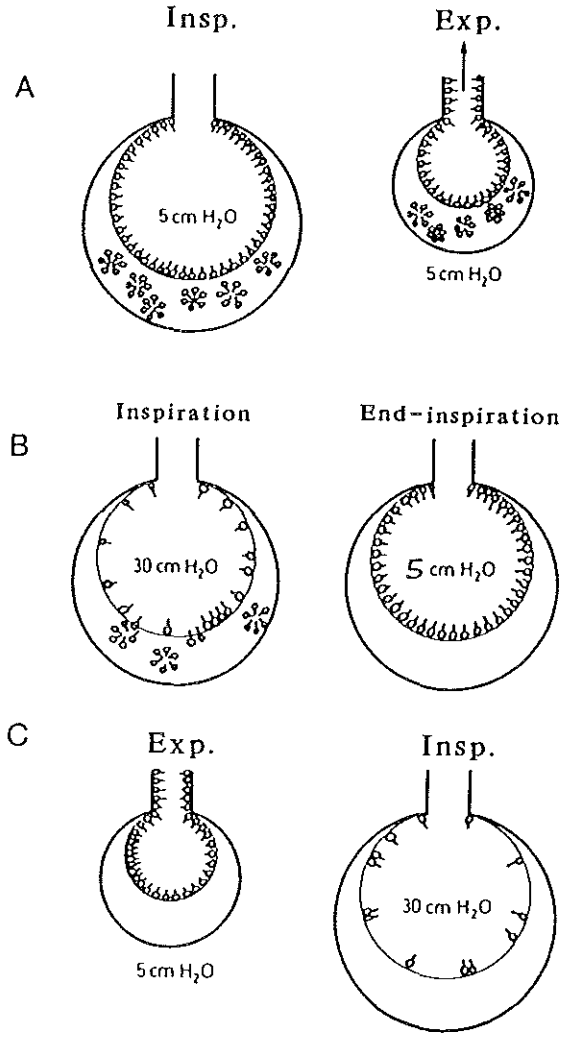

Figure 2. (A) Balance between synthesis, release and consumption of surfactant in the healthy lung. The pressure values given represent the intra-pulmonary pressure needed to open up this alveolus. At the surface and in the hypophase (micelles), there are sufficient molecules of surfactant. These micelles deliver the surfactant necessary to replace the molecules squeezed out during expiration (Exp.). (B) Imbalance between synthesis, release and consumption of surfactant due to mechanical ventilation. At the beginning of inspiration (Insp.), there exists an apparent deficiency of surfactant molecules but 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 (from reference [40]).

When production and secretion of new surfactant molecules keep pace with consumption, no surfactant deficiency will occur, as in a normal healthy lung.

Function of surfactant present in the alveoli can be compromised during mechanical ventilation in several different ways. First, due to the pulmonary interdependence of the alveoli the forces acting on the fragile lung tissue in non-uniformly expanded lungs are not only the applied transpulmonary pressures, but also the shear forces that are present in the interstitium between opened and closed alveoli [44]. These shear forces may well be the major reason for epithelial disruption and the loss of barrier function of the alveolar epithelium, which results in an influx into the alveolar space of plasma proteins, which inhibit surfactant function in a dose-dependent way [45].

Second, the surfactant system consists of surface active large aggregates (LA) and non-surface active small aggregates (SA). In order to preserve an adequate surfactant function, it is necessary that the balance between small and large aggregates is maintained [46]. Studies have shown that the conversion of LA into SA correlates with changes in alveolar surface area. An increase in tidal volume, which increases the magnitude of alveolar surface area changes, has been shown to increase the conversion of LA into SA [47, 48]. The same studies demonstrated that changing the respiratory rate [47] or level of PEEP [48] did not affect the rate of aggregate conversion.

Treatment strategies for ARDS

Exogenous surfactant therapy

Considering its success in neonatal RDS, exogenous surfactant therapy seems very promising in the treatment of ARDS patients. Although these two syndromes are very similar, there is an important difference: neonatal RDS is characterized by a primary surfactant deficiency due to immaturity of the lungs, whereas in ARDS surfactant inactivity is a complication of lung injury.

Although it seems rational to administer exogenous surfactant to ARDS patients, this not yet a clinical reality. At this moment, only a few case reports and results of limited clinical studies are available in which adult patients with ARDS are treated with exogenous surfactant.

Although these reports showed that some patients did not respond, or had an only transient improvement after a single dose of surfactant, better results were seen with higher or multiple surfactant doses. The reason for lack of response, or only transient improvement after exogenous surfactant application, is attributed to the inhibition of the instilled surfactant by plasma components filling the alveolar space. This means that if after surfactant instillation there is no, or only transient, improvement of blood gases in these patients (fibrotic lungs excluded) this does not mean that surfactant treatment does not work, it only means that the concentration of the exogenous surfactant used is too low in relation to the amount of surfactant inhibitors in the lung [45]. Therefore, for treatment of ARDS, a high concentration of surfactant is required to overcome the inhibitory effect of plasma components. This was first postulated by Lachmann [34] after treating the first "adult" patient, and is now confirmed by the results of Gregory and colleagues [49] in a multicenter, randomized pilot study in 59 patients with ARDS of different etiologies. In this latter study, three different dosing strategies were tested and the results showed that maximum improvement of oxygenation, minimum ventilatory requirements, and lowest mortality rate were obtained following treatment with 400 mg surfactant per kg body weight. The surfactant used in Gregory's study was a natural surfactant, as already used in neonates, and was given as a bolus. This study is, to our knowledge, the first controlled trial in which the mortality of ARDS could be decreased (from 43.8% to 18.8%) by a single therapeutic intervention. Walmrath et al. administered a natural surfactant (300 mg/kg) with a bronchoscope to patients with severe ARDS and sepsis, which resulted in an increase in arterial oxygenation [50]. In half of their patients, an additional dose of 200 mg/kg was required to maintain this improvement of gas exchange. In contrast to these results, Anzueto et al. found no effect on mortality and lung function after administration of aerosolized artificial surfactant in a multicenter trial in patients with sepsis-induced ARDS [51]. However, in their study a much lower dose of surfactant was delivered to the lungs (<25 mg/kg) than in the study by Gregory and colleagues, which is speculated to be an insufficient dose to overcome the inhibition by plasma components in the alveolar space.

The exact amount of exogenous surfactant required in ARDS to restore lung surfactant function is not known, but different case reports and pilot studies suggest that a dose between 50 and 400 mg/kg body weight may be appropriate. Because the quantity of inhibitors differs

from patient to patient, an excess of surfactant should always be given or repeatedly be substituted ("titrated") until blood gases improve [45]. Experience in neonates has also learned that exogenous surfactant is more effective when administration takes place in the early stages of RDS with a smaller amount of surfactant inhibitors [52]. Early treatment of ARDS may thus require smaller amounts of surfactant and the outcome results will probably be better.

The optimal method to deliver surfactant to patients with ARDS is unknown and currently under evaluation. The currently used technique of delivering exogenous surfactant is the liquid bolus instillation through the endotracheal tube. This method has been used in most animal studies as well as in neonates with RDS [52]. The advantage of this method of instillation is that it is rapid and able to deliver the large quantities of surfactant. As already mentioned, a large amount of surfactant has to be instilled in ARDS patients to overcome the inhibitory effects of the serum components present in the alveoli. This means that a relatively large amount of fluid has to be instilled into the injured lungs, which may be potentially harmful. Therefore, Lewis et al. [53] investigated surfactant administration as an aerosol. The rationale was that by this method of instillation less volume of liquid will be instilled in the lungs at one time and that the distribution will be more homogenous. Their study demonstrated that the distribution pattern was more homogenous after aerosolized surfactant administration, but it also showed that bolus instillation was superior to aerosolized surfactant in improving blood gases. They suggested that: "the low quantities of aerosolized surfactant deposited in the lungs limited the physiological responses". The same group of investigators also showed, in another study [54], that it was impossible to improve gas exchange after aerosolized surfactant in a non-uniform pattern of lung injury. They found that the less injured areas of the lung received relatively more surfactant than the severely injured areas. They conclude that: "one should be cautious in administering aerosolized surfactant to patients with ARDS who have non-uniform infiltrates on chest radiograph"; this would imply, however, that aerosolized surfactant can not be used in ARDS, since the lungs of an ARDS patient are always injured in a non-uniform way [55].

To improve the response to exogenous surfactant, it could be useful to lavage the lungs of an ARDS patient with a bronchoscope in a lobe-wise fashion, in order to lower the protein content of the alveoli, prior to surfactant administration [56]. However, lung lavages with, for example, saline also remove endogenous surfactant, which leads to a further deterioration of lung

function [57]. To avoid a deterioration of lung function by decreasing the amount of active surfactant, broncho-alveolar lavage can be performed using diluted surfactant, as was successfully shown in an animal model of ARDS [58].

Various surfactant preparations are commercially available, and have been used successfully in worldwide clinical trials in neonates with RDS [52]. The natural surfactants (derived from animal lungs) contain surfactant proteins B and C while the artificial surfactant preparations are protein free. Several animal and clinical studies have shown that the protein-containing surfactants are more effective in improving lung function [59,60]. Also, various studies have demonstrated that surfactant proteins reduce the surfactant inactivation that may be caused by plasma constituents, which is of special importance in ARDS [61,62].

Application of exogenous surfactant in adult patients obviously requires a larger amount of surfactant than in neonates. If the same dose is used, 7-10 g surfactant would be required to treat one adult patient. At current prices, the cost of this treatment would be 30,000-50,000 euro per patient. Thus, the price of surfactant has to be lowered before exogenous surfactant therapy for adult patients can be applied on a larger scale.

The open lung concept

The open lung concept (OLC) is a ventilation strategy that aims to maintain lung aeration during the entire respiratory cycle while preventing ventilation-induced injury to the surfactant system. The physiologic rationale for the open lung concept is found in the LaPlace law ($P=2\gamma/r$, P=pressure to stabilize an alveolus; γ=surface tension at the air-liquid interface in the alveolus; r=radius of the alveolus). From this law it follows that the pressure needed for alveolar expansion increases in conditions associated with a decreased surfactant function as, for example, in ARDS. Experimental studies on surfactant dysfunction show the behaviour of the alveoli to be quantal; that is, they are either open or closed [63]. A critial opening pressure has to be reached until previously collapsed alveoli can be opened (Fig. 3). Once they are opened, they remain open until the pressure drops below a critical level, then immediate collapse follows. Reopening requires the high opening pressure again. Any state between open and closed is unstable and impossible to maintain.

The pressure needed to induce volume changes in an alveolus depends on the initial radius. In other words, to get a certain volume change in larger alveoli, the change in pressure required is much smaller than in alveoli which are collapsed or have a lower volume. It can further be derived from the law of LaPlace that the pressure necessary to keep the alveoli expanded is smaller at a high FRC, since the FRC directly correlates to the amount of open lung units and to their size. Therefore, the PEEP necessary to stabilize the end-expiratory volume can be minimized if the lungs are once totally opened to the FRC of a healthy lung.

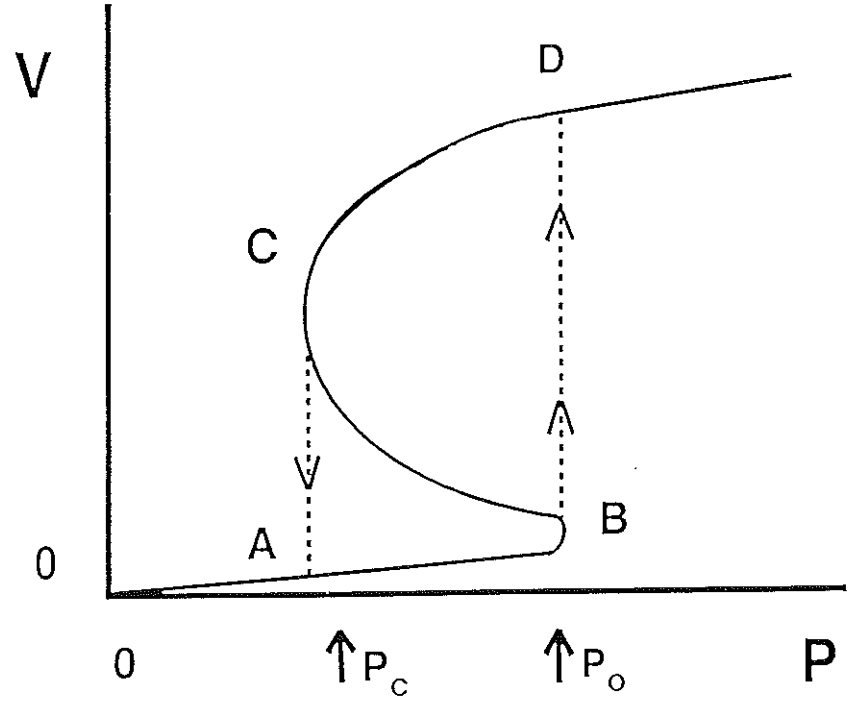

Figure 3. Schematic drawing of a pressure-volume (P/V) curve showing the proposed mechanism of alveolar recruitment and collapse. With the increase of pressure during inspiration the corresponding change in volume is given by the P-V curve (solid line). At A the alveolus is still collapsed. It is not 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 when the alveolus collapses again.

In a clinical study Kesecioglu et al. investigated the effects of pressure-controlled ventilation at different inspiratory/expiratory (I:E) ratios in ARDS patients [8]. They applied an initial peak inspiratory pressure (PIP) of 50 cmH_2O for 10 minutes to reaerate atelectatic lung regions (that is, to open up the lung), and prevented them from end-expiratory collapse by applying a high enough PEEP (that is, they kept the lung open). With this open lung strategy, significant increases in PaO_2 were obtained with increased I:E ratios. In addition, they showed that when the ventilation mode was changed from volume to pressure controlled with an I:E ratio of 4:1, PIP and intrapulmonary pressure amplitude were markedly decreased. Effects on patient outcome were not investigated in this study.

The increase in oxygenation obtained with application of an open lung strategy in patients with severe lung injury was confirmed in a study by Amato et al. [64]. In that study, a ventilation strategy was used that combined permissive hypercapnia, PEEP levels above the lower inflection point of the static pressure-volume curve, and a low tidal volume, together with limited peak inspiratory pressures. Furthermore, the application of this approach resulted in a higher rate of weaning, a faster normalisation of lung function and a trend towards a higher survival. This randomized study was the first one to demonstrate the beneficial effect and the feasibility of opening up previously collapsed alveoli and keeping them open during the whole period of artificial ventilation. In their study, Amato et al. found the effect on oxygenation of the change in ventilation mode to be immediate though not optimal; the improvement in pulmonary compliance was slow and occurred during the days following the change in ventilation strategy. Recently, Amato and colleagues showed that the use of an open lung strategy in ARDS patients results in an improved survival and a lower rate of barotrauma [65].

Partial liquid ventilation

Another rational treatment to compensate for the high retractive forces in an ARDS lung, in which the increased surface tension at the air-liquid interface in the alveoli leads to alveolar collapse, is based on the elimination of the air-liquid interface by filling of the alveoli with fluid. Filling of the lung with fluid thereby results in a marked reduction in the pressure that is required to expand the lungs, as discussed earlier (see Fig. 1). A group of liquids suitable to maintain gas

exchange when they are instilled into the lungs are perfluorocarbons (PFCs). Once the lungs are filled up to the level of FRC, gas ventilation is superimposed on these fluid-filled lungs; this ventilation strategy is called partial liquid ventilation (PLV).

Perfluorocarbons are a group of odorless, colorless, clear liquids that are insoluble in aqueous media. PFCs have a high solubility of both oxygen and carbon dioxide, and a low but constant surface tension. These compounds are very stable and biologically inert, and do not seem to be metabolized by biological systems. When PFCs are administered to the lungs, systemic absorption and distribution of small amounts of PFCs to other tissues has been demonstrated; however, the main elimination of PFCs is through evaporation via the lungs. This elimination is dependent on the vapour pressure of the specific PFC used [66].

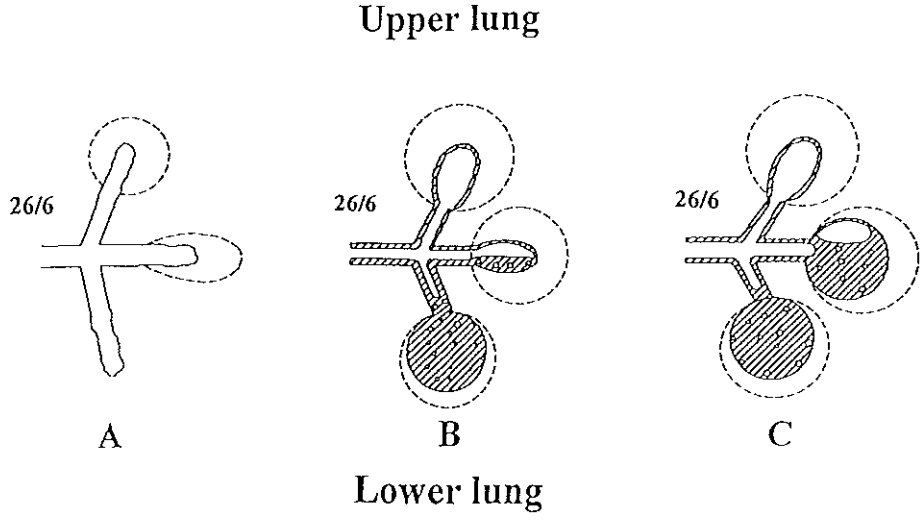

Figure 4. Panel A shows an atelectatic surfactant deficient alveoli at end-inspiration (dashed line) and end-expiration (solid line). Panel B shows what happens after administration of PFC into the lung. Due to its evaporation, a thin film of PFC is formed at the air-liquid interface and due to its low surface tension, pulmonary compliance is improved. This occurs at a low dose of PFC and does not improve at higher doses of PFC. Some dependent alveoli are prevented from end-expiratory collapse by the non-compressable PFC, which improves oxygenation. Panel C shows what happens if more PFC is instilled into the lungs: more alveoli are recruted at end-expiration. Therefore, there is a dose-dependent improvement in oxygenation with PFC during partial liquid ventilation.

The hypothetical mechanism of PLV is explained in Fig. 4. Panel A shows the atelectatic ARDS lung. After administration of a small dose of PFC (3 ml/kg) a thin film of PFC with a low surface tension is formed at the air-liquid interface. Due to evaporation of PFC, this film reaches the whole lung, including the alveoli that do not have direct contact with the instilled PFC (panel B). Due to this PFC film the increased surface tension at the air-liquid interface of the surfactant-deficient lung is decreased. This leads to a reduction in inflation pressures needed to expand the lung, and this pressure cannot be decreased further by administering more PFC. The dose-dependent improvement in oxygenation is attributed to the opening and filling of collapsed alveoli in the dependent lung parts with the non-compressable PFC, thereby preventing them from end-expiratory collapse and thus allowing gas exchange to continue during the entire respiratory cycle (panel C). Administration of more PFC leads to filling of more alveoli, thereby reducing intrapulmonary shunt. This mechanism is supported by results from Quintel et al., who showed that during PLV, PFC is distributed predominantly to the lower lung regions, whereas gas exchange takes place in the upper lung regions [67]. In the normal lung, without PFCs, surfactant is capable of reducing the surface tension at the alveolar air-liquid interface in relation to changes in alveolar radius, thus providing alveolar stability at any alveolar volume, even at very low lung volumes. PFCs do not display this characteristic, since they have a constant surface tension. When surfactant-deficient alveoli are only covered with a PFC film at the air-liquid interface (e.g. in the nondependent area of the lung), end-expiratory collapse can occur in these alveoli during exhalation if the applied PEEP is not high enough to counterbalance the increased retractive forces.

Besides preventing non-fluid-filled alveoli from end-expiratory collapse, a certain level of PEEP is also necessary to prevent bulk movement of the fluid into the airways before instituting the next inspiration [68]. This fluid movement would result in high peak pressures inside the airways during PLV, at volume-constant ventilation. PEEP prevents this movement by pushing the fluid distally, keeping it within the alveoli and thus preventing it from filling the airways during each subsequent expiratory phase.

Partial liquid ventilation is currently being investigated in clinical trials in neonates and adults with RDS. Leach et al. reported a pilot safety and efficacy study in thirteen premature newborns with RDS who failed to respond to conventional therapy and exogenous surfactant

treatment [69]. Instillation of PFC resulted in an increase in PaO_2 and dynamic compliance. There were no serious adverse side effects associated with PLV. Ten infants completed the study, eight of them survived.

Hirschl et al. reported a study in 19 adults, children, and neonates in respiratory failure who were treated with PLV [70]. For safety reasons, gas exchange was supported by extra corporeal life support (ECLS). The primary outcome measure was the alveolar-arterial oxygen difference [(A-a)Do_2] during 10-minute periods of disconnection of ECLS before and during PLV. The (A-a)Do_2 decreased during disconnection of ECLS, and static pulmonary compliance increased over the initial three days of PLV. All patients tolerated PFC administration without hemodynamic compromise. During PLV, six patients had reaccumulation of previous ipsilateral pneumothoraces and new pneumothoraces occurred in three patients. Of the 19 patients, 14 were successfully weaned from ECLS and 11 survivors were well and without evidence of pulmonary or adverse systemic effects from the administration of PFC after 2-12 months of follow-up.

Comparable results were described in other reports from similar studies [71-75]. Repeated bronchoscopy of the PFC-filled lungs did not show any visible evidence of adverse effects of PFC in the airways. In some patients, extravasation of PFC into the pleural space occurred, but radiographs showed that the PFCs slowly resolved without evident injurious effects. A disadvantage of partial liquid ventilation is that PFC may interfere with interpretation of chest radiographs, because the radio-opaque liquid can obscure the position of intravascular catheters and ECLS cannulas in the fluid-filled regions, as well as making routine interpretation of chest radiographs difficult, especially in patients with lung infections [73, 74]. It was also shown that it is necessary to remove pulmonary secretions and exudate, which are immiscible and, therefore, float on the surface of the PFC. They can be removed with suction to avoid blocking the airways. The clinically relevant complications that could be related to PLV described in all studies were pneumothoraces. To date, partial liquid ventilation has not improved survival in these patients; however, these studies were not designed to detect differences in mortality.

Summary

In this chapter a description is given of the central role of surfactant in the pathophysiology of

ARDS. The physiologic rationale for exogenous surfactant therapy, mechanical ventilation according to the open lung concept, and partial liquid ventilation, are presented. These treatment strategies all showed encouraging results in animal studies, and are now undergoing clinical evaluation.

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Chapter 2

At surfactant deficiency application of the "open lung concept" prevents protein leakage and attenuates changes in lung mechanics

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Summary

Objective: To evaluate whether mechanical ventilation using the "open lung concept" during surfactant depletion can attenuate the deterioration in pulmonary function.

Design: Experimental, comparative study.

Setting: Research laboratory of a large university.

Subjects: Eighteen adult male Sprague-Dawley rats, weighing 280-340 g.

Interventions: Twelve rats were anesthetized, mechanically ventilated with 100% oxygen, and randomly divided into two groups (n=6 each). The open lung group underwent six saline lavages at different ventilator settings that prevented alveolar collapse. The settings (expressed as frequency/peak inspiratory pressure/positive end-expiratory pressure/inspiratory:expiratory ratio) were 30/26/6/1:2 during the first lavage, 100/27/10/1:1 during the next two lavages and 100/33/15/1:1 during the last three lavages and during the remaining ventilation period. The ventilated control group underwent six saline lavages with settings at 30/26/6/1:2. After the lavages, peak inspiratory pressure and positive end-expiratory pressure were increased in this group by 2 cm H_2O each for the remaining study period. An additional group of six animals were killed immediately after induction of anesthesia and served as healthy controls. Blood gases were measured before lavage, immediately after the last lavage and thereafter hourly. At the end of the 4-hr study period, we constructed pressure-volume curves from which we determined total lung capacity at a distending pressure of 35 cm H_2O (TLC_{35}). Subsequently, total lung volume at a distending pressure of 5 cm H_2O (V_5) was determined, followed by broncho-alveolar lavage.

Results: In the ventilated control group, PaO_2, V_5 and TLC_{35} were significantly decreased, and protein concentration of broncho-alveolar lavage was significantly increased compared with the healthy control group. In the open lung group, PaO_2 did not decrease after the lavage procedure, and V_5, TLC_{35} and protein concentration of broncho-alveolar lavage were comparable with the healthy controls.

Conclusion: We conclude that application of the open lung concept during surfactant depletion attenuates deterioration in pulmonary function.

Introduction

In acute respiratory distress syndrome (ARDS), dysfunction of the pulmonary surfactant system leads to hypoxemia, decreased functional residual capacity (FRC) and decreased compliance [1]. Despite extensive research the mortality rate of ARDS remains > 50% [2]. New strategies that are currently under clinical investigation include 'open lung' ventilation strategies, which aim to reopen collapsed but recruitable lung units and to keep them open by applying a sufficiently high positive end-expiratory pressure [3-6]. Recent studies show that such strategy improves oxygenation in ARDS patients, and is associated with a decrease in morbidity and mortality [7-9].

Ventilation strategies that prevent repeated alveolar collapse are thought to prevent further damage to the pulmonary surfactant system and progression of lung damage [10-13]. Because surfactant abnormalities are known to be present in patients who are at-risk for ARDS, prophylactic use of such a ventilation strategy might prevent or attenuate the decrease in pulmonary function by protecting the surfactant system [14]. A previous study, in which an open lung concept utilizing an inspiration time of 80% was applied in an animal model during repeated lung lavage, showed that this strategy resulted in better gas exchange, hemodynamics, and oxygen transport and less lung injury [5]; however, lung mechanics, composition, and function of the surfactant system were not assessed.

We hypothesize that when the lungs are kept open in an early stage of lung injury, surfactant function is better preserved resulting in less deterioration of pulmonary function. Therefore, in this study, we applied a ventilation strategy with a high positive end-expiratory pressure and a high frequency during repeated lung lavages, to evaluate whether severity of respiratory distress can be influenced by maintaining a better residual surfactant function, compared with conventional mechanical ventilation.

Materials and methods

The study protocol was approved by the University's Animal Experimental Committee, and the principles of laboratory animal care (National Institutes of Health publication 86-23, revised 1985) were followed.

The study was performed in 18 adult male Sprague-Dawley rats (body weight 280-340

g). After induction of anesthesia with 2% enflurane and 65% nitrous oxide in oxygen, we inserted a polyethylene catheter into a carotid artery to draw arterial blood samples. Before tracheostomy, the animals received 60 mg/kg (i.p.), of pentobarbital sodium (Nembutal, Algin BV, Maassluis, the Netherlands). After tracheostomy, muscle relaxation was induced by 1 mg/kg (i.m.) pancuronium bromide 1 mg/kg (Pavulon, Organon Teknika, Boxtel, the Netherlands) immediately followed by connection to a ventilator. The animals were mechanically ventilated with a Servo Ventilator 300 (Siemens-Elema, Solna, Sweden) in a pressure constant time-cycled mode, at an Fio_2 of 1.0, frequency of 30 breaths/minute, peak inspiratory pressure set at 12 cm H_2O, positive end-expiratory pressure set at 2 cm H_2O, and inspiratory/expiratory ratio of 1:2. Anesthesia was maintained with 40 mg/kg/h (i.p) pentobarbital sodium; muscle relaxation was maintained with 1 mg/kg/h (i.m.) pancuronium bromide. Body temperature was kept within the normal range with a heating pad. Immediately after induction of anesthesia, six animals were killed and served as healthy, non-ventilated controls. The remaining animals subsequently underwent six whole lung lavages with warm saline (37°C), according to Lachmann et al. [15]. During lavage, different ventilator settings were used in both groups, which are shown in Table 1. After lavage, peak inspiratory pressure and positive end-expiratory pressure were increased in the ventilated control group to prevent critical hypoxia and remained unchanged in the open lung group, after which both groups were ventilated for 4 hrs (Table 1). The recovered volume of lavage fluid was recorded, and a phosphorus analysis was performed on the lavage fluid to quantify the amount of surfactant phospholipids washed out during lavage.

Arterial blood gas samples were taken before lavage, after lavage, and hourly for 4 hrs. The samples were analysed for PaO_2 and $PaCO_2$ on a blood gas analyser (ABL 505, Radiometer, Copenhagen, Denmark).

After the animals were killed with an overdose of pentobarbital, we recorded static pressure-volume curves by using the syringe technique. After the thorax and diaphragm were opened, the tracheostomy catheter was connected to a pressure transducer with a syringe attached to it (model DP 45-32, Validyne Engineering, Northridge, CA, USA), and pressures were recorded on a polygraph (model 7B, Grass Instrument, Quincy, MA., USA). First, the lungs were inflated quickly with 100% nitrogen (N_2) from the syringe to an airway pressure of 35 cmH_2O, which was maintained for 5 secs, followed by deflation to an airway pressure of 0 cmH_2O. Then, the lungs

Table 1. Ventilator settings in the two ventilated groups.

	Ventilated control group				Open lung group			
	LAV 1	LAV 2-3	LAV 4-6	>LAV6	LAV 1	LAV 2-3	LAV 4-6	>LAV6
PIP (cmH_2O)	26	26	26	28	26	27	33	33
PEEP (cmH_2O)	6	6	6	8	6	10	15	15
Frequency	30	30	30	30	30	100	100	100
I/E	1/2	1/2	1/2	1/2	1/2	1/1	1/1	1/1

LAV, lung lavage; PIP, positive inspiratory pressure; PEEP, positive end-expiratory pressure; I/E, inspiratory/expiratory ratio.

were re-inflated with N_2 from the syringe in steps of 0.5 ml until an airway pressure of 35 cmH_2O was reached. For each step, the bolus of N_2 was administered quickly, and was followed by a 5-sec pause to allow pressure equilibration. After this, the lungs were deflated likewise, until an airway pressure of 0 cmH_2O was reached. The volume of N_2 left in the syringe was recorded. From the pressure-volume curves we determined total lung capacity (TLC_{35}), which was defined as the lung volume above the collapsed volume at a distending pressure of 35 cmH_2O, and maximal compliance (C_{max}), which was calculated from the steepest part of the deflation limb.

After construction of the pressure-volume curves, the lungs were removed *en bloc* and weighted, and lung volume at an airway pressure of 5 cmH_2O (V_5) was determined by fluid displacement. We chose a positive pressure of 5 cmH_2O to compensate for the loss of transpulmonary pressure in the open chest [16]. The total lung volume at this distending pressure was considered close to FRC.

After we assessed lung mechanics, the lungs were lavaged with 1.5 mmol/L saline-$CaCl_2$. The active surfactant component in the bronchoalveolar lavage fluid was separated from the nonactive surfactant component by differential centrifugation followed by subsequent phosphorus analysis, and the ratio between nonactive and active components (small aggregate to large aggregate ratio or SA/LA ratio) was calculated, as previously described by Veldhuizen and colleagues [17]. We determined the protein concentration of the bronchoalveolar lavage

fluid using the Bradford method (Bio-Rad protein assay, Munich, Germany) [18].

We performed statistical analysis by using the Instat statistical package (GraphPad software, San Diego, CA, USA). For blood gases, we analyzed intergroup comparisons by using the alternate (Welch) Student's *t*-test, and analyzed intragroup comparisons by using repeated-measures analysis of variance (ANOVA). All other data were analysed with ANOVA. If ANOVA resulted in a $p < 0.05$, a Tukey-Kramer posttest was performed. All data are reported as mean ± SD and $p < 0.05$ was considered statistically significant.

Results

In the lavage fluid from the six lavages used to induce lung injury, there were no significant differences in fluid volume recovered (83% ± 0.7% vs. 83% ± 3%) and total amount of phosphorus between the ventilated control group and open lung group (5.58 ± 0.8 μmol vs. 5.28 ± 1.38 μmol), respectively.

Blood gas values before lavage were comparable for both ventilated groups (Table 2). After six lavages, PaO_2 decreased to 102 ± 118 torr [13.6 ± 15.8 kPa] in the ventilated control group, whereas in the open lung group PaO_2 remained >500 torr [67 kPa] ($p<0.001$)(Table 2). In both ventilated groups, PaO_2 did not change during the remaining study period. $PaCO_2$ increased to >60 torr [8 kPa] ($P<0.001$) in the ventilated control group, whereas it remained in the normal range (35-45 torr [4.7-6 kPa]) in the open lung group (Table 2). During the 4-hr ventilation period, none of the animals died.

The pressure-volume curves are shown in Figure 1. TLC_{35} was decreased in the ventilated control group compared with the healthy controls ($p<0.01$), but in the open lung group TLC_{35} was preserved. C_{max} was decreased in the ventilated control group compared with the open lung group (5.4 ± 1.0 mL/cm H_2O/kg vs. 8.6 ± 2.8 mL/cm H_2O/kg; $p<0.05$), but in both ventilated groups C_{max} was decreased compared with the healthy control group (13.4 ± 1.1 mL/cm H_2O/kg, $p< 0.001$ vs. ventilated control, $p<0.05$ vs open lung group). There was no difference between both ventilated groups in total lung volume at a distending pressure of 5 cmH_2O (V_5); however, only in the ventilated control group V_5 was lower than in the healthy control group ($p< 0.001$) (Table. 3).

Table 2. PaO_2 and $PaCO_2$ in the two ventilated groups

	PaO_2		$PaCO_2$	
	Ventilated control	Open lung	Ventilated control	Open lung
Before lavage	585.4 ± 36.2	597.4 ± 38.0	43.6 ± 6.7	42.1 ± 5.1
After lavage	102.3 ± 118.2[§¶]	621.3 ± 31.7	64.8 ± 16.4[§¶]	33.2 ± 8.0[¶]
1 hr	109.4 ± 120.5[§¶]	599.3 ± 36.7	62.7 ± 19.2[§¶]	40.0 ± 8.9
2 hrs	104.8 ± 125.5[§¶]	602.9 ± 30.7	65.5 ± 20.7[§¶]	43.9 ± 11.1
3 hrs	100.5 ± 112.0[§¶]	598.2 ± 37.3	66.9 ± 22.0[§¶]	44.1 ± 11.6
4 hrs	100.8 ± 114.7[§¶]	600.5 ± 41.6	68.8 ± 24.6[§¶]	44.0 ± 9.8

Values are mean ± SD, expressed in torr. To convert torr to kPa, multiply the value by 0.1333

[§] $p< 0.05$ vs. open lung group; [¶] $p< 0.05$ vs. before lavage.

Table 3. V_5 and lung weight.

	Healthy control group	Ventilated control group	Open lung group
V_5 (mL/kg)	17.0 ± 3.7	7.4 ± 3.5*	12.1 ± 3.4
Lung weight (g)	1.9± 0.2	4.5 ± 0.3*	4.5 ± 0.3*

Values are mean ± SD. V_5, total lung volume at a distending pressure of 5 cmH_2O. * $p<0.05$ vs. healthy control group.

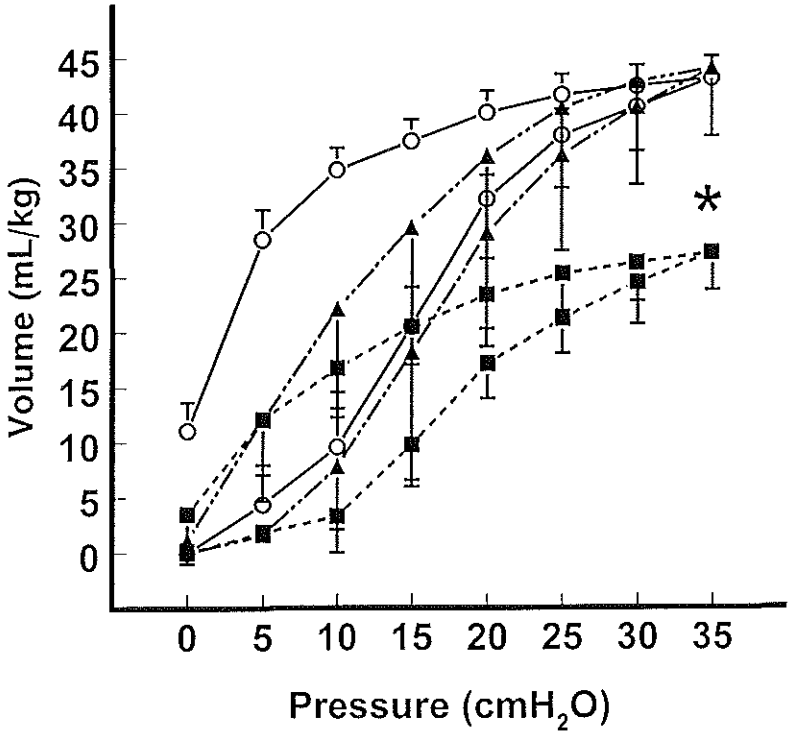

Figure 1. Pressure-volume curves, mean ± SD. Volume (mL/kg) is lung volume above functional residual capacity.*$p< 0.01$ vs. healthy control group. Maximal compliance C_{max} was decreased in the ventilated control group compared with the open lung group (5.4 ± 1.0 vs. 8.6 ± 2.8 mL/cm H_2O/kg; $p< 0.05$), but in both ventilated groups C_{max} was significantly decreased compared to the healthy control group (13.4 ± 1.1 mL/cm H_2O/kg, $p<0.001$ vs ventilated control, $p<0.05$ vs open lung group). *Filled squares,* ventilated control group; *filled triangles,* open lung group; *open circles,* healthy controls.

The concentration of protein in the bronchoalveolar lavage fluid was increased in the ventilated control group ($p< 0.001$), whereas it was not increased in the open lung group (Fig. 2). The total amount of phosphorus in the bronchoalveolar lavage fluid, which we measured to quantify the phospholipid-containing surfactant system, was obviously decreased in both lavaged groups, but there were no differences between these groups (Table 4). The ratio between nonactive and active surfactant components (SA/LA ratio) was significantly increased in both ventilated groups compared with the healthy control group ($p< 0.001$) (Table 4).

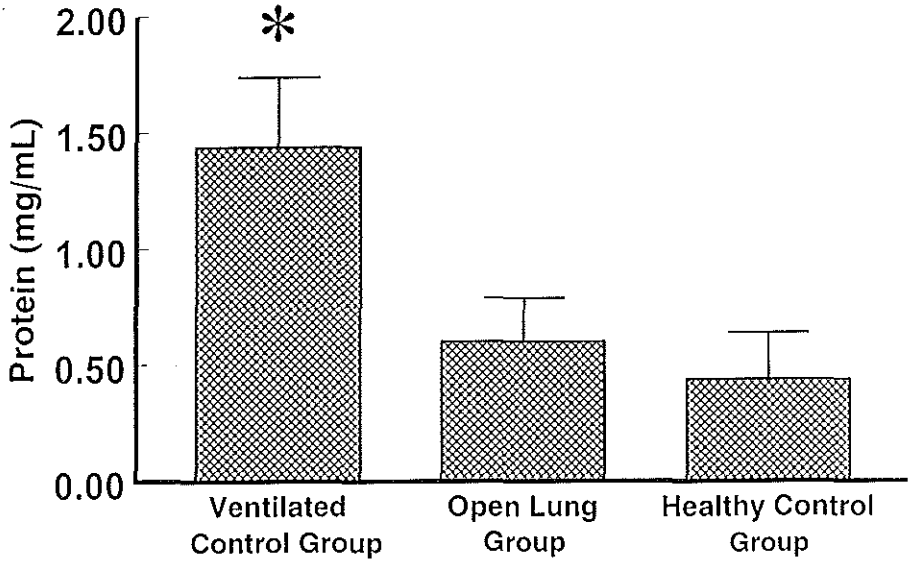

Figure 2. Protein concentration (mean ± SD) (mg/mL) of the bronchoalveolar lavage fluid of the three study groups. * $p < .05$ vs. healthy control group.

Table 4. Pulmonary surfactant data: Total phosphorus, and small aggregate to large aggregate ratio (SA/LA ratio) in the three study groups.

	Healthy control group	Ventilated control group	Open lung group
Total phosphorus (μmol/mL)	0.14 ± 0.06	0.07 ± 0.03*	0.06 ± 0.01*
SA/LA ratio	0.3 ± 0.1	1.6 ± 0.6 *	1.5 ± 0.5 *

Values are mean ± SD. *$p<0.05$ vs. Healthy control group

Discussion

In this study, we defined the lungs to be open when PaO_2/FiO_2 was above 500 torr [67 kPa], indicating that there is no intrapulmonary shunting. We previously showed that surfactant-deficient lungs can be opened and kept open when the open lung concept that applies a high positive end-expiratory pressure and high frequency is used [5, 6, 19]. Therefore, we used these same settings in the present study to preserve oxygenation during surfactant depletion.

In the open lung group, gas exchange and total lung capacity were preserved, and protein leakage into the alveoli was prevented, compared with the healthy control group. In the ventilated control group, however, all these variables deteriorated. Compared with healthy controls, lung volume at a distending pressure of 5 cm H_2O (V_5), which we took to be FRC, was significantly decreased only in the ventilated control group, although we found no significant differences in V_5 between the open lung and ventilated control groups.

An important determinant of protein transport across the alveolar-capillairy membrane is integrity of the alveolar epithelium. Repeated alveolar collapse has been shown to compromise the integrity of the alveolar epithelium because of the occurrence of shear forces [20, 21]. Application of the open lung concept in an animal model after lung lavage previously was demonstrated to decrease protein leakage [19; for review, see Ref. 21]. In addition, application of positive end-expiratory pressure has been shown to favor the shift of fluid from the alveoli to the interstitium by decreasing the pressure gradient across the alveolar-capillary membrane [22]. This may explain the decrease in protein leakage that was found in the open lung group, in which end-expiratory alveolar collapse was prevented by application of a positive end-expiratory pressure of 15 cmH_2O.

We found no differences in surfactant quantity and quality (SA/LA ratio) between the open lung group and the ventilated control group (Table 4). It has been demonstrated that the alveolar area cycling, which depends on the pressure difference between inspiration and expiration, is responsible for converting active LA into nonactive SA [17]. The difference in pressure amplitude was comparable between both ventilated groups, which may explain the absence of any difference in SA/LA ratio between both ventilated groups. However, we found significant differences between both ventilated groups in lung mechanics (TLC_{35} and maximal compliance). We attribute this discrepacy between lung mechanics and surfactant parameters to

the difference in protein leakage, because it has been established that plasma proteins inhibit surfactant function in a dose-dependent way [23]. Therefore, we conclude that prevention of protein leakage during and after surfactant depletion is important to protect the remaining surfactant function.

Recruitment of collapsed alveoli requires inspiratory airway pressures that overcome the critical opening pressure of these alveoli, which implies brief application of a high inspiratory airway pressure. However, airway pressures can be decreased once the lungs have been opened, as has been pointed out previously, with reference to the law of LaPlace [3]. From this law ($P=2\gamma/r$, where P=alveolar pressure; γ=surface tension at the alveolar air-liquid interface; and r=alveolar radius) it follows that the pressure necessary to keep alveoli open and the pressure difference to induce volume changes in the alveoli are smaller at a high FRC level (i.e. larger alveolar radius). Therefore, when the lungs are opened, gas exchange can be maintained with a lower positive end-expiratory pressure and smaller pressure difference, and hence, a lower peak inspiratory pressure, than before alveolar recruitment. If positive end-expiratory pressure is kept above the critical closing pressure, alveolar collapse will not occur and reperated application of higher peak inspiratory pressure will not be necessary.

Although direct translation to the clinical situation is difficult, our results are in contrast to those of a clinical study by Pepe et al. [24], who found that early application of positive end-expiratory pressure in high-risk patients did not affect the incidence of ARDS. However, those authors used a positive end-expiratory pressure of 8 cmH_2O, and removed the positive end-expiratory pressure for 8 mins when taking blood samples, thus allowing alveolar collapse. In a more recent study, Steward et al. [25] evaluated the use of a pressure- and volume limited ventilation strategy in patients at high risk for ARDS. Steward et al. [25], who reported no reduction in mortality and possibly an increase in morbidity, also used an average positive end-expiratory pressure of <10 cmH_2O. We hypothesize that the positive end-expiratory pressure levels used in these latter studies were not high enough to prevent alveolar collapse, which may have increased protein leakage. This hypothesis is supported by results from Gattinoni et al. [26], who in a computed tomography study of ventilated ARDS patients, showed that reinflated lung tissue could only be kept open at end-expiration at positive end-expiratory pressure levels of 15 cmH_2O and higher. This might explain the results by Kirby et al. [27] and Douglas and Downs

[28], who showed an improvement in pulmonary function in patients with acute respiratory failure after application of a PEEP in excess of 25 cmH_2O, and more recently by Amato et al. [9], who demonstrated that application of an open lung approach in ARDS patients resulted in improved survival and reduction of barotrauma.

We conclude that the application of the open lung concept during surfactant depletion prevents a decrease in gas exchange, attenuates the deterioration in lung mechanics, and prevents an increase in protein leakage. The prevention of protein leakage is of special importance in surfactant-deficient lungs, because the low amount of remaining surfactant makes it more vulnerable to inhibition of its function. We speculate that the prevention of end-expiratory collapse during mechanical ventilation in the early phase of acute respiratory failure may decrease morbidity and mortality in patients.

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Chapter 3

Comparison of exogenous surfactant therapy, mechanical ventilation with a high end-expiratory pressure and partial liquid ventilation in an animal model of acute lung injury

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Summary

We have compared three treatment strategies, that aim to prevent repetitive alveolar collapse, for their effect on gas exchange, lung mechanics, lung injury, protein transfer into the alveoli and surfactant system, in a model of acute lung injury. In adult rats, the lungs were ventilated mechanically with 100% oxygen and a PEEP of 6 cm H_2O, and acute lung injury was induced by repeated lung lavage to obtain a PaO_2 <13 kPa. Animals were then allocated randomly (n=12 in each group) to receive exogenous surfactant therapy, or ventilation with high PEEP (18 cmH_2O), partial liquid ventilation, or ventilation with low PEEP (8 cmH_2O) (ventilated controls). Blood-gas values were measured hourly. At the end of the 4 h study, in six animals per group pressure-volume curves were constructed and broncho-alveolar lavage (BAL) was performed, whereas in the remaining animals lung injury was assessed. In the ventilated control group, arterial oxygenation did not improve and protein concentration of BAL and conversion of active into non-active surfactant components increased significantly. In the 3 treatment groups, PaO_2 increased rapidly to >50 kPa and remained stable over the next 4 h. The protein concentration of the BAL fluid increased significantly only in the partial liquid ventilation group. Conversion of active to non-active surfactant components increased significantly in the partial liquid ventilation group and in the group ventilated with high PEEP. In the surfactant group and partial liquid ventilation group, less lung injury was found compared with the ventilated control group and the group ventilated with high PEEP. We conclude that although all three strategies improved PaO_2 to >50 kPa, their impact on alveolar protein transfer into the alveoli, surfactant system, and lung injury differs markedly.

Introduction

Acute lung injury (ALI) is a condition of acute respiratory failure, in which lack of active surfactant leads to alveolar collapse, resulting in severe hypoxia [1]. Available treatments include mechanical ventilation with high inspiratory oxygen concentration and high peak alveolar pressures with large distending tidal volumes, but these are known to induce lung damage [2]. Ventilation strategies that prevent repeated alveolar collapse are thought to prevent further progression of lung damage [3]. Therefore, new treatment strategies that aim to prevent repetitive alveolar collapse during ALI are under investigation.

These new strategies include: 1) 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 [4, 5]. 2) Partial liquid ventilation, in which ventilation is superimposed on lungs that are filled with perfluorocarbons thus preventing expiratory collapse [6, 7]. 3) Exogenous surfactant therapy, in which the lost active surfactant is replaced [8, 9].

Studies have shown that these strategies improve oxygenation while diminishing the effects on lung injury in animal models of ALI [4, 8, 10]. All three strategies are currently under investigation for clinical use, and although some of the results are promising, they have not been compared directly [11-15]. In this study, we have compared these three techniques for their efficacy on improving arterial oxygenation and lung mechanics in rats who underwent bronchoalveolar lavage, and assessed their impact on transfer of protein into the alveoli, the surfactant system and on lung injury.

Methods

The study was approved by the University's Animal Experimental Committee, and the care and handling of the animals conformed with European Community guidelines (86/609/EC). The study was performed in 60 adult male Sprague-Dawley rats (body weight 270-330 g). After induction of anaesthesia with 2% enflurane and 65 % nitrous oxide in oxygen, a polyethylene catheter was inserted into a carotid artery for drawing arterial blood samples. Before tracheostomy, the animals received pentobarbital (pentobarbitone) 60 mg/kg (i.p.) (Nembutal,

Algin BV, Maassluis, the Netherlands). After tracheostomy, neuromuscular block was produced with pancuronium 1 mg/kg (i.m.) (Pavulon, Organon Teknika, Boxtel, the Netherlands) followed immediately by connection to a ventilator. The animals underwent mechanically ventilated with a Servo Ventilator 300 (Siemens-Elema, Solna, Sweden) in a pressure constant time-cycled mode, at an inspired oxygen concentration (FiO_2) of 1.0, frequency of 30 bpm, peak inspiratory pressure (PIP) of 12 cm H_2O, positive end-expiratory pressure (PEEP) of 2 cm H_2O, and inspiratory/expiratory (I/E) ratio of 1:2. Anaesthesia was maintained with pentobarbital 40 mg/kg/h (i.p.) and neuromuscular block was maintained with pancuronium 1 mg/kg/h (i.m.) Body temperature was maintained within normal range using heating pad. Immediately after induction of anaesthesia 12 animals were killed and served as healthy controls.

Acute lung injury was induced by repeated broncho-alveolar lavage (BAL) (32 ml/kg) with warm saline (37°C), according to Lachmann and colleagues [16]. BAL was repeated as often as necessary to produce a PaO_2<13 kPa at a PIP and PEEP of 26 and 6 cm H_2O, respectively. Within 10 min of the last lavage, the animals were allocated randomly to one of the following groups (n=12 each). In the first group, the lungs were opened by increasing PIP to 40 cm H_2O and PEEP to 20 cm H_2O, and the I/E ratio was set at 1:1. After 2 to 3 min, PIP was decreased to 35 cm H_2O and PEEP to 18 cm H_2O, and arterial blood-gas values were obtained. Ventilator setting remained unchanged for the rest of the study. The second group received an intra-tracheal bolus dose of perfluorocarbon 15 ml/kg (APF-175A; Perfluoro-dimethyldecalin, Fluoro-Seal Inc, Round Rock, USA) after disconnection from the ventilator. APF-175A is a perfluorocarbon with a density of 1.98 g/ml, a vapour pressure of 0.09 kPa, a surface tension of 20.5 dynes/cm and an oxygen solubility of 35 ml O_2 per 100 ml perfluorocarbon per atmosphere of oxygen pressure (all values at 25°C). During the study, evaporational losses of perfluorocarbon were compensated for by administering substitution doses. The substitution doses were based on our previous experience with this model, and aimed at maintaining PaO_2 constant during the rest of the study. The third group received exogenous surfactant at a dose of 120 mg/kg. The surfactant used was isolated from minced pig lungs, prepared as described previously [17]. The freeze-dried material was suspended in warm saline to a concentration of 40 mg/ml, and administered intra-tracheally, after disconnection 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 followed immediately by re-connection to the ventilator. In the fourth group, ventilator pressures were increased by 2 cmH_2O (PIP/PEEP of 28/8 cm H_2O) to prevent critical hypoxia and remained unchanged throughout study. This group served as ventilated controls.

Arterial blood-gas samples were obtained before lavage, after lavage, and hourly for 4 h. Samples were analysed for arterial oxygen tension (PaO_2) and arterial carbon dioxide tension ($PaCO_2$) using an electrochemical blood-gas analyser (ABL 505, Radiometer, Copenhagen, Denmark).

At the end of the experiment, the animals were killed by an overdose of pentobarbital. Six animals from each group were selected randomly for histopathologic examination. The lungs of these animals were fixated, sectioned and stained as described previously [18]. A semi-quantitative morphometric analysis of lung injury was performed under blinded conditions by a pathologist (R.S.), who scored atelectasis, oedema, vascular wall thickening and leucocyte infiltration as none, light, moderate or severe (score 0, 1, 2 or 3, respectively). Lung injury score was defined as the average from all parameters for each group.

The remaining animals from each group were used to assess lung mechanics. Static pressure-volume curves were recorded using conventional techniques [16]. Total lung capacity (TLC_{35}) was defined as lung volume at inflation with a distending pressure of 35 cmH_2O. After pressure-volume recordings, BAL was performed five times with saline-$CaCl_2$ 1.5 mmol/litre. The active surfactant component in the BAL fluid was separated from the non-active surfactant component by differential centrifugation followed by subsequent phosphorus analysis, and the ratio between non-active and active components (small aggregate to large aggregate ratio (SA/LA) ratio) was calculated, as described previously [19]. Protein concentration of BAL fluid was determined using the Bradford method (Bio-Rad protein-assay, Munich, Germany) [20].

Statistical analysis was performed using the Instat statistical package. Inter-group comparisons were analysed with ANOVA and intra-group comparisons were analysed with repeated measures ANOVA. If ANOVA resulted in a $p < 0.05$ a Tukey-Kramer post-test was performed. All data are reported as mean ± SD and $p < 0.05$ was considered statistically significant.

Results

Blood gas values before and immediately after lavage were comparable for all groups (Fig.1, Table 1). None of the animals died during the 4 h observation period. In the ventilated control group PaO_2 did not improve whereas it increased to pre-lavage values and remained stable during the 4 h observation period in the surfactant treated group and the group ventilated with high PEEP (Fig. 1). In the partial liquid ventilation group, instillation of a bolus dose of perfluorocarbon 15 ml/kg resulted in a significant improvement of PaO_2 but pre-lavage values were not reached (Fig. 1). Perfluorocarbon needed to be substituted periodically to compensate for the evaporational loss and the substitution dose of perfluorocarbon was 1.1 ± 0.4 ml/kg/h.

$PaCO_2$ data are give in Table 1. $PaCO_2$ decreased significantly in both the surfactant treated group and the partial liquid ventilation group, and was significantly lower in the surfactant treated group compared to the other groups (Table 1).

Figure 2 shows the deflation limbs of the pressure-volume curves. At deflation less than 15 cm H_2O, lung volume in the healthy controls exceeded lung volume in all other groups, other than the group treated with surfactant. TLC_{35} was significantly decreased in the ventilated control group, but not in the three treatment groups, compared with the healthy controls.

The protein concentration of BAL fluid was significantly increased in both the partial liquid ventilated group and the ventilated control group compared with healthy control animals (Fig. 3). SA/LA ratio, the ratio between non-active and active surfactant components, was significantly increased in the ventilated control group, the group ventilated with high PEEP, and the partial liquid ventilation group, but not in the surfactant treated group (Fig. 4). Compared with healthy control animals, the total amount of phosphorus in BAL fluid, measured to quantify the phospholipid-containing surfactant system, was significantly lower in the ventilated control group, the group ventilated with high PEEP, and the partial liquid ventilation group (Table 2).

Table 1. $PaCO_2$ (mean (SD), kPa) for all treatment groups. Before lavage (Healthy), immediately after lavage (Lav) and 1, 2, 3 and 4 hours after lavage. § $p<0.05$ compared with SURF group. * $p<0.05$ compared with CONTR group. Intra-group comparisson: ¶ $p<0.05$ compared to Lav. CONTR = ventilated controls with low PEEP; H-PEEP = high PEEP; PLV = partial liquid ventilation; SURF = exogenous surfactant therapy.

	Healthy	Lav	1h	2 h	3 h	4 h
CONTR	5.8 (0.9)	9.0 (1.4)	7.5 (1.7)§ ¶	7.7 (1.7)§ ¶	7.8 (1.8)§ ¶	8.0 (2.1)§
H-PEEP	5.2 (0.6)	8.6 (1.3)	7.7 (1.2)§	6.9 (1.5)§ ¶	6.4 (1.7)§ ¶	6.8 (1.8)§ ¶
PLV	5.5 (1.1)	9.9 (1.5)	6.8 (1.6)§ ¶	6.6 (1.7)§ ¶	6.2 (1.8)§ ¶	6.1 (1.9)* ¶
SURF	5.2 (1.2)	8.5 (1.5)	4.7 (0.6)¶	4.4 (0.5)¶	4.3 (0.6)¶	4.5 (0.7)¶

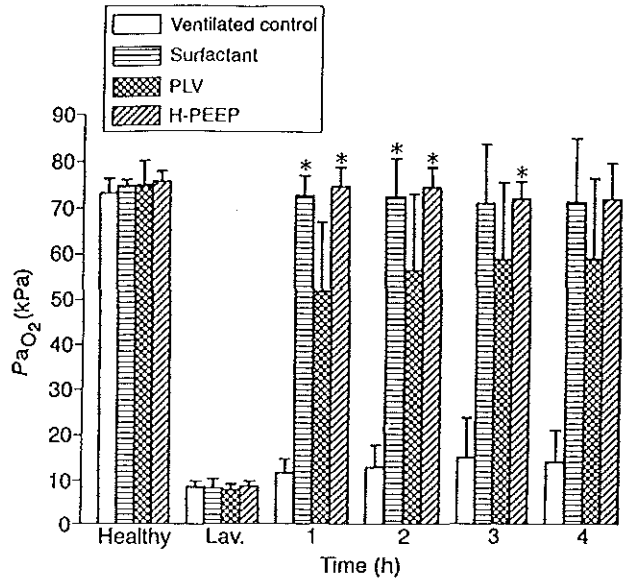

Figure 1. PaO_2 values (mean ± SD, kPa) in the ventilated control, surfactant-treated, partial liquid ventilation (PLV) and high (H) PEEP groups. * $p < 0.05$ vs. PLV group.

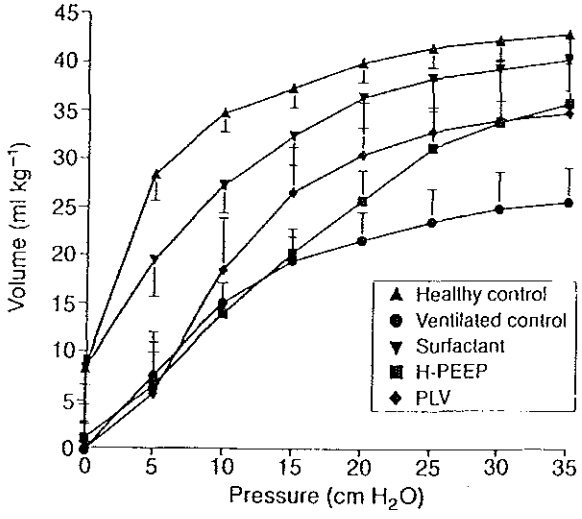

Figure 2. Deflation limbs from the pressure-volume curves, (mean ± SD) in healthy control, ventilated control, surfactant-treated, partial liquid ventilation (PLV) and high (H) PEEP groups. Volume is lung volume above FRC. At deflations less than 15 cmH_2O, lung volume in the healthy controls exceeded lung volume in all other groups, except the surfactant-treated group. TLC_{35} was decreased only in the ventilated control group compared with the healthy control group.

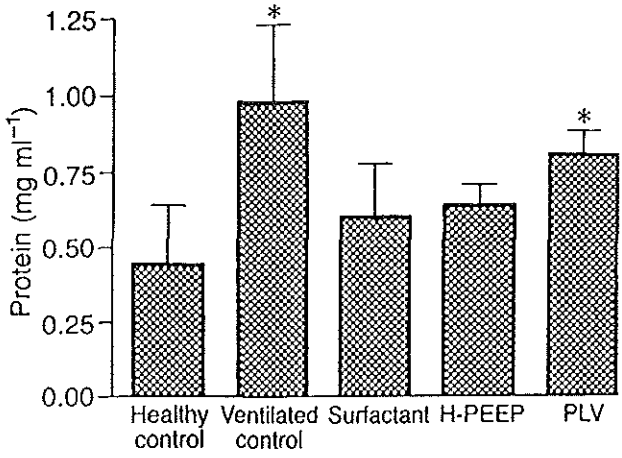

Figure 3. Protein concentration (mean ± SD, mg/ml) of BAL fluid in healthy control, ventilated control, surfactant-treated, partial liquid ventilation (PLV) and high (H) PEEP groups. * $p < 0.05$ vs. healthy control group.

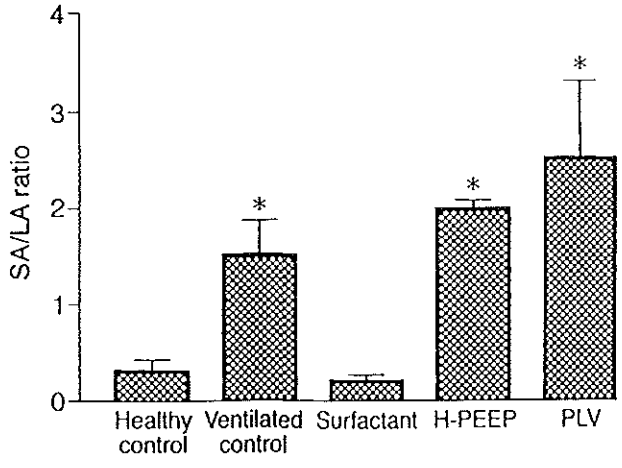

Figure 4. Ratio between non-active and active surfactant components (mean ± SD) in healthy control, ventilated control, surfactant-treated, partial liquid ventilation (PLV) and high (H) PEEP groups. (SA/LA, small to large aggregates). * $p < 0.05$ vs. healthy control group.

Semi-quantitative lung injury analysis showed that in both the surfactant and the partial liquid ventilation groups, lung injury was significantly lower than in the two groups that were ventilated only (high and low PEEP, Figure 5). However, only in the surfactant group, lung injury was not significantly increased compared with the healthy controls.

Table 2. Total phosphorus recovered from bronchoalveolar lavage fluid (mean ± SD, μmol/ml). § $p<0.05$ vs. healthy controls (Healthy); ¶$p<0.05$ vs. all other groups. Control = ventilated controls with low PEEP; H-PEEP = high PEEP; PLV = partial liquid ventilation; surfactant = exogenous surfactant therapy.

	Total phosphorus (μmol/ml)
Healthy	0.14 ± 0.06
Control	0.05 ± 0.01 §
H-PEEP	0.05 ± 0.01 §
PLV	0.05 ± 0.01 §
Surfactant	0.45 ± 0.05¶

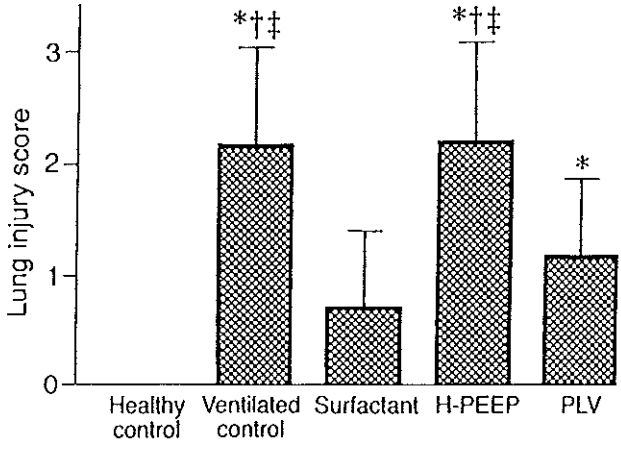

Figure 5. Lung injury score (mean ± SD) in healthy control, ventilated control, surfactant-treated, partial liquid ventilation (PLV) and high (H) PEEP groups. * $p < 0.05$ vs. healthy control group; † $p < 0.05$ vs. surfactant group; ‡ $p < 0.05$ vs. partial liquid ventilation group.

Discussion

We have shown that although exogenous surfactant therapy, ventilation with high PEEP and partial liquid ventilation increased PaO_2 to greater than 50 kPa, the impact on transfer of proteins into the alveoli, lung injury, and on the surfactant system differed markedly. Ventilation with high PEEP and exogenous surfactant therapy prevented transfer of proteins into the alveoli, whereas partial liquid ventilation did not. Conversion of active to non-active surfactant aggregates was increased in both the partial liquid ventilation group and the group ventilated with high PEEP, but not in the surfactant treated group. Lung injury score was reduced in both the partial liquid ventilation and the surfactant groups compared with the groups that were ventilated only.

The sustained improvement in PaO_2 to pre-lavage values in the group ventilated with high PEEP indicates that the applied PIP and PEEP were sufficient to open the lungs and to keep them open (Fig. 1). That alveolar recruitment and stabilization in this group was a result of mechanically counterbalancing the increased retractive forces and not recovery of the endogenous surfactant system, was evident by the lack of improvement in surfactant variables of BAL fluid determined at the end of the study (Table 2). However, protein concentration of BAL fluid was not increased during the 4-h ventilation period with high PEEP. This is important as plasma proteins are known to inhibit surfactant function in a dose-dependent manner [21]. Therefore, protein leakage may mediate the destructive chain of events that lead to further progression of lung injury. The clinical significance of the findings in this high PEEP group remains to be determined, but studies by Kesecioglu, Tibboel and Lachmann [11], and recently by Amato and colleagues [12], have shown an improvement in PaO_2 in patients when using an 'open lung' strategy, and provided the first results indicating that the technique is associated with a decrease in morbidity and mortality [22].

In surfactant-deficient lungs, partial liquid ventilation with perfluorocarbons has been shown to provide adequate gas exchange, which was confirmed in our study (Fig. 1) [10, 23]. However, despite the high PaO_2 values which indicate that the lungs were kept open, we found that transfer of proteins into the alveoli was increased after partial liquid ventilation for 4 hours (Fig. 2). The mechanism responsible for this is not known. It is hypothesized that the improvement of gas exchange with partial liquid ventilation results from filling the collapsed

atelectatic alveoli in the dependent part of the lung with the non-compressible, high-density perfluorocarbons thus preventing them from end-expiratory collapse. In the non-dependent part of the lung, a thin film of perfluorocarbons is formed at the air-liquid interface because of evaporation of perfluorocarbons from the lower lung regions [23]. We speculate that as a result of the low constant surface tension of perfluorocarbon, the retractive forces in the non-dependent part of the lung are reduced, resulting in large volume changes at small increments in pressure, making the lungs prone to epithelial overstreching, which has been shown to damage the alveolar-capillary membrane leading to increased transfer of proteins into the alveoli [24] (for review see Dreyfuss and Saumon [25]). This mechanism is supported by a study of Cox and colleagues [26] who showed that during partial liquid ventilation perfluorocarbon is distributed predominantly to the lower lung regions, whereas gas ventilation takes place in the upper lung regions. Furthermore, several pathology studies have demonstrated a significant variance in lung injury between the non-dependent and dependent lobes after partial liquid ventilation, with greater non-dependent lobe damage [27, 28].

In our study, the partial liquid ventilation and the surfactant groups underwent ventilation with the same PIP and PEEP pressures, but the protein concentration of the BAL fluid from the surfactant group was not increased (Fig. 2). Pulmonary surfactant has the unique property of reducing surface tension in parallel with a decrease in alveolar radius, thus keeping the ratio of surface tension/radius of the alveolus constant and thus preventing epithelial overstretching. Furthermore, as seen in the surfactant-treated group, a lower lung injury score was found in the partial liquid ventilation group despite the increased transfer of protein into the alveoli. This probably reflects a direct effect of perfluorocarbon on inflammation processes, as *in vitro* evidence suggests a decrease in alveolar macrophage and neutrophil adherence, chemotaxis, phagocytosis and superoxide release [29, 30].

Total lung capacity on a distending pressure of 35 cm H_2O (TLC_{35}) was decreased only in the ventilated control group (Fig. 3). As substances with surface tension lowering properties were administered into the lungs of the partial liquid ventilation group and the surfactant treated group, it is not surprising that TLC_{35} was preserved in these groups. However, preservation of TLC_{35} in the group ventilated with high PEEP, but decreased TLC_{35} in the ventilated control group is striking as there were no differences in recovery of pulmonary surfactant between these

groups (Fig. 4, Table 2). We speculate that the difference in TLC_{35} is explained by the difference in transfer of protein into the alveoli between these two groups, because of inhibition of the already compromised surfactant function by plasma proteins, as mentioned above.

In our study we used the lung lavage model which has been studied extensively and is considered a reliable model of acute lung injury [16]. Repeated whole-lung lavage produced an acute quantitative surfactant deficiency and, together with conventional mechanical ventilation leading to severe lung injury with impaired gas exchange, decreased lung compliance and FRC, increased permeability changes of the alveolo-capillairy membrane with oedema, and sustained pulmonary hypertension [16, 17, 31]. Despite the fact that the lung injury in this study is not representative of the pathology in humans with ALI, this model is ideal for testing interventions which may prove therapeutic for acute lung injury [4, 6, 17].

In summary, we have shown that although exogenous surfactant therapy, mechanical ventilation with high PEEP and partial liquid ventilation opened up the lungs and kept them open, as indicated by the high PaO_2 values, the impact on pulmonary function differed markedly. Only with exogenous surfactant therapy there was improvement in all variables. Some studies have reported physiological and pathological benefits of partial liquid ventilation or ventilation with high PEEP in combination with exogenous surfactant, but whether either one of such hybrid techniques has advantages over the use of exogenous surfactant alone remains yet to be confirmed.

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Chapter 4

Improvement of lung mechanics by exogenous surfactant: effect of prior application of high positive end-expiratory pressure

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Summary

Application of a ventilation strategy which aims to recruit collapsed alveoli and to prevent them from repeated collapse by applying a high PEEP, has shown to decrease alveolar protein influx in experimental acute lung injury (ALI). This could be important for the pulmonary response to exogenous surfactant, since plasma proteins are known to inhibit surfactant function. This study was performed to evaluate the effect of exogenous surfactant on lung mechanics after 4 hours of mechanical ventilation with either high or low PEEP. Twenty-two adult male Sprague-Dawley rats were anaesthetized, tracheotomized and submitted to pressure controlled mechanical ventilation with 100% oxygen. One group served as healthy controls (n=6). In the remaining animals acute lung injury was induced by repeated lung lavages to obtain a PaO_2<13 kPa at a peak inspiratory pressure (PIP) of 26 cmH_2O and a PEEP of 6 cmH_2O. After steady state, animals were randomly divided to receive either ventilation with high PEEP (n=8; 100 breaths/min., I:E=1:1, PIP of 35 cmH_2O, PEEP of 18 cmH_2O), or to receive conventional mechanical ventilation (PIP of 28 cmH_2O, PEEP of 8 cmH_2O; n=8; control group). After four hours of ventilation, all animals received exogenous surfactant (120 mg/kg) and ventilation was continued for 15 minutes. At the end of the study pressure-volume curves were constructed from which total lung capacity at 35 cmH_2O (TLC_{35}) and maximal compliance (C_{max}) were calculated, and thereafter broncho-alveolar lavage was performed to assess alveolar protein influx. After lavage, PaO_2 remained around 13 kPa in the ventilated control group and improved to >66 kPa in the high PEEP group. After surfactant, PaO_2 was >53 kPa in both groups. In the ventilated control group alveolar protein influx was higher, and TLC_{35} and C_{max} were lower than in the high PEEP group. We conclude that the pulmonary response to exogenous surfactant after mechanical ventilation in experimental ALI is improved when a ventilation strategy with high PEEP is used.

Introduction

Mechanical ventilation strategies that prevent repeated alveolar collapse are thought to prevent further progression of lung injury during ventilation of surfactant deficient lungs [1, 2]. Application of a high PEEP was previously shown to decrease accumulation of lung water, protein leakage and prevent intra-alveolar edema in experimental acute lung injury (ALI) [3-5]. A decrease in protein transfer into the alveoli has important consequences for pulmonary function, since proteins are known to inhibit surfactant function in a dose dependent way [6]. In previous studies in an animal model of ALI, we showed that ventilation with a high PEEP attenuated the decrease in lung mechanics, which was attributed to a decrease in protein transfer into the alveoli [3, 7].

Exogenous surfactant is successfully administered in the respiratory distress syndrome (RDS) of the premature newborn, resulting in improvement of gas exchange, lung mechanics, and outcome [8]. Since hypoxia and deterioration in lung mechanics in ALI are also caused by a lack of active surfactant, the efficacy of surfactant replacement in ALI is currently evaluated in both experimental and clinical studies [9-11]. An important problem with surfactant therapy in adults is the high costs and non-availability of the large amounts of surfactant that are needed. A decrease in ventilation-induced protein influx in ALI patients who may benefit from exogenous surfactant might reduce the surfactant dose required to improve pulmonary function, which could make surfactant replacement in ALI more feasible.

We hypothesize that the improvement in lung function that is obtained by surfactant replacement in experimental ALI after 4 h of mechanical ventilation, is better when during ventilation a high PEEP is used to decrease alveolar protein influx. We therefore ventilated surfactant-depleted rats with either a high or a low PEEP, followed by surfactant replacement, and evaluated gas exchange and lung mechanics.

Methods

The study protocol was approved by the University's Animal Experimental Committee, and the care and handling of the animals conformed with European Community guidelines (86/609/EC). The study was performed in twenty-two adult male Sprague-Dawley rats (body weight 270-330 g). After induction of anaesthesia with 2% enflurane and 65% nitrous oxide in oxygen, a

polyethylene catheter was inserted into a carotid artery for drawing arterial blood samples. Before tracheostomy, the animals received 60 mg/kg pentobarbital sodium, i.p. (Nembutal, Algin BV, Maassluis, the Netherlands). After tracheostomy, muscle relaxation was induced by pancuronium bromide 1 mg/kg, i.m. (Pavulon, Organon Teknika, Boxtel, the Netherlands) immediately followed by connection to a ventilator. The animals were mechanically ventilated with a Servo Ventilator 300 (Siemens-Elema, Solna, Sweden) in a pressure constant time-cycled mode, at an inspired oxygen concentration (FiO_2) of 1.0, frequency of 30 breaths per minute (bpm), peak inspiratory pressure (PIP) of 12 cm H_2O, positive end-expiratory pressure (PEEP) of 2 cmH_2O, and inspiratory/expiratory (I/E) ratio of 1:2. Anaesthesia was maintained with pentobarbital sodium 40 mg/kg/h, i.p.; muscle relaxation was maintained with pancuronium bromide 1 mg/kg/h, i.m. Body temperature was kept within normal range by means of a heating pad. Six animals were killed immediately after induction of anaesthesia and served as healthy controls.

In the remaining animals acute lung injury was induced by repeated broncho-alveolar lavage (BAL) (32 ml/kg) with warm saline (37° C), according to Lachmann and colleagues [12]. BAL was repeated as often as necessary to produce a PaO_2<13 kPa at a PIP and PEEP of 26 and 6 cmH_2O, respectively. Within 10 min after the last lavage, the animals were randomised to one of two study groups (each group n=8). In the first group, mechanical ventilation according to the open lung concept was applied. We have previously shown that this strategy, in which collapsed alveoli are recruited by applying a high PIP and kept open with a high PEEP, PaO_2 can be kept >65 kPa in surfactant-deficient rats [3]. In the present study, we used identical ventilator settings. First, the lungs were opened by increasing PIP to 40 cmH_2O and PEEP to 20 cmH_2O, and the I/E ratio was set at 1:1. After 2 to 3 min, PIP was decreased to 35 cmH_2O and PEEP to 18 cmH_2O, and arterial blood gas samples were taken. Ventilator setting were kept unchanged for the remainder of the study period. In the second group, that served as ventilated controls, ventilator pressures were increased by 2 cmH_2O (PIP/PEEP of 28/8 cmH_2O). These ventilator settings were chosen based on the results from a previous study using this model, where it was found that at these settings PaO_2 remained stable at a low value of about 13 kPa for 4 h at an FiO_2 of 1.0, indicating that large parts of the lungs remain atelectatic [3]. Protein transfer into the alveoli was found to be increased with these settings. In the present study, both ventilated

groups were treated with exogenous surfactant at a dose of 120 mg/kg after 4 hours of ventilation. The surfactant used was isolated from minced pig lungs, that were processed as previously described [13]. 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 re-connection to the ventilator. After surfactant administration, ventilation was continued for 15 min, while ventilator settings were unchanged in both groups. This short interval was chosen since maximal improvement of lung function by exogenous surfactant occurs 2-5 min after administration, and because surfactant inactivation by proteins is an event that occurs instantaneously.

Arterial blood gas samples were taken prior to lavage, after lavage, hourly for 4 h after lavage, immediately and 15 min after surfactant administration. The samples were analysed for arterial oxygen tension (PaO_2) and arterial carbon dioxide tension ($PaCO_2$) on an electrochemical blood gas analyser (ABL 505, Radiometer, Copenhagen, Denmark).

At the end of the experiment, the animals were killed by an overdose of pentobarbital. Static pressure-volume curves were recorded using conventional techniques. Total lung capacity (TLC_{35}) was defined as lung volume at inflation with a distending pressure of 35 cmH_2O. Maximal compliance (C_{max}) was defined as the steepest part of the deflation limb of the pressure-volume curve. After the pressure-volume recordings, BAL was performed five times with saline-$CaCl_2$ 1.5 mmol/litre. The active surfactant component in the BAL fluid was separated from the non-active surfactant component by differential centrifugation followed by subsequent phosphorus analysis, and the ratio between non-active and active components (small aggregate to large aggregate ratio = SA/LA ratio) was calculated, as previously described by Veldhuizen and colleagues [14]. The protein concentration of the BAL fluid was determined using the Bradford method (Bio-Rad protein-assay, Munich, Germany) [15].

Statistical analysis was performed using the Instat 2.0 biostatistics package (GraphPad software, San Diego, CA, USA). For blood gases inter-group comparisons were analysed using the alternate (Welch) t-test, while intra-group comparisons were analysed using repeated measures ANOVA. All other data were analysed with ANOVA. If ANOVA resulted in a $p < 0.05$

a Tukey-Kramer post-test was performed. All data are reported as mean ± SD and $p < 0.05$ was considered statistically significant.

Results

Blood gases before and immediately after lavage were comparable for both ventilated groups (Fig.1 and Table 1). None of the animals died during the 4 h and 15 min observation period. In the group ventilated with high PEEP, PaO_2 increased to pre-lavage values and remained stable during the 4 h ventilation period, but decreased to 61.8 ± 5 kPa after surfactant administration. In the control group, PaO_2 did not improve until surfactant was administered, after which PaO_2 increased to 54.0 ± 17 kPa.

$PaCO_2$ data are given in Table 1. In both ventilated groups $PaCO_2$ increased after lavage, and remained unchanged during the entire study period (Table 1).

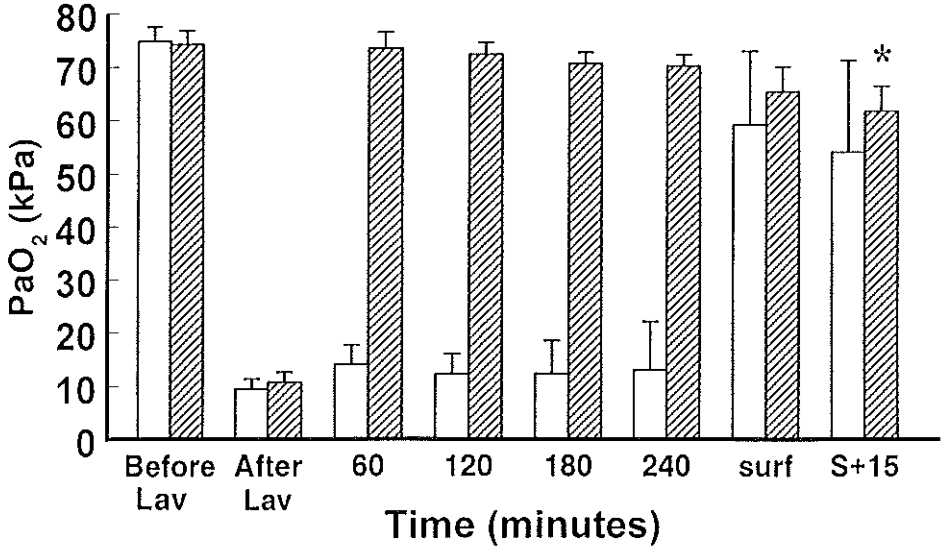

Figure 1. PaO_2 values (mean ± SD, kPa) of the ventilated groups. Striped bars=high PEEP group; open bars=ventilated control group with low PEEP; Before Lav=before lavage; After Lav=after lavage; surf=after surfactant administration; S+15=15 minutes after surfactant administration. * $p<0.05$ vs. 240 minutes.

Table 1. $PaCO_2$ values (mean ± SD), kPa) for the ventilated groups, before lavage (Healthy), immediately after lavage (Lav), 60, 120, 180 and 240 minutes after lavage, immediately after surfactant administration (Surf), and 15 minutes after surfactant (Surf+15'). *$p<0.05$ vs. Healthy. H-PEEP=high PEEP; Control=ventilated controls with low PEEP.

	H-PEEP	Control
Healthy	4.9 ± 1.0	5.0 ± 0.8
Lav	6.7 ± 0.9*	7.2 ± 1.3*
60'	7.6 ± 1.8*	6.7 ± 1.6*
120'	7.1 ± 1.5*	7.0 ± 1.8*
180'	7.5 ± 1.6*	7.3 ± 1.9*
240'	7.6 ± 2.0*	7.6 ± 1.6*
Surf	7.1 ± 2.4*	7.7 ± 1.8*
Surf+15'	7.8 ± 2.6*	7.8 ± 2.1*

The protein concentration of BAL fluid was significantly increased in both ventilated groups compared to healthy control animals (Fig. 2). However, in the ventilated control group protein concentration was significantly higher than in the high PEEP group.

There were no differences in SA/LA ratio between the three groups (Table 2). In comparison with healthy control animals, the total amount of phosphorus in the BAL fluid (that was measured to quantify the phospholipid-containing surfactant system) was significantly higher in both groups that received exogenous surfactant (Table 2).

Table 2. Data on maximal compliance (C_{max}, ml/cm H_2O/kg), total phosphorus (μmol/ml) recovered from the BAL fluid, μmol/ml, and SA/LA ratio, all values mean ± SD). * p <0.05 vs. healthy controls (Healthy), † p <0.05 vs. high PEEP.

	C_{max} (ml/cm H_2O/kg)	Total phosphorus (μmol/ml)	SA/LA ratio
Healthy	13.4 ± 1.1	0.14 ± 0.06	0.31 ± 0.12
High PEEP	10.0 ± 2.6	0.32 ± 0.08*	0.36 ± 0.17
Control	6.8 ± 1.2*†	0.29 ± 0.06*	0.47 ± 0.19

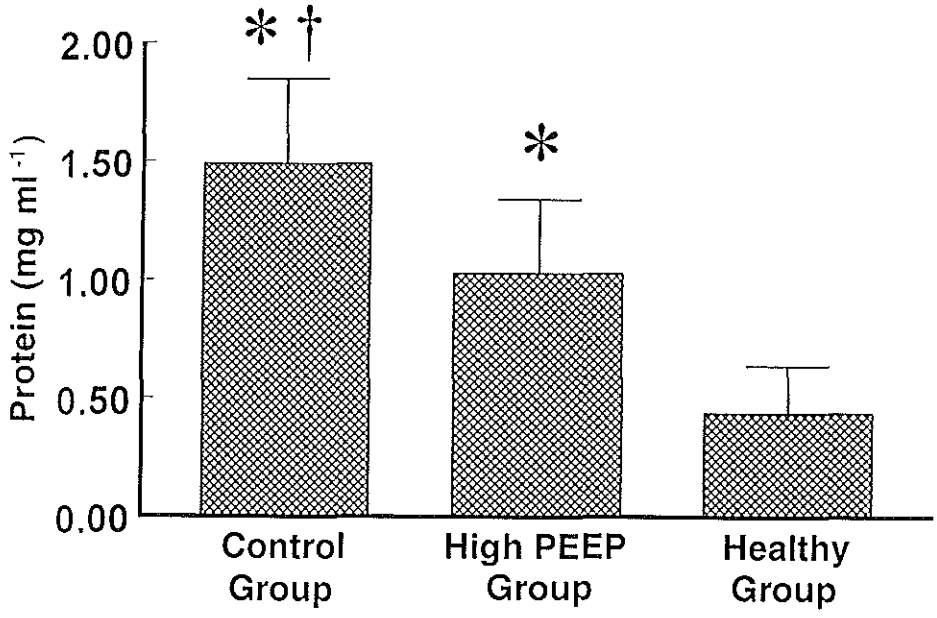

Figure 2. Mean protein concentration ± SD, mg/ml, of the BAL fluid of the different groups. * $p < 0.05$ *vs* healthy controls (Healthy); † $p < 0.05$ *vs* high PEEP group.

Figure 3 shows the pressure-volume curves. On deflation to 15 cmH_2O, lung volume is decreased only in the ventilated control group. Below 15 cmH_2O, lung volume in both ventilated groups is decreased compared to the healthy controls. Maximal compliance (C_{max}) was decreased in the ventilated control group compared to the high PEEP group, and to the healthy controls (Table 2).

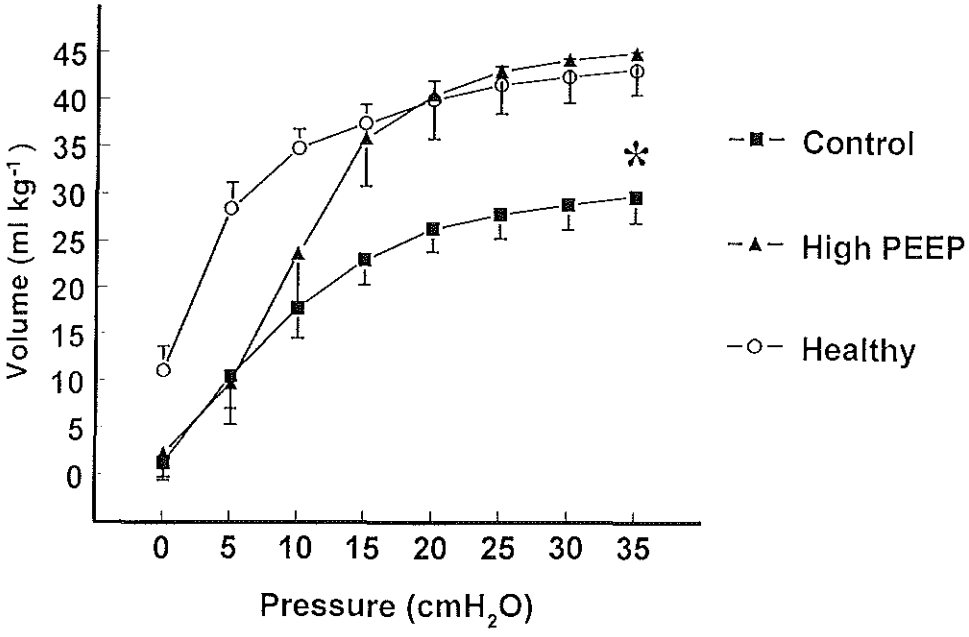

Figure 3. Deflation limbs from the pressure-volume curves, mean ± SD. Volume is lung volume above FRC. On deflation to 15 cmH_2O, lung volume is decreased only in the ventilated control group. Below 15 cmH_2O, lung volume in both ventilated groups is decreased compared to the healthy controls. * p <0.05 vs healthy controls (Healthy).

Discussion

This study shows that the efficacy of exogenous surfactant therapy, administered after 4 h of mechanical ventilation, to improve total lung capacity at a distending pressure of 35 cmH_2O and maximal compliance is less attenuated after ventilation with a high PEEP compared to ventilation with a low PEEP. Protein concentration in the BAL fluid of the high PEEP group was lower compared to the ventilated controls with low PEEP.

In the ventilated control group, TLC_{35} and C_{max} were decreased compared to the healthy controls, but not in the high PEEP group (Table 2 and Fig. 3). This indicates that function of the

exogenous surfactant was better in the high PEEP group compared to the ventilated controls. Surfactant has the unique property of reducing surface tension at the air-liquid interface in the alveoli in parallel with the decrease in alveolar radius, thus improving lung distensibility and alveolar stability. It was previously shown that plasma proteins decrease the capability of surfactant to decrease surface tension [6]. We therefore speculate that the decreased improvement of lung mechanics in the low PEEP group after surfactant administration is caused by the increased protein transfer into the alveoli in this group. These results are consistent with previous results obtained in this model, where mechanical ventilation with high PEEP resulted in a decrease in protein transfer into the alveoli, and better lung mechanics [3, 7].

Preservation of the integrity of the alveolar epithelium by prevention of the occurrence of shear forces that are caused by repeated alveolar collapse is considered an important factor in decreasing protein influx [4]. Application of PEEP has also been shown to lead to a shift of fluid from the alveoli to the interstitium by decreasing the pressure gradient across the alveolar-capillary membrane [16]. Furthermore, there is evidence that PEEP prevents the loss of surfactant from the alveoli, which prevents an increase in surface tension mediated suctioning across the alveolar capillary membrane that increases alveolar protein influx [5].

Before surfactant administration, PaO_2 was >66 kPa in the high PEEP group, indicating that the applied ventilator settings were sufficient to open up the lungs and keep them open, which confirms previous experience in this model (Fig. 1) [3, 7]. The decrease in oxygenation which is observed after surfactant administration in this group is attributed to the bolus of liquid in which the surfactant was suspended, that partly filled up the alveoli causing a decrease in diffusion [9].

No differences were found between the ventilated groups in non-active and active surfactant aggregates (SA/LA ratio). This is in accordance with data from Veldhuizen et al., who showed that the conversion of LA into SA is dependent on alveolar area cycling and time [17]. Alveolar cycling is determined by tidal volume, which was comparable for both ventilated groups.

An important factor in the successful application of exogenous surfactant in RDS of the premature infant is the fact that the surfactant is administered shortly after birth, thus reducing the time in which proteins can transfer into the surfactant-deficient alveoli. In the adult ALI

patient, where the surfactant deficiency is secondary to lung injury, protein concentration in the alveoli is increased [18]. It was previously shown that mixing exogenous surfactant with plasma proteins decreases the capability of surfactant to improve gas exchange, and that larger amounts of surfactant are necessary to overcome the inhibitory effects [6]. The results of the present study show that the efficacy of exogenous surfactant can be improved by attenuating the increase in protein transfer into the alveoli. It has previously been suggested that a decrease in alveolar protein concentration, for example by lobe-wise lung lavage, could improve the effect of exogenous surfactant on pulmonary function [19]. Our study implicates that when the lungs are kept open by application of a high PEEP during the ventilation period which usually precedes exogenous surfactant administration in patients, the efficacy of surfactant replacement can be improved. Given the dose-dependent inhibition of surfactant by proteins, a reduction in protein influx during mechanical ventilation prior to surfactant administration is important since this could lead to a decrease in the exogenous surfactant dose that is necessary to improve pulmonary function in ALI patients. Thereby, a reduced protein influx could reduce costs and improve outcome in ALI.

In conclusion, this study shows that the improvement in lung function that is obtained by surfactant replacement in experimental ALI after 4 h of mechanical ventilation, is better when during ventilation a high PEEP is used to decrease alveolar protein influx. Future clinical studies are necessary to evaluate if application of a high PEEP in ALI patients that could benefit from exogenous surfactant therapy can improve efficacy of surfactant replacement and improve outcome.

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Chapter 5

High-frequency oscillatory ventilation is not superior to conventional mechanical ventilation in surfactant-treated rabbits with lung injury

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Summary

The aim of this study was to compare high-frequency oscillatory ventilation (HFOV) with conventional mechanical ventilation (CMV) with and without surfactant in the treatment surfactant-deficient rabbits. A previously described saline lung lavage model was used. The efficacy of each therapy was assessed by evaluating gas exchange, lung deflation stability and lung histopathology. Arterial oxygenation did not improve in the CMV group without surfactant, but increased rapidly to prelavage values in the other three study groups. During deflation stability, mean PaO_2 values decreased to post-lavage values in the group that received HFOV alone but not in both surfactant treated groups (HFOV and CMV). The HFOV group without surfactant showed more cellular infiltration and epithelial damage compared with both surfactant-treated groups (HFOV and CMV). There was no difference in gas exchange, lung deflation stability, and lung injury between HFOV and CMV after surfactant therapy.

It is concluded that the use of surfactant therapy in combination with high-frequency oscillatory ventilation is not superior to conventional mechanical ventilation in improving gas exchange, lung deflation stability and in the prevention of lung injury, if the lungs are kept expanded. This indicates that achieving and maintaining alveolar expansion (*i.e.* open lung) is of more importance than the type of ventilator.

Introduction

In the first reported high-frequency oscillatory ventilation (HFOV) trial, a low distending airway pressure was used in order to minimize the risk of barotrauma [1]. However, experimental studies have shown that alveoli should be actively opened and that relatively high airway pressure has to be used to stay above the closing pressure to avoid hypoxemia and lung injury [2, 3]. Results of recent pilot studies in neonates with respiratory distress syndrome (RDS) applying this high-lung volume strategy are encouraging [4-6].

To date, very few studies have been published on the combined use of surfactant and HFOV in animals or humans [4, 6-9]. Those studies demonstrated that after surfactant therapy HFOV was superior to conventional mechanical ventilation (CMV) in improving pulmonary function and reducing lung injury [4, 6-9]. However, in those studies, HFOV was used in combination with the high-lung volume strategy whereas CMV was not. Recently, Froese and colleagues [8] compared HFOV to CMV after surfactant therapy at low- and high-lung volume and confirmed that HFOV at high-lung volume was superior to the alternatives in improving gas exchange and lung mechanics in lung-lavaged rabbits. Surprisingly, these authors were not able to maintain oxygenation above 46.7 kPa (350 mmHg), despite the high-lung volume strategy, after surfactant therapy with the use of CMV [8]. This finding is in contrast to earlier results of CMV with surfactant therapy in lung-lavaged rabbits in which oxygenation increased rapidly to prelavage values after surfactant instillation and was kept stable for hours [10-12]. The purpose of the present study was to compare the use of HFOV to CMV with and without surfactant therapy in the management of acute lung injury caused by lung lavage in adult rabbits.

Materials and methods

Animal preparation

This 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/EEG) [13]. A total of 27 adult New Zealand White rabbits (IFFA-Credo, Brussels, Belgium) with a mean body weight of 2.7 ± 0.3 kg were anaesthetized with pentobarbital sodium (50 mg/kg body weight) via an auricular vein and then placed in a supine position. An endotracheal tube (i.d. 3.5 mm) was inserted via tracheostomy and mechanical ventilation was

initiated with a Servo Ventilator 900C (Siemens-Elema AB, Solna, Sweden) in a pressure-control mode, indicating time-cycled ventilation with decelerating flow, with the following ventilator settings: FiO_2 of 1.0, positive end-expiratory pressure (PEEP) of 2 cm H_2O, frequency of 30 breaths/min, inspiratory/expiratory ratio of 1:2 and a peak inspiratory pressure of 10-14 cm H_2O to keep $PaCO_2$ within normal range. An infusion of 2.5% glucose was continuously administered via the auricular vein as a maintenance fluid (5 ml/kg body weight /h). Anaesthesia was maintained by hourly injection of pentobarbital sodium (5 mg/kg body weight /h, i.v.); muscle paralysis was achieved by hourly injection of pancuronium bromide (0.1 mg/kg body weight /h, i.m.).

A carotid artery was cannulated for continuous blood pressure measurements and for intermittent blood sampling. Arterial samples were analysed for blood gases, pH and haemoglobin using conventional methods (ABL-505 and Osm-3; Radiometer, Copenhagen, Denmark). Core temperature was monitored with an esophageal thermistor (Elektrolaboratoriet, Copenhagen, Denmark) and maintained within normal range by a heating pad.

Induction of lung injury

In all animals, respiratory insufficiency was induced by repeated whole-lung lavage according to the technique described by Lachmann et al. [14]. Each lavage was preformed with saline (30 ml/kg body weight) heated to 37 °C. Lung lavage was repeated 5-8 times at 2-5 min intervals to achieve a PaO_2 <11.3 kPa (< 85 mmHg) at a peak pressure of 26 cm H_2O and a PEEP of 6 cm H_2O (other ventilator settings were not changed).

Study design

After reaching steady state, 24 animals were divided randomly into four groups of six animals. In the first group, animals received a bolus (100 mg/kg body weight, 25 mg/ml) of a natural surfactant intratracheally (Alveofact®; Thomae, Biberach, Germany) and immediately followed by connection to a high-frequency oscillator (type OHF-1, S.A. Dufour, Villeneuve d'Ascq, France). During HFOV, a frequency of 10 Hz was used and mean airway pressure (MAwP) was increased until PaO_2 was above 46.7 kPa (350 mmHg) to guarantee the high-lung volume strategy (i.e. open lungs) [4, 5]. When PaO_2 was above 73.3 kPa (550 mmHg) during the study

period, MAwP was decreased by steps of 1 cm H_2O. Pressure amplitude was initiated with 30 cm H_2O and was altered as necessary to keep $PaCO_2$ within normal range (4.7-6.0 kPa (35-45 mmHg)). The second group received exogenous surfactant intratracheally (Alveofact®; 100 mg/kg body weight) and conventional mechanical ventilation (CMV) with the Servo Ventilator 900C was continued (ventilator settings were not changed). For instillation of surfactant, the animals were disconnected from the ventilator and received the surfactant suspension (25 mg/ml) directly into the endotracheal tube via a syringe followed by immediate reconnection to the ventilator. The other two groups served as controls and received HFOV or CMV alone in the same way as described above.

After 4 h of ventilation, MAwP was first decreased to 12 cm H_2O for 10 min in those animals that received a MAwP of ≥ 12 cm H_2O and then to 9 and 6 cm H_2O during the same period of time. Finally, all study groups were restarted on CMV at the same settings as used during lung lavage (PIP/PEEP=26/6 cm H_2O). Ten minutes later, blood samples were taken for blood gases.

During the observation period (4 h and 40 min), arterial blood samples were collected at the following times: before lavage; 5 and 10 min after the last lavage; 5, 15, 30 min after surfactant or HFOV application; every 30 min for 4 h; 10 min after the three reduction steps of MAwP; and finally 10 min after CMV with the same settings as during the lavage procedure. Continuous blood pressure monitoring enabled observation of changes of mean arterial blood pressure. Volume expansion (Isodex®; 5 ml/kg body weight, i.v.) was indicated when mean arterial pressure was below 6.7 kPa (50 mmHg).

Pathological evaluations

At the end of the observation period, lungs were ventilated with air with no changes in the ventilatory parameters. The abdomen of the rabbit was opened and the diaphragm was inspected for evidence of pneumothorax. The inferior vena cava was cannulated and perfused with a solution, consisting of saline saturated with 95% O_2 and 5% CO_2, 2.2 mM $CaCl_2$, 0.5% procaine and 1% heparin, was then infused at a rate of 50 ml/min. The abdominal aorta was cut and the infusion was stopped when clear fluid flowed from the aorta. Thereafter, the peak pressure was lowered to 1 cm H_2O, that was maintained while the lungs were fixated by infusing ±100 ml of

a fixation solution, consisting of 3.6% formaldehyde and 0.25% glutaraldehyde, via the inferior vena cava. After fixation, the trachea was clamped at a pressure of 6 cm H_2O, the thorax was opened and the lungs were removed en bloc and stored in the fixation solution. The lungs were numbered and histopathologic examinations of the lungs were performed blindly. The lungs of the three remaining animals were fixed 10 min after the lavage procedure as described above and histopathologic examinations were performed. These animals were used to study the influence of the lavage procedure itself on morphological changes.

The lungs were then embedded in paraffin, sectioned and stained with the haematoxylin and eosin (HE) and elastica-van Gieson (EvG) technique. A semi-quantitative morphometric analysis of lung injury was performed under blinded conditions by a pathologist who scored atelectasis, edema, vascular wall thickening and leucocyte infiltration as none, light, moderate or severe (score 0-3). Lung injury score was defined as the average from all parameters for each group. For transmission electron microscopic examination, lung tissue was pre-contrasted with 2% osmium acid, dehydrated, and embedded in epoxy resin (Epon 812). Semi-thin sections (1.5 μm) and ultrathin sections (0.5 μm) were produced. Semi-thin sections were stained with 2% methylene blue and 3% alkaline fuchsin. Ultrathin sections were counterstained with uranyl acetate and lead citrate. The specimens for the scanning electron microscope were dehydrated with increasing lines of alcoholic solutions, dried with the critical point method, and sputtered with gold.

Statistical analysis

All data are expressed as mean ± SD. Analysis of variance (ANOVA) was used to assess whether there was an overall difference within or between the two groups. If a difference was found, a post hoc test was used (Student-Newman-Keuls' multiple comparison procedure). Lung injury data were analysed using the Kurskal-Wallis nonparametric ANOVA test, followed by Dunn's multiple comparisons test if a difference was found. Statistical significance was accepted at p-values <0.05.

Results

Blood gases before lavage and directly after lavage were comparable in all animals. In one

animal of the HFOV group which developed a pneumothorax at 210 min, measurements were discontinued but the lungs were fixated for histologic examinations.

Mean PaO_2 values in both HFOV groups (with or without surfactant) were kept above 46.7 kPa (350 mmHg) in the first 4 h by using appropriate MAwP (Fig. 1, Table 2). In the CMV group with surfactant, PaO_2 increased from 8.3 ± 2.1 kPa (62 ± 16 mmHg) to 53.3 ± 10.7 kPa (400 ± 80 mmHg) within 5 min and remained stable during the subsequent 4 h without changing the ventilator settings (Fig. 1).

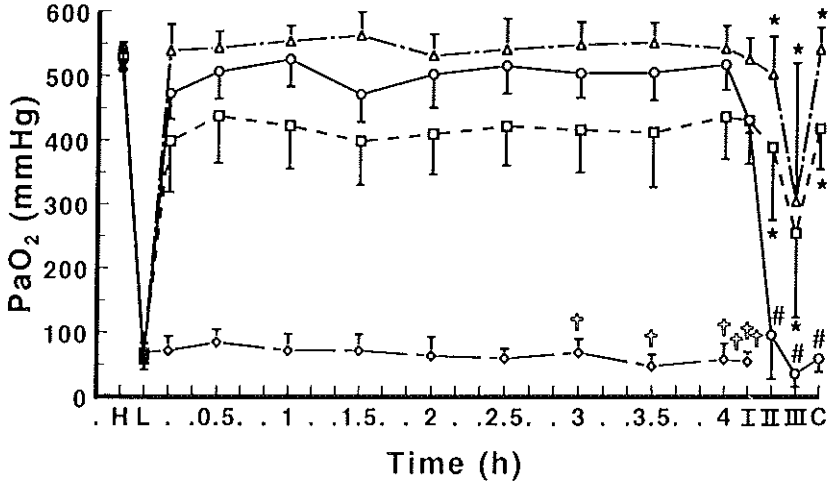


Figure 1. Change in mean arterial oxygen tension (PaO_2) (±SD) of the four study groups before lung lavage (H; healthy), after lavage (L; 10 min after the last lavage), and during the subsequent 4 h and 40 min observation period. I, II and III are the 3 reduction steps of MAwP to: I = 12 cm H_2O; II = 9 cm H_2O; III = 6 cm H_2O. C, pressure-controlled ventilation with the same settings as during the lavage procedure, indicating a MAwP of ±12 cm H_2O. O, animals (n=6) that received HFOV without surfactant; ◊, animals (n=6) that received CMV without surfactant; □, animals (n=6) that received CMV and surfactant (100 mg/kg body weight); Δ, animals (n=6) that received HFOV and surfactant (100 mg/kg body weight). H, healthy. L, 10 min after the last lavage. *, $p < 0.05$ versus HFOV group. #, $p < 0.05$ versus PaO_2 values at 4 h; †: death of one animal. 1 mmHg=0.133 kPa.

Table 1. Data on $PaCO_2$ (mmHg) and MAwP (cm H_2O) of the different study groups. Values are given as mean ± SD.

Group	H	L	5'	0.5 h	1 h	2 h	3 h	4 h	I	II	III	C
						$PaCO_2$						
CMV	31±4	44±10	44±9	47±11	45±11	51±9*	55±8*†	68±9*††	58±11*†	-††	-	-
HFOV	27±3	40±4	42±8	41±5	42±6	38±2	35±3	34±3	34±2	41±5	49±5	44±7
CMV+ Surf.	29±6	45±5	38±11	37±6	45±5	35±2	35±5	36±4	38±6	40±9	48±12	47±14
HFOV+ Surf.	31±5	40±9	40±4	40±4	36±9	32±4	29±2	30±3	29±3	30±3	38±3	35±2
						MAwP						
CMV	6.1±0.7	12.1±0.8	12.3±1.6*	12.8±2.3*	12.3±1.4*	12.9±1.8	13.1±1.8	13.3±2.1	12	9	6	-
HFOV	5.3±0.8	11.3±0.8	20	18.8±1.0	16.5±3.4	16.7±3.4	16.8±3.2	15.4±1.5	12	9	6	13.4±0.9
CMV+ Surf.	5.8±0.8	11.8±0.8	11.3±2.6*	11.8±2.3*	12.3±2.4	12.3±2.3	11.7±1.8*	12.3±2.3	12	9	6	11.2±1.2
HFOV+ Surf.	6.3±0.5	11.9±0.3	19.0±2.0#	16.6±2.6	15.2±2.8	12.6±1.9	11.6±2.2	10.4±2.6*	9.8±2.3	8.4±0.9	6	12.1±0.8

*, $p \leq 0.05$ vs. HFOV group. #, $p \leq 0.05$ vs. group CMV+Surf. HFOV, group that received high-frequency oscillatory ventilation without surfactant. CMV, group that received pressure-controlled ventilation without surfactant. CMV+Surf, group that received surfactant with pressure-controlled ventilation. HFOV+Surf, group that received high-frequency oscillatory ventilation with surfactant. H, healthy. L, 10 min after the last lavage. MAwP, mean airway pressure. I, II and III are the 3 reduction steps of MAwP to: I = 12 cm H_2O; II = 9 cm H_2O; III = 6 cm H_2O. C, pressure-controlled ventilation with the same settings as during the lavage procedure. †, death of one animal.

In the CMV group without surfactant, mean PaO_2 values gradually decreased over time and all animals died after reduction of the MAwP to 9 cm H_2O (Fig. 1). Mean PaO_2 values were comparable up to a MAwP of 12 cm H_2O between the following groups: HFOV without surfactant; HFOV with surfactant; and CMV with surfactant (Fig. 1). Mean PaO_2 values of the animals ventilated with HFOV without surfactant dropped to post-lavage values after MAwP was lowered to 9 and 6 cm H_2O, respectively. Mean PaO_2 values did not improve when those HFOV animals were switched to CMV at the end of the observation period. However, in both surfactant groups (HFOV and CMV) mean PaO_2 values were significantly higher at a MAwP of 9 and 6 cm H_2O compared to the group HFOV without surfactant (Fig. 1). Furthermore, mean PaO_2 values restored to the PaO_2 levels at time point 4 h in both surfactant-treated groups (HFOV and CMV) after CMV for 10 min with the same settings as used during the lavage procedure, indicating a MAwP of ±12 cm H_2O (Fig. 1).

The mean carbon dioxide tension in arterial blood ($PaCO_2$) values gradually increased in the group CMV without surfactant (Table 2). In the other three groups, mean $PaCO_2$ values were maintained at 4.7-6.0 kPa (35 and 45 mmHg). MAwP data are shown in Table 2. In both groups that received HFOV (with or without surfactant), MAwP was initially increased to 18-20 cm H_2O and could be decreased significantly during the subsequent 4 h while PaO_2 remained stable. At time point 4 h, MAwP of the group HFOV with surfactant was significantly lower compared to the HFOV group without surfactant (Table 2).

All animals showed evidence of pneumonitis that was composed mainly of eosinophils with some neutrophils. The three animals that were lavaged only and ventilated with CMV for 10 min showed also a pneumonitis that was similar in extent and distribution as the animals of the 4 study groups. The pneumonitis was similar to that originally described by Lachmann et al. [15] and the presence of the pneumonitis even in the lavage control animals suggested that a chemical pneumonitis is induced by the lavage process itself [4].

Figure 2 shows the lung injury score of the different groups. Animals treated with surfactant combined with HFOV or CMV had significantly less lung injury than both groups without surfactant (HFOV and CMV) at the end of the ventilation period. The extent of lung injury of both surfactant-treated groups was comparable with that of the animals that were lavaged only. Representative photomicrographs are shown in Figures 3 and 4. More detailed

quantitative comparisons between both surfactant treated groups (HFOV and CMV) were not made.

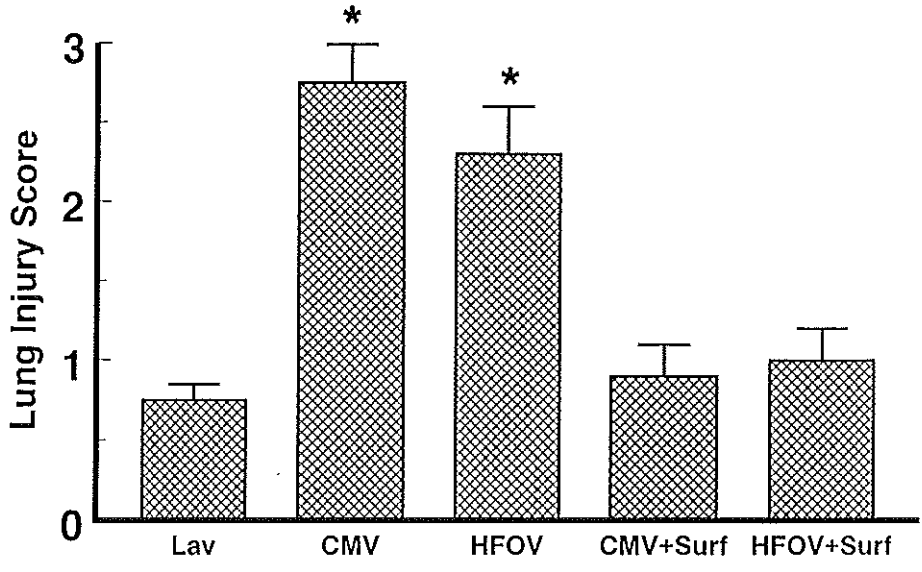
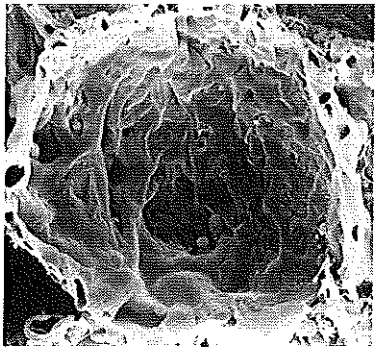


Figure 2. Semi-quantitative lung injury score for all groups. Lav: animals that were lavaged only; CMV: conventional mechanical ventilation; HFOV: high-frequency oscillatory ventilation; CMV+Surf: animals that received CMV and surfactant; HFOV+Surf: animals that received HFOV and surfactant. *, $p \leq 0.05$ vs. animals that were lavaged only. Data are presented as mean ±SD.

A



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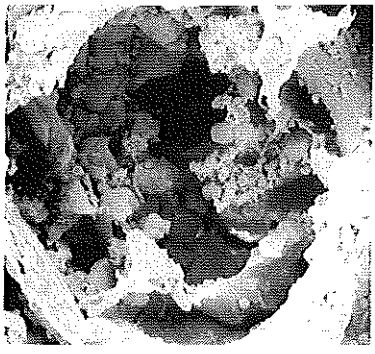

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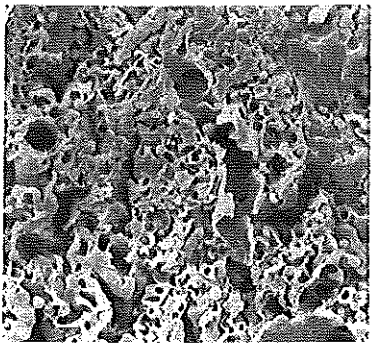
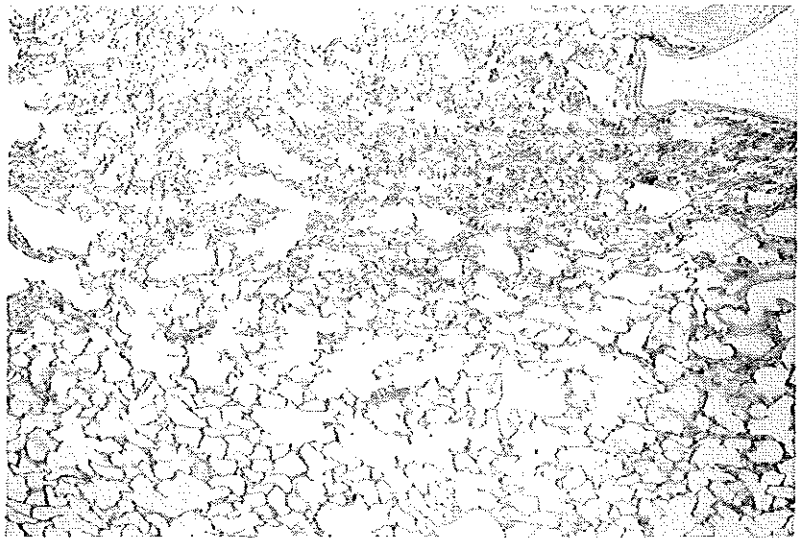


Figure 3. (A) Half of the lung tissue is atelectatic in the group that was lavaged only. No cellular reaction exists and the septa are structured regularly. (B) The lung tissue is evenly aerated and only focally atelectatic (group conventional mechanical ventilation and surfactant). Few granulocytes are situated next to the bronchioli and small vessels. (C) Aerated lung tissue with focal atelectasis (group high-frequency oscillatory ventilation +Surfactant). Collapse of the alveoli is accompanied by a slight interstitial and intra-alveolar infiltrate. Light microscopy, haematoxylin eosin stain, original magnification x 40.

A



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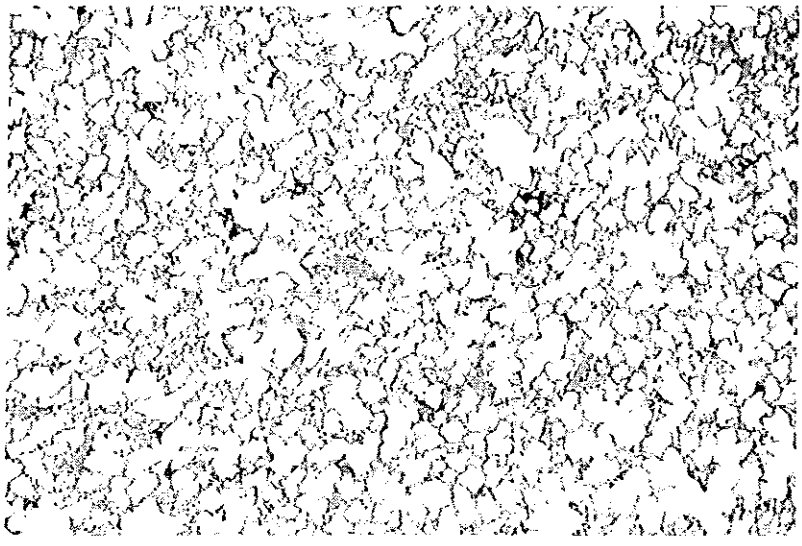

Figure 4. Representative alveoli of the group that received (A) conventional mechanical ventilation for only 10 min after the lavage procedure and of the group that received (B) high-frequency oscillatory ventilation (HFOV) without surfactant for 4.5 h. The alveolus of the HFOV group contain fibrin, leucocytes, erythrocytes, and macrophages. Scanning electron microscopy, magnification x 750.

Discussion

In the present study we used the lung lavage model that has proved to be a consistent and convenient model of acute lung injury [10-12, 14]. Despite the use of adult animals, it has been postulated that this model reflects a primary surfactant deficiency, as seen in neonatal RDS [15]. Several investigators have confirmed the direct relationship in this model between arterial oxygenation and lung volume [2, 3, 11, 16]. It has been demonstrated that arterial oxygenation increased with increasing lung volume as alveoli re-expanded and shunt flow decreased [16]. In the present study, we therefore used arterial oxygenation as a reflection of lung volume.

The results of this study demonstrate that after surfactant therapy there is no difference between the use of HFOV and CMV in improving gas exchange, lung deflation stability and prevention of lung injury in lung-lavaged rabbits. These results are in contrast to the results of Froese *et al.* [10] who demonstrated that the effect of exogenous surfactant on arterial oxygenation remained stable with HFOV, whereas it decreased significantly during the 4 h study period with CMV at high-lung volume. In their study, the high-lung volume strategy with CMV was performed by a gradual increase of PIP and PEEP but without an active volume recruitment manoeuvre as used with HFOV [8]. Furthermore, CMV was used with high tidal volumes (20 ml/kg body weight) which is known to increase the conversion from active into non-active surfactant subfractions; this leads to a shortage of 'active' surfactant at alveolar level [17,18]. In the present study, an active volume recruitment manoeuvre was not performed with CMV after surfactant therapy, but arterial oxygenation increased to above 46.7 kPa (350 mmHg) within 5 min (without change of ventilator settings) and kept stable during the subsequent 4 h. In contrast to the study of Froese *et al.* [8], this study utilized normal tidal volumes (10 ml/kg body weight) and installed the surfactant at a higher concentration (100 mg/kg body weight) and as one bolus, that is known to improve the surfactant distribution and its efficacy [12].

Surfactant metabolism and turnover is known to be strongly influenced by ventilation and some authors suggested that secretion of surfactant is increased with HFOV [19-21]. In the present study, it was confirmed that in lung-lavaged rabbits optimal gas exchange can be obtained with HFOV without surfactant, by using the high-lung volume strategy. However, lung function does not improve over time, as shown by the results that mean PaO_2 values at the end of the observation period were comparable with the post-lavage values at the same ventilatory

support (Fig. 1). This indicates that the reduced end-expiratory stability, due to the repeated lung lavages (i.e. surfactant deficiency), was apparently not improved by HFOV. This confirms the results by Meredith *et al.* [22], who showed excellent gas exchange using HFOV with the high-lung volume strategy in premature baboons, but no beneficial effect on lung volume at zero pressure (functional residual capacity) determined at 24 h. This indicates that optimization of alveolar expansion with HFOV markedly improves oxygenation but does not influence alveolar stability as long as the underlying cause, i.e. surfactant deficiency, is not reversed. This will occur by gradual synthesis of endogenous surfactant over time, or after exogenous surfactant instillation [15].

Morphologic changes were more pronounced in the animals that received CMV or HFOV alone compared to the animals that were lavaged only (Fig. 2). In surfactant-deficient lungs, high shear forces between open and closed alveoli are, to a great extent, held responsible for the damage caused by artificial ventilation [7]. Therefore, alveoli should be actively opened and kept open during the entire respiratory cycle in order to minimize additional lung damage [2, 3]. Studies in lung-lavaged rabbits demonstrated that HFOV had beneficial effects on preventing development of lung injury due to mechanical ventilation when arterial oxygenation was kept above 46.7 kPa (350 mmHg) (indicating alveolar expansion) and not when arterial oxygen tensions were maintained at 9.3-13.3 kPa (70-100 mmHg) [2, 3]. This may explain the higher degree of lung injury as seen in the present study in the group HFOV without surfactant. In that group, mean PaO_2 values dropped to <13.3 kPa (<100 mmHg) for only 30 min, after MAwP was decreased to ≤9 cm H_2O at the end of the observation period (Fig. 1). In contrast, mean PaO_2 values remained >46.7 kPa (350 mmHg) almost for the entire observation period, despite the reduction of the MAwP, in both surfactant-treated groups (HFOV and CMV). Also, histopathology examinations of both groups that received surfactant (HFOV and CMV) showed no additional structural lung damage in comparison with the animals that were lavaged only (Fig. 2). This result is supported by earlier experimental studies in which surfactant therapy has shown to improve uniform alveolar expansion and end-expiratory alveolar stability and thereby effectively prevents the progression of ventilator-induced lung injury [7,23]. In the CMV group without surfactant, mean PaO_2 values were <13.3 kPa (<100 mmHg) during the entire study period and evidenced the high lung injury score in this study (Fig. 2). In contrast to HFOV, the

CMV group did not receive a high-lung volume strategy. In pilot studies, McCulloch et al. [3] and Kolton et al. [24] tried to have a CMV group at high-lung volume but found that this was not possible. They concluded that the severity of lung lavage lesion necessitated such high ventilator pressures with CMV that fatal barotrauma terminated all such attempts [2].

In contrast to Jackson *et al.* [9], the current study found no difference in the prevention of lung damage between HFOV and CMV after surfactant therapy (Fig. 2). Further, it was shown that the HFOV group without surfactant showed more cellular infiltration and epithelial damage than the HFOV group with surfactant (Fig. 2). This indicates that achieving and maintaining alveolar expansion (i.e. open lung) is of more importance than the type of mechanical ventilation (HFOV vs. CMV). The importance of an open lung strategy is supported by the results of Amato and colleagues [25] who recently demonstrated in adults with ARDS that CMV with an open-lung approach had, for the first time, a significant impact on survival and barotrauma. Therefore, it was concluded that surfactant therapy with CMV is equally effective to prevent ventilator-induced lung injury as HFOV combined with surfactant, as long as alveoli are opened and kept open to avoid high shear stress. This can be achieved by the use of a PEEP level that sufficiently counterbalances the retractive forces or by higher and/or repeated doses of exogenous surfactant to reduce the retractive forces.

It is concluded that after surfactant therapy the use of high-frequency oscillatory ventilation was not superior to conventional mechanical ventilation in improving gas exchange, lung deflation stability and reducing lung injury, if lungs are kept expanded independently of the mode of ventilation. Furthermore, it was confirmed that high-frequency oscillatory ventilation with the high-lung volume strategy markedly improves blood gases but without improvement of lung function, in particular arterial oxygenation at low mean airway pressure, in surfactant-depleted rabbits. This indicates that the high mean airway pressure used with high-frequency oscillatory ventilation only counterbalances the increased collapse tendency due to surfactant deficiency and therefore exogenous surfactant therapy is still required.

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Chapter 6

Improved oxygenation by nitric oxide is enhanced by prior lung reaeration with surfactant, rather than positive-end expiratory pressure, in lung-lavaged rabbits

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Summary

Objective: The inhalation of nitric oxide increases oxygenation by improving ventilation-perfusion ratios in neonates with respiratory distress syndrome and those ratios in adults with acute respiratory distress syndrome. There is evidence that inhaled nitric oxide is ineffective when the lung remains atelectatic and poorly inflated. This study aimed to enhance nitric oxide delivery by improving lung aeration by means of exogenous surfactant or by increasing positive end-expiratory pressure.

Design: Experimental, comparative study.

Setting: Research laboratory of a large university.

Subjects: Twenty-eight adult New Zealand white rabbits (2.7 ± 0.3 kg).

Interventions: Lung injury was induced by repeated whole-lung lavage with saline. The animals were mechanically ventilated with a tidal volume of 10 ml/kg, an FiO_2 of 1.0 and a PEEP of 6 cm H_2O. Forty-five minutes after the last lavage, animals were randomly assigned to five groups. In two groups, lung aeration was first increased either by instillation of a low dose of exogenous surfactant (25 mg/kg) or by increasing the positive end-expiratory pressure to 10 cm H_2O, before inhalation of nitric oxide was started. In each of these animals, five different nitric oxide concentrations (4-20 parts per million) were inhaled for 30 min, followed by a 30 min washout period. The other three groups served as controls and received only one treatment protocol: nitric oxide (4-20 parts per million), or surfactant (25 mg/kg), or positive end-expiratory pressure (10 cm H_2O).

Measurements and Main Results: Before and after lavage, blood gases and lung mechanics were measured every 30 min. Both strategies to increase lung aeration improved PaO_2 values from 61 ± 13 mmHg (8.1 ± 1.7 kPa) to 200 to 300 mmHg (26.6 to 39.9 kPa) in 30 min. After inhalation of nitric oxide, additional increases of oxygenation were seen only in the animals that received a low dose (25 mg/kg) of surfactant. The control group that inhaled nitric oxide showed no significant change in oxygenation, and four of the six animals did not survive the observation period. In the two groups in which positive end-expiratory pressure was increased to 10 cm H_2O, half of the animals developed a pneumothorax during the observation period.

Conclusions: These data indicate that inhaled nitric oxide is able to improve arterial oxygenation after alveolar recruitment by means of a low dose of exogenous surfactant and not by elevation

of PEEP from 6 to 10 cm H_2O, in lung-lavaged rabbits.

Introduction

In 1987, nitric oxide, the endothelium-derived relaxing factor synthesized from L-arginine by the enzyme nitric oxide synthase, was identified as an important endogenous vasodilator [1]. 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 (RDS) and adults with acute respiratory distress syndrome (ARDS) [2-7]. It is assumed that inhaled nitric oxide rapidly diffuses across the alveolar barrier to vascular smooth muscle causing relaxation of the vascular smooth muscle which, in turn, causes vasodilation of the pulmonary vessels [8]. Excess nitric oxide that reaches the bloodstream binds rapidly and avidly to hemoglobin; this binding to hemoglobin eliminates the availability of nitric oxide for causing systemic vasodilation [8].

Recently, Kinsella and colleagues [9] reported that the inhalation of nitric oxide resulted in a limited success in improving blood gases, especially in newborns with reduced lung compliance. It has been suggested that the reduced lung volume will probably contribute to decreased efficacy of inhaled nitric oxide by decreased effective delivery of nitric oxide to the pulmonary vasculature [9,10]. Therefore, it could be expected that after reaeration of atelectatic lung regions by either exogenous surfactant or by increasing the positive end-expiratory pressure, the effect of inhaled nitric oxide on oxygenation will be enhanced, as long as a ventilation/perfusion mismatch is present.

To test this hypothesis, a study was designed in which we investigated the effects of inhaled nitric oxide combined either with a low dose of exogenous surfactant or increased positive end-expiratory pressure, on blood gases in surfactant-depleted rabbits.

Materials and methods

This 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/EEG). A total of 28 adult New Zealand white rabbits (IFFA-Credo, Brussels, Belgium) with a mean body weight of 2.7 ± 0.3 kg were anesthetized with intravenous pentobarbital

sodium (50 mg/kg) via an auricular vein. Tracheostomy was performed, and an uncuffed endotracheal tube was introduced into the trachea. Mechanical ventilation with an FiO_2 of 1.0 was performed using a ventilator (Servo 300, Siemens-Elema, Solna, Sweden) with volume-controlled mode, tidal volume of 10 ml/kg, positive end-expiratory pressure of 2 cm H_2O, frequency of 30 breaths/min, inspiration time of 25% and a pause time of 10%. An infusion of 2.5% glucose was continuously administered via the auricular vein as a maintenance fluid (5 ml/kg/h). Anesthesia was maintained with intermittent injection of pentobarbital sodium (5 mg/kg/h, i.v.); muscle paralysis was achieved with pancuronium bromide (0.1 mg/kg/h, i.m.). A femoral artery was cannulated with a polyethylene catheter for continuous blood pressure measurements and for intermittent blood sampling. Arterial samples were analysed for blood gases, hemoglobin and methemoglobin saturation using conventional methods (ABL-505 and Osm-3; Radiometer, Copenhagen, Denmark). Core temperature was measured using an esophageal thermistor (Elektrolaboratoriet, Copenhagen, Denmark) and maintained at 99 ± 1 °F (37 ± 0.5 °C) by a heating pad.

In all animals, respiratory insufficiency was induced by repeated whole-lung lavage (30 ml/kg) according to Lachmann *et al.* [11]. After the first lavage, positive end-expiratory pressure was increased to 6 cm H_2O (other ventilator settings were not changed) and whole-lung lavage was repeated until PaO_2 was <80 mmHg (<10.6 kPa). After the last lavage, all animals were ventilated for 45 min, and after blood gases were measured, the animals were randomly divided into five groups. The first group (n=6), received a low dose of surfactant (25 mg/kg) intratracheally which, 30 min later, was followed by inhalation of nitric oxide. Five different concentrations of nitric oxide (4, 8, 10, 16 and 20 parts per million [ppm]) were each inhaled for 30 min. The sequence of the five nitric oxide concentrations was randomized for each animal. After each nitric oxide inhalation for 30 min, blood gases were measured and nitric oxide was turned off for 30 min to get a new baseline blood gas value. In another group (n=6), positive end-expiratory pressure was increased from 6 to 10 cm H_2O, and, after 30 min, inhaled nitric oxide was given in the same way as described above. The other three groups served as controls and received the following treatments: (a) nitric oxide alone in the same way as described above (n=6); (b) 25 mg/kg surfactant alone (n=5); or (c) a positive end-expiratory pressure of 10 cm H_2O alone (n=5).

The surfactant used in this study was a freeze-dried natural surfactant isolated from minced pig lungs as previously described [12]. For instillation of the surfactant, animals were disconnected from the ventilator and received the surfactant suspension (25 mg/ml) directly into the endotracheal tube via a syringe. The animals were then immediately reconnected to the ventilator (ventilator settings were not changed).

A new prototype of a ventilator was used (Siemens-Elema, Solna, Sweden), with a built in, computerized, nitric oxide delivery system, consisting of an additional digital-controlled nitric oxide valve. An on-line electrochemical sensor was used to continuously measure expiratory nitric oxide and nitric dioxide concentrations. This system has been used in adult patients with ARDS and has proven to be reliable [5].

Data were collected at the following times: before lavage; 5 and 45 min after the lavage procedure; and every 30 min for 5.5 h. In the control group, that received nitric oxide only, data were collected for only 5 h because there was no lung aeration improvement procedure. At each data collection point, PaO_2, $PaCO_2$, methemoglobin, blood pressures, peak airway pressure, positive end-expiratory pressure and mean airway pressures were measured.

Statistical analysis of the data was performed using the SAS statistical package (SAS Users Guide, 1990, SAS Institute Inc., Cary NC). Between-group differences for PaO_2, $PaCO_2$, mean arterial pressure, mean airway pressure and peak pressure were tested with an analysis of variance (ANOVA) for repeated time measurements using the general linear models procedure. In addition, a paired *t*-test was performed to test the effect of each nitric oxide concentration on PaO_2 within one group. When nitric oxide was switched on for 30 min, the mean PaO_2 value was compared with the mean baseline PaO_2 value that was defined as the mean PaO_2 of both washout periods before and after nitric oxide was switched on. Differences were accepted as significant at $p<0.05$. For the nitric oxide effects, no adjustments were made for multiple comparisons.

Results

In a preliminary study [unpublished observations], it was found that giving a dose of 25 mg surfactant per kg body weight or increasing the positive end-expiratory pressure level from 6 to 10 cm H_2O after the lavage procedure, led to an improvement of arterial oxygenation to 50% of the prelavage PaO_2 values (PaO_2/FiO_2 from 60 to 80 mmHg [8.0 to 10.7 kPa] to 200-300 mmHg

[26.7 to 40.0 kPa]).

In the two groups receiving exogenous surfactant (25 mg/kg), mean PaO_2 values increased from 58 ± 11 mmHg (7.7 ± 1.5 kPa) to 283 ± 64 mmHg (37.6 ± 8.5 kPa) in 30 min (Figure 1). After additional nitric oxide inhalations, PaO_2 further increased and decreased when nitric oxide was switched off. The mean PaO_2 values of the group receiving the combination of surfactant and nitric oxide were significantly higher compared with the group that received surfactant only (Figure 1). In this last group, PaO_2 was maximal at 30 min after surfactant instillation and decreased over time (Figure 1). $PaCO_2$ and peak airway pressure decreased directly after surfactant instillation but slowly increased during the following observation period (Table 1).

In the two groups in which positive end-expiratory pressure was increased from 6 to 10 cm H_2O, mean PaO_2 values increased from 62 ± 15 mmHg (8.2 ± 2.0 kPa) to 239 ± 48 mmHg (31.8 ± 6.4 kPa) in 30 min (Figure 1). After nitric oxide was inhaled by one of these groups, no significant difference in mean PaO_2 values was seen compared with the group that received a positive end-expiratory pressure of 10 cm H_2O only (Figure 1). In the group receiving the combination of positive end-expiratory pressure of 10 cm H_2O and inhaled nitric oxide, mean PaO_2 values improved over time but did not decrease when nitric oxide was switched off. Peak airway pressure and mean airway pressure were significantly higher in the two groups that received a positive end-expiratory pressure of 10 cm H_2O as compared with the two surfactant treated groups; half of the animals in both positive end-expiratory pressure groups developed a pneumothorax during the observation period (Table 1).

There was no change in mean PaO_2 values after inhalation of nitric oxide in the group that received nitric oxide only (Figure 1). In this group, $PaCO_2$ and peak airway pressure increased over time and 4 of the 6 animals did not survive the observation period (Table 1). For the three groups that received nitric oxide, the mean changes of PaO_2 per nitric oxide concentration are given in Figure 2. The PaO_2 response was higher in the group receiving the combination of surfactant and nitric oxide for each nitric oxide concentration but there was no difference in PaO_2 response to the different nitric oxide concentrations used (Figure 2). Non-response (defined as an increase of PaO_2 of < 10% above the baseline PaO_2 value) was 27% in group surfactant and nitric oxide; 93% in group positive end-expiratory pressure and nitric oxide;

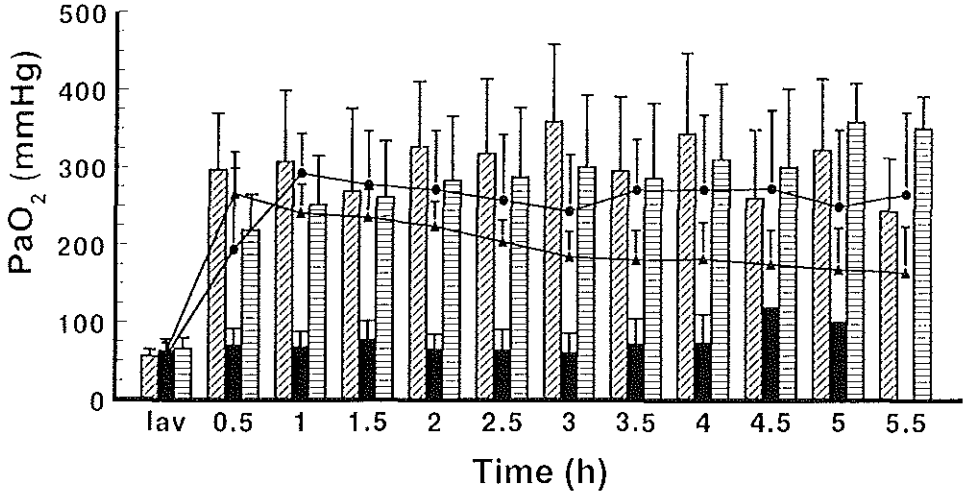

Figure 1. Mean PaO_2 values (±SD) of all 5 groups. *Diagonal-striped bar*: animals (n=6) treated with a low dose of exogenous surfactant (25 mg/kg) and inhaled nitric oxide; *horizontal-striped bar*: animals (n=6) treated with a positive end-expiratory pressure of 10 cm H_2O and inhaled nitric oxide; *solid bar*: animals (n=6) treated with inhaled nitric oxide alone. The *solid lines* are two control groups: *triangles*, animals (n=5) treated with a low dose of exogenous surfactant (25 mg/kg) alone; *circles*, animals (n=5) treated with a positive end-expiratory pressure of 10 cm H_2O. *Lav*, 45 min after the last lavage. To covert mmHg to kPa multiply the value by 0.1333.

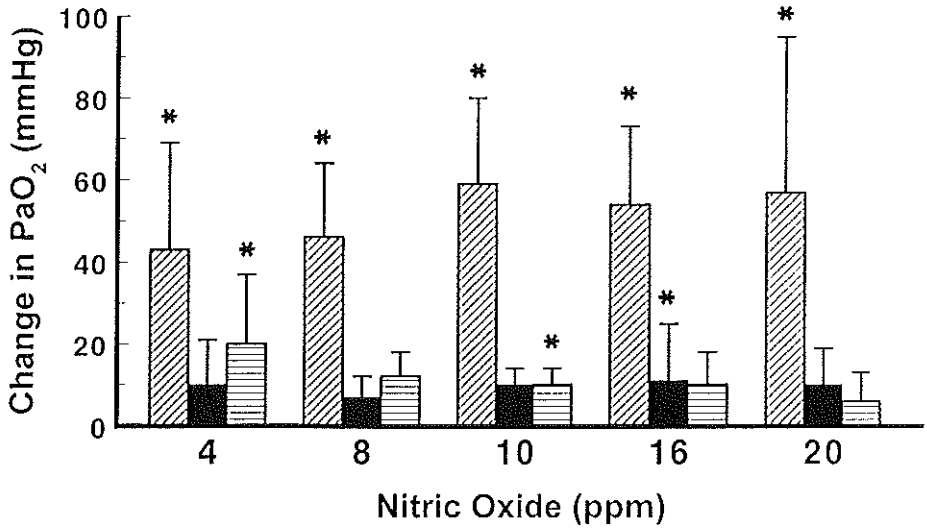

Figure 2. Mean change in PaO_2 values (±SD) per nitric oxide concentration for the three different groups that inhaled nitric oxide. *Diagonal-striped bar*: animals (n=6) treated with a low dose of exogenous surfactant (25 mg/kg) and inhaled nitric oxide; *horizontal-striped bar*: animals (n=6) treated with a positive end-expiratory pressure of 10 cm H_2O and inhaled nitric oxide; *solid bar*: animals (n=6) treated with inhaled nitric oxide alone. * = significant improvement of PaO_2 due to inhaled nitric oxide (evaluated with a paired *t*-test by comparing the mean PaO_2 value when nitric oxide was switched on for 30 min with the baseline PaO_2 value). The baseline blood gas value was defined as the mean PaO_2 value of both wash out periods before and after nitric oxide was switched on. To convert mmHg to kPa, multiply the value by 0.1333.

and 58% in the group that received nitric oxide only.

Mean arterial pressure did not change after inhaling any nitric oxide in the different groups (Table 1). Methemoglobin concentration remained low (0.3 ± 0.1%) and there was no increase after any nitric oxide inhalation in any of the groups. The expired nitric dioxide concentration was never > 1 ppm during any nitric oxide inhalation in any of the groups The expired nitric dioxide concentrations are given in Table 2.

Table 1. $PaCO_2$ (mmHg), MAP (mmHg), mean airway pressure (MAwP) and peak pressure (cm H_2O) in the five study groups (mean ± SD).

	Prelavag	Group	Lavage	30'	60'	90'	120'	150'	180'	210'	240'	270'	300'	330'
$PaCO_2$	36±3	SURF+NO	58±10	46±6	45±3	48±6	48±7	48±7	48±8	48±9	49±10	50±11	50±11	50±10
	37±3	PEEP+NO	57±7	54±6	57±6	58±4	60±5	58±5	57±7	55±6	55±4	55±5§§§	53±2	51±1
	34±5	NO	52±6	51±10	54±12	55±14	57±13	60±14	60±18†	55±19†	53±17†	54±2†	54±2	
	32±6	SURF	55±6	48±3	49±6	49±2	48±3	50±3	50±5	51±5	51±6	51±6	52±7	52±6
	32±4	PEEP	46±4	45±4	46±7	47±3	48±4	49±6	53±3§	53±3	55±3§	54±2	57±1§	54±4
MAP	97±9	SURF+NO	90±10	82±11	81±7	81±11	81±7	83±7	87±9	88±6	90±10	89±9	91±10	89±9
	108±11	PEEP+NO	101±14	87±9	86±12	89±13	86±14	82±12	78±10	78±11	78±9	77±8§§§	76±6	70±15
	99±8	NO	88±13	82±17	83±14	85±10	88±10	89±15	74±18	77±23†	82±9†	87±3†	85±4	
	108±7	SURF	91±9	90±14	89±13	90±14	89±12	89±14	94±13	88±13	91±17	86±15	83±25	80±19
	104±11	PEEP	94±10	89±5	91±11	89±5	85±5	91±9	90±9§	88±1	85±5§	84±5	90±4§	76±16
MAwP	5±3	SURF+NO	11±1	10±1	10±1	10±1	10±1	10±1	10±1	10±1	10±1	11±1	11±1	11±1
	6±1	PEEP+NO	12±1	17±2	17±2	17±2	17±2	17±2	17±2	17±2	17±2	17±2§§§	18±2	18±2
	5±0	NO	11±1	12±1	12±1	12±1	12±1	12±1	12±1	12±1†	12±2†	12±2†	12±2	
	5±0	SURF	11±1	10±1	11±1	11±1	11±1	11±1	11±1	11±1	11±1	11±1	11±1	11±1
	6±1	PEEP	12±2	16±1	16±1	16±1	16±1	16±1	16±1§	16±1	16±2§	16±2	15±1§	15±1
Ppeak	11±1	SURF+NO	24±2	22±2	22±2	22±3	23±3	23±3	23±3	23±3	23±2	23±2	23±2	24±2
	13±2	PEEP+NO	24±2	28±1	27±1	27±1	28±1	28±1	28±1	28±1	28±2	28±1§§§	27±1	27±1
	13±2	NO	25±1	26±2	26±2	28±2	28±1	29±2	29±2	30±1†	30±1†	30±1†	30±1	
	12±1	SURF	25±2	24±2	24±2	24±2	25±2	25±3	25±3	25±3	25±3	25±3	25±3	25±3
	12±1	PEEP	26±2	29±2	30±2	29±1	29±1	30±1	31±1§	31±1	32±1§	32±1	32±1§	31±1

SURF+NO, represents the animals treated with surfactant and nitric oxide; PEEP+NO, represents the animals treated with a PEEP of 10 cmH_2O and nitric oxide; NO, represents the animals treated with nitric oxide only; SURF, represents the animals treated with surfactant only; PEEP, represents the animals treated with PEEP of 10 cm H_2O only; MAP, mean arterial pressure; MAwP, mean airway pressure; Ppeak, peak inspiratory pressure; †, death of one animal; §, pneumothorax of one animal.

Table 2. Expiratory nitric oxide (NO) and nitric dioxide (NO_2) concentrations measured with an on-line electrochemical sensor (mean±SD).

NO (ppm)	NO-expired (ppm)	NO_2-expired (ppm)
4	3.7±0.6	0.25±0.04
8	7.2±0.6	0.34±0.05
10	8.8±0.8	0.38±0.06
16	14.3±0.7	0.60±0.05
20	17.7±0.7	0.78±0.07

Discussion

The application technique limits the pharmacologic effect of nitric oxide to the aerated regions of the lungs [8]. Therefore, progressive atelectasis, as seen in severe RDS or ARDS, decreases effective delivery of this inhalational agent to its site of action in the terminal lung units [7,9,10]. This hypothesis was confirmed by the results of the present study, in which inhalation of nitric oxide was less efficacious in improving arterial oxygenation in the control group that received inhaled nitric oxide only, as compared with the group with prior administration of exogenous surfactant (Figure 2). In congenital diaphragmatic hernia lambs, Karamanoukian *et al.* [13] reported that the combination of exogenous surfactant and inhaled nitric oxide is beneficial to improving arterial oxygenation. They [13] found that inhalation of 80 ppm of nitric oxide for 10 min did not improve oxygenation without prior administration of surfactant (50 mg/kg) [13]. From experimental and clinical studies [12,14] in neonatal RDS, it is known that instillation of 100 to 200 mg/kg of surfactant resulted in recruitment of atelectatic lung regions with maximal improvement of arterial oxygenation. In the present study, we administered a dose of only 25

mg/kg surfactant, and the results showed that PaO_2 improved to 50% of the prelavage values within 30 min and decreased over time due to diminished surfactant function (Figure 1). After treatment of late-stage RDS and ARDS with a low dose of surfactant, transient improvement of PaO_2 is attributed to inhibition of the exogenous surfactant by plasma-derived proteins that are filling the alveolar space due to leakage of the alveoli-capillary membrane [14-16]. In addition, in the present study, arterial oxygenation was significantly higher during the whole observation period in animals that received a combination of surfactant and inhaled nitric oxide, compared with the group that received a low dose (25 mg/kg) of surfactant only (Figure 1). This result may imply that inhaled nitric oxide had a therapeutic effect on the lung injury. In patients with acute lung injury, Benzing and colleagues [17] showed that inhalation of 40 ppm nitric oxide decreased pulmonary transvascular albumin flux. The exact mechanism is not yet known, but the authors [17] suggested that the decreased pulmonary capillary pressure due to inhaled nitric oxide reduced transvascular filtration. Therefore, we speculate that in our study, inhaled nitric oxide may have decreased the influx of plasma proteins into the alveolar space, and thereby decreased the inhibition of the low dose (25 mg/kg) of exogenous surfactant, leading to higher PaO_2 values in lung-lavaged rabbits.

Other strategies designed to recruit atelectatic lungs, such as increased positive end-expiratory pressure, may be as beneficial as surfactant therapy in the delivery of inhaled nitric oxide to the target cells. In the present study, arterial oxygenation improved after positive end-expiratory pressure was increased from 6 to 10 cm H_2O but no additional effect of inhaled nitric oxide was seen on PaO_2 (Figure 1). Furthermore, half of the animals of both positive end-expiratory pressure groups developed a pneumothorax during the observation period indicating that the used peak airway pressures were high (Table 1). From clinical experience, it is known that one of the benefits of surfactant therapy includes lower peak airway pressures with reduced risk of barotrauma [14,16], which is confirmed by the results of this study (Table 1). Putensen *et al.* [18] demonstrated that in dogs with oleic acid-induced lung injury, adequate recruitment of the lung by a positive end-expiratory pressure of 10 cm H_2O was essential to get an increase in oxygenation after inhaled nitric oxide compared with a control group without positive end-expiratory pressure. Also, in adult patients with ARDS, Puybasset *et al.* [7] reported that the effect of nitric oxide on PaO_2 was potentiated by the application of 10 cm H_2O positive end-

expiratory pressure. This potentiation occurred only in patients in whom positive end-expiratory pressure had induced a significant alveolar recruitment. Thus, it seems realistic to conclude that alveolar recruitment by positive end-expiratory pressure can also improve the efficacy of inhaled nitric oxide. However, we speculate that in the present study, the airway pressures used were too high, leading to high intrathoracic pressures that made vasodilation of the pulmonary vasculature impossible due to inhaled nitric oxide. Therefore, we suggest that alveolar recruitment induced by exogenous surfactant is more beneficial than increased positive end-expiratory pressure for improving arterial oxygenation due to inhaled nitric oxide because of the use of lower airway pressures.

The results of previous clinical observations are controversial concerning the dose-dependency of inhaled nitric oxide [5,6,19,20]. In the present study, there was no dose-dependent effect of the different used nitric oxide concentrations (4 to 20 ppm) (Figure 2). Most of the animals had a different nitric oxide concentration by which the change in arterial oxygenation was maximal. It appears that each lung has its own 'optimal' nitric oxide concentration that probably depends of the severity of the disease process (i.e., atelectasis, edema). In the present study, a low doses of nitric oxide (<20 ppm) were used because of the demonstrated efficacy of low doses in animal experiments and patients, and to minimize toxicity [21]. In the presence of oxygen, nitric oxide is rapidly oxidized to nitrite, or nitrates that can induce tissue damage [22]. Also, nitric oxide reacts with superoxide anions to produce peroxynitrite [22]. Haddad and colleagues [23,24] demonstrated *in vitro* that peroxynitrite inhibits pulmonary surfactant function by lipid peroxidation and damaging surfactant proteins. In the present study, there was no evidence that the surfactant function was more decreased after inhalation of nitric oxide when compared with the control group that only received surfactant (Figure 1). Furthermore, severe methemoglobinemia due to inhalation of nitric oxide has been reported [25]. However, in the present study, no changes in methemoglobin concentration were observed with the used nitric oxide concentrations that were used during the 30 min inhalation periods.

In the present study, we used the lung lavage model which has proved to be a consistent and convenient model of acute lung injury [11]. Repeated whole-lung lavage produces an acute quantitative surfactant deficiency. This deficiency, together with conventional mechanical ventilation, leads to severe lung injury with impaired gas exchange, decreased lung compliance

and functional residual capacity, increased permeability changes of the alveoli-capillary membrane with edema, and sustained pulmonary hypertension [11,12,26]. In the present study, animals were first ventilated for 45 min after the last lavage before treatment was started to induce a more severe lung injury. Although an untreated control group was not used in this study, we [27] have previously demonstrated with the same model that following the lavage procedure, there is no spontaneous improvement in oxygenation or lung mechanics over a 6 h period. Despite the fact that the lung injury in this study is not representative of the pathology as seen in humans with ARDS, this model is ideal for testing various therapeutic interventions that may prove therapeutic for acute lung injury [12,27-32].

We conclude that in lung lavaged rabbits, the effect of nitric oxide on improving oxygenation is superior when lung aeration is increased with exogenous surfactant rather than with positive end-expiratory pressure. In neonates with RDS and patients with ARDS, it has been shown that both exogenous surfactant and nitric oxide increase PaO_2 by improving the ventilation /perfusion match [2-6,14,16]. Whereas the inhalation of nitric oxide improves perfusion of the ventilated areas of the lung, instillation of exogenous surfactant leads to improvement of the ventilation by reaeration of atelectatic regions. Therefore, combined therapy of exogenous surfactant and nitric oxide inhalation could be clinically important, especially in patients with ARDS. Nevertheless, in terms of our goal of improving oxygenation while diminishing lung injury, it remains unclear whether a low dose of surfactant plus inhaled nitric oxide is more optimal than a high dose surfactant.

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Chapter 7

Combining partial liquid ventilation with nitric oxide to improve gas exchange in acute lung injury

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Summary

Objective: To assess the effects of increasing concentrations of inhaled nitric oxide (NO) during incremental dosages of partial liquid ventilation (PLV) on gas exchange, hemodynamics and oxygen transport in pigs with induced acute lung injury (ALI).

Design: prospective experimental study.

Setting: Experimental intensive care unit of a university.

Subjects: 6 pigs with induced ALI.

Interventions: Animals were surfactant depleted by lung lavage to a partial pressure of oxygen in arterial blood (PaO_2) < 100 mmHg. They then received four incremental doses of 5 ml/kg perflubron (LiquiVent). Between each dose the animals received 0, 10, 20 , 30, 40 and 0 parts per million (ppm) NO.

Measurements and Main Results: Blood gases, hemodynamic parameters, and oxygen delivery were measured after each dose of perflubron as well as after each NO concentration. Perflubron resulted in a dose-dependent increase in PaO_2. At each perflubron dose, additional NO inhalation resulted in a further significant (ANOVA $p<0.05$) increase in PaO_2, with a maximum effect at 30 ± 10 ppm NO. The 5 ml/kg perflubron dose led to a significant decrease in mean pulmonary artery pressure which decreased further with higher NO concentrations.

Conclusions: PLV can be combined with NO administration and results in an cumulative effect on arterial oxygenation and to a decrease in pulmonary artery pressure, without having any deleterious effect on measured systemic hemodynamic parameters.

Introduction

The presence of an increased surface tension at the alveolar air-liquid interface during acute lung injury (ALI) leads to end-expiratory alveolar collapse, atelectasis, right-to-left shunt and a decrease in the partial pressure of oxygen in arterial blood (PaO_2), finally resulting in hypoxemia [1]. Rational therapies to treat this condition are: first, counterbalancing the increased tendency for collapse by applying positive end-expiratory pressure (PEEP) to prevent end-expiratory collapse, and/or decreasing alveolar surface tension by application of exogenous surfactant. Another option could be elimination of the air-liquid interface by filling the lung with a fluid that is able to maintain gas exchange. Such a fluid could be perfluorocarbon (PFC), which is capable of dissolving large amounts of respiratory gases. Recently, the use of PFC was modified by filling the injured lung up to the functional residual capacity (FRC) level with PFC, and ventilating the lung with normal gas ventilation superimposed on the fluid-filled lung. This type of ventilation is called partial liquid ventilation (PLV), or perfluorocarbon associated gas exchange (PAGE). This technique was used in animals suffering from respiratory failure of different etiologies showing an improvement in gas exchange [2-9]. In some of these studies, there was a clear dose dependent effect on oxygenation, creating the possibility for titrating the amount of PFC to its effect. The results of a clinical pilot study on the use of PAGE have recently been published [10].

Besides hypoxemia due to atelectatic regions in the lung, other serious problems complicating the treatment of ALI are disabled ventilation/perfusion matching and pulmonary hypertension. Administration of inhaled nitric oxide (NO) in adult patients with acute respiratory distress syndrome (ARDS) has been shown to cause selective vasodilation of the pulmonary vasculature of ventilated lung regions leading to an improved oxygenation due to a decrease in pulmonary right-to-left shunt [11-13].

Based on these findings we hypothesized that, after increasing the area of gas exchange at end-expiration by PLV, the administration of NO by inhalation may further enhance oxygenation and result in decreased pulmonary artery pressures. To test this hypothesis we investigated the effects of increasing concentrations of NO during incremental dosages of PFC on gas exchange, hemodynamics and oxygen transport in pigs with induced ALI.

Materials and methods

Animal preparation

The study protocol was approved by the University's animal experimental committee. Anesthesia was induced in 6 female Yorkshire pigs (weight 7 ± 1 kg) with ketamine (10 mg/kg) and midazolam (0.5 mg/kg), and was maintained with a continuous infusion of ketamine (80 µg/kg/min) and midazolam (9 µg/kg/min). All animals were tracheotomized, intubated with a 6.0 mm endotracheal tube fitted with a Filtraflux heat-moisture exchanger with built-in bacterial filter (ICHOR AB, Bromma, Sweden) and cannulated with a carotid artery catheter, a 5 Fr pulmonary artery catheter (SP51055H Viggo-Spectramed, Wiltshire, UK), a continuous blood gas monitoring sensor (Paratrend 7, Pfizer, Biomedical Sensors, High Wycombe, UK) placed in the left femoral artery, and a central venous catheter. During animal preparation, volume controlled ventilation with a Servo 300 ventilator equipped with a built-in NO administration module (Siemens, Solna, Sweden) (set at frequency 20 /min, inspiratory time 25%, pause time 10%, inspiratory rise time 5%, PEEP 5 cm H_2O and 100% oxygen) was used. Muscle relaxation was achieved by a continuous infusion of pancuronium bromide (2.5 µg/kg/min). Minute ventilation was set to deliver tidal volumes of 10 ml/kg body weight. These ventilator settings were maintained during the entire study period.

All animals were surfactant depleted according to Lachmann et al. [14] by repeated lung lavage with warm saline (38°C, 30 ml/kg) to reduce PaO_2 below 100 mmHg. Subsequently, all animals were ventilated for 1 hour to obtain stable baseline values. Following this baseline period all animals received four intratracheal doses of 5 ml/kg perflubron (LiquiVent; Alliance Pharmaceutical Corporation, San Diego, USA); in between these doses a sequence of different concentrations of NO (0, 10, 20, 30, 40 and 0 ppm NO) was added to the inspiratory gas. Each concentration of NO was administered for period of about 10 min.

Measurements

Arterial and mixed venous samples were analyzed for blood gases, pH and mixed venous oxygen saturation (SvO_2) and hemoglobin concentration by conventional methods (ABL-505 OSM-3 combination, Radiometer, Copenhagen, Denmark). This combination was used to calculate base excess (BE), intrapulmonary shunt, arterial oxygen content (C_aO_2), oxygen delivery and

arteriovenous oxygen content difference. Additionally blood gases were monitored continuously by means of a bloodgas monitor.

Using Statham P23XL transducers (Spectramed, Oxnard, CA, USA), systolic, diastolic and mean arterial pressure as well as systolic (SysPAP) diastolic (DiaPAP) and mean pulmonary artery pressure (MPAP), pulmonary capillary wedge pressure and central venous pressure were recorded in all animals. Cardiac output was measured in triplicate using the thermodilution technique with 5 ml saline, using a Sirecust 1280 monitor (Siemens, Danvers, MA, USA) that also traced heart rate. This monitor was also used to calculate pulmonary vascular resistance and systemic vascular resistance.

All measurements were recorded just prior to a change in PFC and/or NO concentration. At the end of the study period all animals were sacrificed with an intracardiac overdose of KCl.

Statistical analysis

Statistical analyses were performed using the Instat 2.0 biostatistics package (GraphPad Software, San Diego, USA). For each PFC dose and subsequent NO concentrations intra-group comparisons were made with repeated measures ANOVA. If ANOVA resulted in a $p < 0.05$ a Dunnett post test was performed. This post test used the data measured after each increment of perflubron with the first 0 ppm NO setting as control value. A p value of 0.05 was taken as significance level. All data are reported as mean values ± standard error of the mean (SEM).

Results

Before and after lung lavage in all animals all data for blood gases and hemodynamics were comparable ($p > 0.05$, ANOVA). No improvement in blood gases was observed during the 1-h postlavage period. All animals survived the study period.

Gas exchange parameters (Table 1)

Administration of increments of perflubron of 5 ml/kg resulted in an increase in PaO_2 of 22.0, 55.3, 47.5 and 51.2 mmHg (i.e. 35, 58, 28 and 24% resp.); supplemental NO inhalation at each dose of perflubron resulted in an additional significant increase in PaO_2 with a maximum effect of NO at 20-30 ppm NO (Fig 1a). The incremental increases in perflubron dose did not result

in a statistically significant improvement of $PaCO_2$ and pH values; however, these $PaCO_2$ and pH values showed a dose-dependent significant improvement when combined with additional NO administration at the 15 and 20 ml/kg body weight perflubron dose. There were no significant changes in BE during the study period.

The first dose of 5 ml/kg BW perflubron resulted in a significant improvement in SvO_2 from 48.5 ± 5.3% to 54.9 ± 9.6%. Additional NO inhalation at the 15 and 20 ml/kg perflubron doses significantly improved SvO_2 at 10-30 ppm NO.

Shunt was reduced at each increment of perflubron reaching statistical significance at 10-20 ml/kg perflubron. At each dose of perflubron additional NO administration further reduced shunt, although these changes were not significant.

Each increment in perflubron resulted in an increase in mean CaO_2; at the 5 and 10 ml/kg perflubron doses CaO_2 was significantly increased by additional NO administration. At each dose of perflubron, DO_2 was not significantly changed by NO administration.

Online blood gas recordings showed a time-related effect of NO on blood gases. Figure 2 shows the dose-dependent improvement in gas exchange in one animal at a dose of 10 ml/kg perflubron. Figure 3 shows a rapid (< 3 min) decrease in PaO_2 from 140 to 89 mm Hg, as a result of switching from 40 to 0 ppm NO in an other animal at a perflubron dose of 5 ml/kg.

Hemodynamics (Table 2)

Administration of 5 ml/kg perflubron to a total dose of 10 ml/kg resulted in a significant decrease in SysPAP (-14%) and MPAP (-11%). All other decreases in pulmonary artery pressures were observed during additional NO administration: SysPAP showed a significant decrease with additional 30 ppm NO (15 and 20 ml/kg perflubron), DiaPAP showed a significant decrease with additional 30-40 ppm NO inhalation (15 ml/kg perflubron) and MPAP values (Fig 1b) showed a significant dose-dependent decrease at the 10, 15 and 20 ml/kg BW perflubron doses.

Stopping NO administration resulted in a significant rebound hypertension in MPAP at a dose of 5 ml/kg BW perflubron, with a simultaneous increase in pulmonary vascular resistance.

This was reduced significantly at a dose of 10 ml/kg BW perflubron with 40 ppm NO and at a dose of 15 ml/kg BW perflubron with 20 ppm NO.

There was a significant decrease in HR at the 10 ml/kg perflubron dose with additional 40 ppm NO. There were no significant changes in any other measured or calculated hemodynamic parameters.

Table 1 Data on gas exchange parameters in pigs ($n = 6$) with ALI following a combination of perflubron (LiquiVent) and NO. [a] Values are mean ± SEM

	pCO_2 (mmHg)				pH			
Baseline:[b]	55.2 ± 3.1	60.8 ± 7.5	58.6 ± 6.6	56.1 ± 6.2	7.27 ± 0.031	7.25 ± 0.044	7.23 ± 0.059	7.26 ± 0.054
LiquiVent:	5 ml/kg	10 ml/kg	15 ml/kg	20 ml/kg	5 ml/kg	10 ml/kg	15 ml/kg	20 ml/kg
0 ppm NO	52.8 ± 2.5	53.2 ± 4.5	61.0 ± 8.0	57.3 ± 6.4	7.26 ± 0.028	7.25 ± 0.051	7.22 ± 0.061	7.25 ± 0.052
10 ppm NO	50.2 ± 3.2	53.0 ± 5.7	56.7 ± 6.6	52.5 ± 4.6	7.29 ± 0.036	7.26 ± 0.056	7.26 ± 0.058*	7.27 ± 0.048
20 ppm NO	49.2 ± 2.9	53.1 ± 6.0	55.5 ± 6.6*	51.2 ± 4.9*	7.29 ± 0.040	7.26 ± 0.057	7.27 ± 0.056*	7.28 ± 0.046*
30 ppm NO	49.9 ± 3.5	54.5 ± 5.9	54.5 ± 5.8*	51.5 ± 4.9*	7.28 ± 0.047	7.25 ± 0.058	7.27 ± 0.055*	7.29 ± 0.044*
40 ppm NO	50.4 ± 3.7	55.5 ± 6.4	53.4 ± 6.0*	50.7 ± 4.6*	7.28 ± 0.043	7.25 ± 0.057	7.27 ± 0.055*	7.29 ± 0.040*
0 ppm NO	60.8 ± 7.5	58.6 ± 6.6	56.1 ± 6.2	52.2 ± 4.5	7.25 ± 0.044	7.23 ± 0.059	7.26 ± 0.054	7.28 ± 0.036
	Shunt (%)				Mixed venous O_2 saturation (%)			
Baseline:	43.4 ± 7.1	42.3 ± 7.3*	32.4 ± 5.4*	26.5 ± 3.7*	48.5 ± 5.3*	54.9 ± 9.6	65.7 ± 4.7	71.2 ± 3.9
LiquiVent:	5 ml/kg	10 ml/kg	15 ml/kg	20 ml/kg	5 ml/kg	10 ml/kg	15 ml/kg	20 ml/kg
0 ppm NO	37.2 ± 6.2	32.8 ± 6.0	25.6 ± 3.7	19.2 ± 1.9	60.2 ± 7.5	58.9 ± 7.0	67.5 ± 5.0	73.0 ± 3.3
10 ppm NO	32.7 ± 5.6	25.7 ± 3.9	21.5 ± 2.7	19.5 ± 1.9	63.5 ± 7.5	64.6 ± 6.2	73.1 ± 3.6	77.6 ± 4.3*
20 ppm NO	28.7 ± 4.6	25.7 ± 3.5	20.6 ± 2.1	16.4 ± 1.1	63.9 ± 6.7	64.9 ± 5.2	80.6 ± 5.6*	75.1 ± 3.2
30 ppm NO	27.4 ± 4.1	24.1 ± 3.1	21.4 ± 2.5	16.2 ± 1.1	63.4 ± 7.1	66.8 ± 5.9	76.2 ± 3.3*	76.4 ± 2.9
40 ppm NO	28.4 ± 4.2	24.1 ± 3.1	22.7 ± 2.5	16.2 ± 1.2	62.9 ± 7.0	70.0 ± 4.4	75.7 ± 3.8	76.0 ± 3.7
0 ppm NO	42.3 ± 7.3	32.4 ± 5.4	26.5 ± 3.7	19.4 ± 2.3	54.9 ± 9.6	65.7 ± 4.7	71.2 ± 3.9	72.5 ± 3.8
	Arterial oxygen content (ml/dl)							
Baseline:	12.1 ± 0.9	13.7 ± 1.1	15.7 ± 1.2	16.3 ± 1.2				
LiquiVent:	5 ml/kg	10 ml/kg	15 ml/kg	20 ml/kg				
0 ppm NO	13.3 ± 0.9	15.1 ± 1.0	16.5 ± 1.3	17.3 ± 1.0				
10 ppm NO	15.1 ± 0.9*	16.5 ± 1.1*	16.8 ± 1.1	17.0 ± 1.0				
20 ppm NO	15.4 ± 0.8*	16.2 ± 1.1*	17.1 ± 1.1	17.1 ± 0.9				
30 ppm NO	15.8 ± 0.9*	16.3 ± 0.9*	16.7 ± 1.2	17.2 ± 0.9				
40 ppm NO	16.0 ± 0.9*	16.4 ± 0.9*	16.7 ± 1.0	16.9 ± 1.0				
0 ppm NO	13.7 ± 1.1	15.7 ± 1.2	16.3 ± 1.2	16.8 ± 1.0				

* $p < 0.05$

[a] Intragroup comparisons ANOVA with Dunnett posttest if ANOVA $p < 0.05$, using the data after each increment of LiquiVent as control

[b] Baseline represents the data prior to an increase in LiquiVent dose

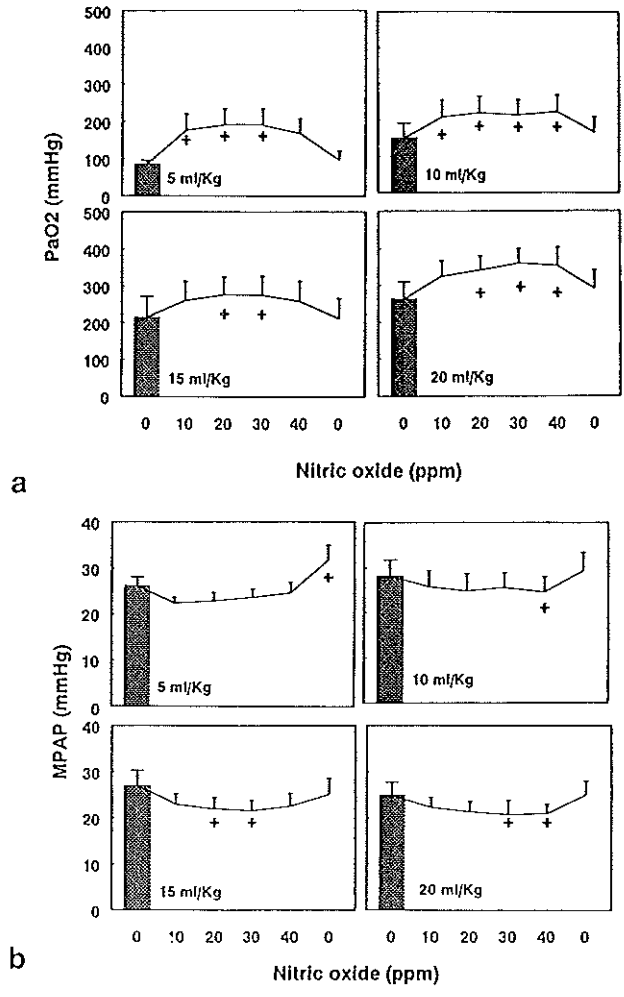

Figure 1. Arterial oxygen tension (PaO_2) (a), and mean pulmonary artery pressure (MPAP) (b) in relation to the amount of LiquiVent followed by different NO concentrations in parts per million (ppm) in pigs (n=6) with acute lung injury. *Bars* represent increments of perflubron. Data are means ± SEM. Intra-group comparisons ANOVA with Dunnet post-test if ANOVA ($p<0.05$), using the data after each increment of perflubron with the first setting 0 ppm NO as control

+ = p <0.05.

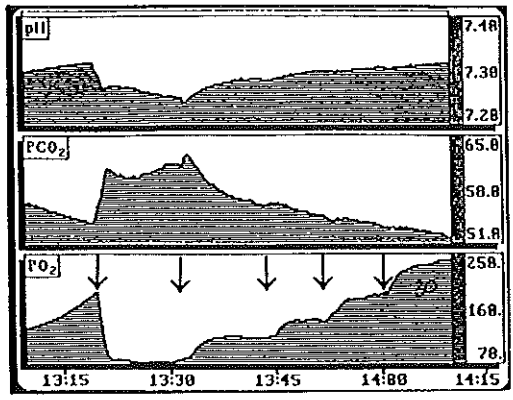

Figure 2. Print-out from the online blood gas monitor, showing the effect of different doses of inhaled NO (0, 10, 20, 30 ppm) on pH, PCO_2 and PO_2 in one pig ventilated with 10 ml/kg LiquiVent.

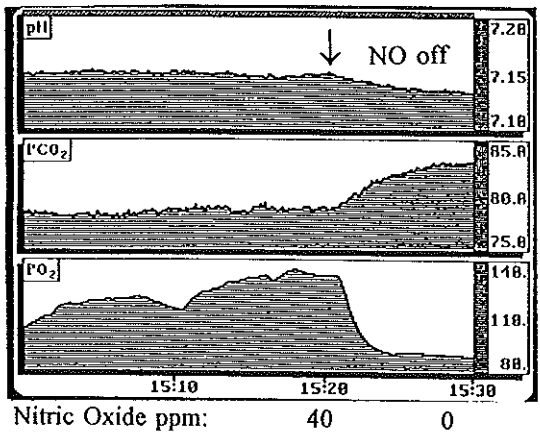

Figure 3. Print-out from the on-line blood gas monitor, showing the effect on pH, PCO_2 and PO_2 when switching from 40 to 0 ppm NO in one pig ventilated with 5 ml/kg LiquiVent.

Table 2 Data on hemodynamic parameters in pigs ($n = 6$) with ALI following the combination of perflubron (LiquiVent) and NO.[a] Values are mean ± SEM

	Mean arterial pressure (mmHg)				Systolic pulmonary pressure (mmHg)			
Baseline[b]:	96.2 ± 5.4	91.0 ± 6.8	95.5 ± 5.1	91.7 ± 3.8	37.8 ± 3.4	39.4 ± 3.6*	37.6 ± 4.8	33.4 ± 4.1
LiquiVent:	5 ml/kg	10 ml/kg	15 ml/kg	20 ml/kg	5 ml/kg	10 ml/kg	15 ml/kg	20 ml/kg
0 ppm NO	94.0 ± 5.0	97.8 ± 4.7	95.7 ± 6.7	93.5 ± 4.4	32.0 ± 1.7	33.8 ± 4.2	33.4 ± 3.6	31.8 ± 3.1
10 ppm NO	96.8 ± 5.5	98.0 ± 6.6	93.0 ± 6.3	89.3 ± 3.8	27.0 ± 0.8	32.2 ± 3.6	28.0 ± 2.5	28.4 ± 2.5
20 ppm NO	95.8 ± 6.0	92.5 ± 5.0	93.3 ± 5.4	90.7 ± 3.9	28.0 ± 1.9	31.0 ± 4.0	27.4 ± 2.1	28.2 ± 2.5
30 ppm NO	91.3 ± 7.7	93.2 ± 7.3	93.8 ± 5.1	87.7 ± 5.9	28.2 ± 2.5	32.2 ± 3.7	26.8 ± 3.0*	27.0 ± 3.4*
40 ppm NO	93.8 ± 5.2	94.7 ± 6.2	90.5 ± 3.1	89.0 ± 7.3	30.4 ± 2.9	31.4 ± 4.1	30.0 ± 3.0	27.8 ± 2.6
0 ppm NO	91.0 ± 6.8	95.5 ± 5.1	91.7 ± 3.8	90.0 ± 6.4	39.4 ± 3.6	37.6 ± 4.8	33.4 ± 4.1	30.6 ± 3.7
	Diastolic pulmonary pressure (mmHg)				Central venous pressure (mmHg)			
Baseline:	24.0 ± 1.6	24.4 ± 3.4	22.8 ± 4.1	17.6 ± 3.0	4.5 ± 1.1	4.8 ± 1.0	4.5 ± 1.4	5.3 ± 1.2
LiquiVent:	5 ml/kg	10 ml/kg	15 ml/kg	20 ml/kg	5 ml/kg	10 ml/kg	15 ml/kg	20 ml/kg
0 ppm NO	20.6 ± 2.0	22.0 ± 3.3	20.8 ± 3.2	17.6 ± 3.0	4.2 ± 1.1	4.3 ± 1.1	5.0 ± 1.4	5.2 ± 1.2
10 ppm NO	18.0 ± 1.8	17.0 ± 5.4	17.0 ± 2.5	15.8 ± 1.8	4.0 ± 1.4	5.8 ± 1.0	4.7 ± 1.3	3.7 ± 1.0
20 ppm NO	18.6 ± 2.0	19.4 ± 3.9	16.6 ± 2.7	14.6 ± 1.9	3.3 ± 1.0	5.2 ± 1.1	5.2 ± 1.3	6.3 ± 0.9
30 ppm NO	18.8 ± 1.8	19.8 ± 3.6	15.2 ± 1.7*	14.2 ± 2.6	3.5 ± 1.1	4.8 ± 1.0	5.5 ± 1.5	4.8 ± 0.6
40 ppm NO	19.4 ± 2.2	17.8 ± 3.3	15.2 ± 2.5*	14.2 ± 1.6	4.0 ± 1.2	4.3 ± 1.1	4.7 ± 1.3	6.3 ± 0.5
0 ppm NO	24.4 ± 3.4	22.8 ± 4.1	17.6 ± 3.0	19.4 ± 2.7	4.8 ± 1.0	4.5 ± 1.4	5.3 ± 1.2	6.2 ± 0.4
	Heart rate (beats/min)				CO (l/min)			
Baseline:	152.5 ± 10.2	156.7 ± 10.0	143.7 ± 8.1	148.7 ± 8.6	1.1 ± 0.1	0.9 ± 0.1	1.0 ± 0.2	1.1 ± 0.1
LiquiVent:	5 ml/kg	10 ml/kg	15 ml/kg	20 ml/kg	5 ml/kg	10 ml/kg	15 ml/kg	20 ml/kg
0 ppm NO	152.0 ± 10.4	153.7 ± 9.7	144.7 ± 10.2	152.8 ± 10.4	1.0 ± 0.1	0.9 ± 0.2	1.0 ± 0.1	1.1 ± 0.1
10 ppm NO	150.7 ± 8.5	149.8 ± 9.2	144.8 ± 8.7	152.2 ± 9.8	1.0 ± 0.2	1.0 ± 0.2	1.1 ± 0.1	1.1 ± 0.2
20 ppm NO	155.5 ± 11.3	148.8 ± 8.1	145.7 ± 8.0	149.2 ± 10.0	1.0 ± 0.1	1.1 ± 0.2	1.1 ± 0.1	1.0 ± 0.2
30 ppm NO	155.8 ± 11.4	144.2 ± 10.3	146.2 ± 8.1	149.2 ± 9.6	0.9 ± 0.2	1.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.1
40 ppm NO	152.3 ± 10.4	139.2 ± 8.4*	149.2 ± 8.3	152.8 ± 11.7	0.9 ± 0.2	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
0 ppm NO	156.7 ± 10.0	143.7 ± 8.1	148.7 ± 8.6	140.8 ± 6.7	0.9 ± 0.1	1.0 ± 0.2	1.1 ± 0.1	1.1 ± 0.2
	Pulmonary wedge pressure (mmHg)				PVR (dynes.s/cm^5)			
Baseline:	6.2 ± 0.5	6.6 ± 1.3	5.4 ± 1.0	5.2 ± 1.0	1828 ± 249	2452 ± 418	2092 ± 414	1603 ± 315
LiquiVent:	5 ml/kg	10 ml/kg	15 ml/kg	20 ml/kg	5 ml/kg	10 ml/kg	15 ml/kg	20 ml/kg
0 ppm NO	5.2 ± 0.2	6.0 ± 1.0	5.6 ± 0.7	4.8 ± 0.9	1743 ± 223	2407 ± 586	1871 ± 408	1649 ± 339
10 ppm NO	4.0 ± 0.6	5.6 ± 0.9	5.2 ± 1.1	4.8 ± 1.0	1754 ± 428	1999 ± 505	1401 ± 234	1461 ± 270
20 ppm NO	6.0 ± 0.9	5.0 ± 0.8	5.2 ± 1.2	5.0 ± 0.8	1546 ± 272	1859 ± 557	1353 ± 225*	1456 ± 294
30 ppm NO	6.0 ± 0.9	5.0 ± 1.0	4.6 ± 1.0	4.6 ± 1.0	1742 ± 324	1913 ± 435	1414 ± 232	1356 ± 284
40 ppm NO	5.6 ± 1.0	5.4 ± 1.0	4.4 ± 1.1	5.4 ± 0.7	1946 ± 433	1732 ± 369*	1388 ± 260	1299 ± 225
0 ppm NO	6.6 ± 1.3	5.4 ± 1.0	5.2 ± 1.0	4.6 ± 0.9	2452 ± 418*	2092 ± 414*	1603 ± 315	1755 ± 364

* $p < 0.05$

[a] Intragroup comparisons ANOVA with Dunnett post-test if ANOVA ($p < 0.05$), using the data after each increment of LiquiVent as control

[b] Baseline represents the data prior to an increase in LiquiVent dose

Discussion

The model of induced ALI used in the present study leads to decreased arterial oxygenation as a result of end-expiratory collapse due to surfactant deficiency, and is comparable to the changes found in ARDS [14-16].

The results of the present study show that PLV is an effective therapy, leading to a dose-dependent improvement in gas exchange in surfactant-depleted pigs. These findings confirm the results from previous reports on PLV [5, 8]. The beneficial effect of PLV on gas exchange is mediated through the physical presence of PFC in the alveoli preventing them from expiratory collapse. This collapse usually accounts for two-thirds of the time of the respiratory cycle. The high dissolved volume of oxygen (perflubron; 55 ml/100 ml at 37°C and at 1 atmosphere of pressure) continues to oxygenate the blood during the expiratory period, resulting in improved arterial oxygenation. Furthermore, PFC enhances alveolar recruitment in the surfactant-deficient, atelectatic lung [17].

Pulmonary hypertension due to pulmonary vasoconstriction and/or widespread vascular obstruction is a common finding in severe ALI or ARDS [18]. Due to an increased microvascular filtration pressure pulmonary hypertension can increase the accumulation of extravascular lung water [19]. This extravascular lung water may lead to worsening oxygenation, resulting in further increase in hypoxic vasoconstriction. Furthermore, pulmonary hypertension can cause right ventricular dysfunction [20]. Because of its selective pulmonary vasodilating properties inhaled NO was suggested as a therapy for pulmonary hypertension [13]. NO-therapy is feasible because NO is short acting, is easily titrated to its effect and can readily be removed from the lungs [21].

Our study also shows that administration of NO, after pretreatment with PFC, results in a further improvement in gas exchange, with a simultaneous decrease in pulmonary artery pressures. The successful combination of NO and PFC was first reported in a hypoplastic congenital diaphragmatic hernia lamb model [9]. However, this lamb model is a neonatal model in which the pathophysiology of respiratory failure is different from that of ALI in adults.

Figure 1a shows that the maximal effect of additional NO inhalation resulted in a more than 100% increase in PaO_2 values (48.9 ± 10.6 to 177.1 ± 42.1 mmHg) at the 5 ml/kg

perflubron dose. A similar increase in PaO_2 was obtained with the next increment of 5 ml/kg perflubron alone. At the higher doses of perflubron, additional NO inhalation resulted in a smaller increase (30-50%) in PaO_2 values, whereas each subsequent increment in perflubron showed an equal increase in PaO_2 as obtained with NO administration at the previous perflubron dose. These effects indicate that, after an initial improvement in PaO_2 by administration of perflubron a further increase in oxygenation can be obtained by either a low dose of perflubron in combination with NO or a higher dose of perflubron without NO.

The well-known finding that high NO concentrations decrease PaO_2 by diffusing to nonventilated lung regions, dilating the vessels in these areas, resulting in an increase in right-to-left shunt [22], was observed in our experiments only at the 5 ml/kg perflubron dose. This effect, however, was not significant.

The improvement in CO_2 elimination by administration of perflubron was enhanced by additional NO inhalation resulting in an improvement in pH. The findings proved to be statistically significant; however, we can not contribute this effect to additional NO inhalation alone. Because of the fact that during the study period minute ventilation was not changed, baseline $PaCO_2$ values varied widely. Therefore we question the significance of these findings.

Perflubron has a high solubility for respiratory gases. To date there is no information available on the solubility of NO in PFC, nor were we able to measure the amount of NO dissolved. However, online bloodgas monitoring showed that changes in NO concentration resulted in rapid changes in PaO_2. (Fig 2 and). This rapid response to NO was similar to the time-response curves reported by Gerlach et al. (1-2 min)[21]. The rapid response to changes in NO concentration in our study shows that there is no clinically important effect of PFC on the time-response effect of NO, indicating that the total amount of NO dissolved in perflubron must be low. When applying partial liquid ventilation with perflubron higher concentrations of inspired oxygen are needed to maintain a high oxygen diffusion gradient, in order to adequately oxygenate the blood. Higher inspired concentrations of oxygen are known to react rapidly with NO to form toxic redox forms of NO [23]. In the present study no measurements were made to quantify the transformation of NO. However methaemoglobin concentration, as well as mixed expired NO_2 concentration both remained low (<5% and <5 ppm respectively). Further

discussion of the safety of inhaled NO alone or in combination with PFC is beyond the scope of this paper. The toxicity of nitrogen oxides has recently been reviewed [23].

In conclusion: This study showed that the combination of perflubron and NO has an cumulative effect in increasing gas exchange and lowering pulmonary artery pressures. Due to its additive effect it may even be possible to reduce the amount of PFC's by adding NO to get a certain oxygenation which normally results after higher doses of PFC. However, before this combination may be used routinely to treat patients, more basic information is required about, for example, what is happening with NO in the PFC, is there any accumulation of possible radicals in the PFC, or what is the solubility of NO in PFC.

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Chapter 8

Comparing the effects of four different perfluorocarbons on gas exchange and lung mechanics in an animal model of acute lung injury

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Summary

Partial liquid ventilation (PLV), a hybrid technique that superimposes gas ventilation on lungs that are filled with perfluorocarbons (PFCs), has been shown to improve oxygenation in animal models of acute lung injury (ALI). Several physico-chemical properties of perfluorocarbons are considered important in their capability to improve pulmonary function. This study was performed to allow a direct comparison of four perfluorocarbons with different physico-chemical properties during PLV with respect to gas exchange, lung mechanics, alveolar protein influx and surfactant system. Thirty-six adult male Sprague-Dawley rats were anesthetized, tracheotomized and submitted to pressure controlled mechanical ventilation at an FiO_2 of 1.0, 30 breaths/min, I/E of 1:2, a positive inspiratory pressure (PIP) of 26 cmH_2O, and a PEEP of 6 cmH_2O. Acute lung injury was induced by repeated lung lavages to obtain a $PaO_2 < 100$ mmHg. After steady state, animals were randomly divided (each group n=6) to receive either APF-140, APF-215, APF-175A or FC 3280 intratracheally, in a dose of 15 ml/kg body weight. One group received no PFCs, but was ventilated with a PIP of 28 cmH_2O and a PEEP of 8 cmH_2O (ventilated control group). Gas exchange was determined hourly during a 4 h observation period. In the groups that received PFCs, evaporational losses were compensated for. Instillation of APF 175A resulted in a significant and sustained improvement in gas exchange, an increase in total lung capacity, and prevented an increase in alveolar protein influx. Instillation of APF 140 caused a transient increase in oxygenation and prevented an increase in alveolar protein influx, but did not increase total lung capacity. In the other two perfluorocarbon treated groups, gas exchange did not improve and no differences were found compared with the ventilated controls. We conclude that the efficacy of perfluorocarbons to improve pulmonary function cannot be predicted based on their physico-chemical properties.

Introduction

In the last few years, several studies have investigated the effect of partial liquid ventilation (PLV) in animal models of acute lung injury (ALI). Filling the lungs up to the level of functional residual capacity with perfluorocarbons, followed by gas ventilation, has been shown to improve gas exchange and lung mechanics, and to result in less lung injury [1-4]. In clinical studies, some of these beneficial effects have been confirmed in patients with acute respiratory distress syndrome (ARDS) [5, 6, for review see 7].

The exact mechanisms by which perfluorocarbons improve gas exchange and lung mechanics, and the way in which these are influenced by the physico-chemical properties of the perfluorocarbons, have not yet been elucidated. Different perfluorocarbons with a wide range of physico-chemical properties are available for experimental use, thus allowing evaluation of these different profiles and their efficacy. One of these perfluorocarbons, FC 3280 has shown to improve gas exchange and lung mechanics, and to attenuate lung injury [8, 9]. Others perfluorocarbons have not yet been tested for use in partial liquid ventilation.

The aim of the present study was to compare four perfluorocarbons with different physico-chemical properties on their efficacy in improving pulmonary function. We performed the study in lung lavaged rats, which were submitted to partial liquid ventilation under well standardized experimental conditions. We used the rat model of surfactant deficiency which has proven to be a convenient and consistent model of ALI. Gas exchange was assessed during 4 hours of partial liquid ventilation, after which lung mechanics, alveolar protein influx, and several parameters characterizing the surfactant system were determined.

Methods

The study protocol was approved by the University's animal experimental committee, and the care and handling of the animals conformed with European Community guidelines (86/609/EC). The study was performed in 36 adult male Sprague-Dawley rats (body weight 270-330 g). After induction of anaesthesia with 2% enflurane and 65% nitrous oxide in oxygen, a polyethylene catheter was inserted into a carotid artery for drawing arterial blood samples. Before tracheostomy, the animals received 60 mg/kg pentobarbital sodium, i.p. (Nembutal®, Algin BV, Maassluis, the Netherlands). After tracheostomy, muscle relaxation was induced by

pancuronium bromide 1 mg/kg, i.m. (Pavulon®, Organon Teknika, Boxtel, the Netherlands) immediately followed by connection to a ventilator. The animals were mechanically ventilated with a Servo Ventilator 300 (Siemens-Elema, Solna, Sweden) in a pressure constant time-cycled mode, at an inspired oxygen concentration (FiO_2) of 1.0, frequency of 30 breaths per minute (bpm), peak inspiratory pressure (PIP) of 12 cm H_2O, positive end-expiratory pressure (PEEP) of 2 cmH_2O, and inspiratory/expiratory (I/E) ratio of 1:2. Anaesthesia was maintained with pentobarbital sodium 40 mg/kg/h, i.p.; muscle relaxation was maintained with pancuronium bromide 1 mg/kg/h, i.m. Body temperature was kept within normal range by means of a heating pad. Immediately after induction of anaesthesia one group of animals (n=6) was killed to serve as healthy controls.

Acute lung injury was induced in the remaining animals by repeated broncho-alveolar lavage (BAL) (32 ml/kg) with warm saline (37°C), according to Lachmann and colleagues [10]. BAL was repeated as often as necessary to produce a PaO_2<100 mmHg at a PIP and PEEP of 26 and 6 cmH_2O, respectively. Within 10 min after the last lavage, 30 animals were randomised to receive an intra-tracheal bolus of 15 ml/kg of one of four perfluorocarbons, with different physico-chemical properties (Table 1), or to serve as ventilated controls (each group n=6). For administration of the bolus of perfluorocarbons, the animals were disconnected from the ventilator, immediately followed by re-connection to the ventilator. To compensate for evaporational losses of perfluorocarbon during the study period, an hourly check was made to establish whether a meniscus was visible just above the tracheostomy-cannula at disconnection from the ventilator. If this was not the case, perfluorocarbon was administered in boluses of 3 ml/kg until the meniscus was visible. In the ventilated control group, which did not receive perfluorocarbons, ventilator pressures were increased by 2 cmH_2O (PIP/PEEP of 28/8 cmH_2O) to maintain PaO_2 above 50 mmHg. These ventilator settings were not changed throughout the remaining study period; this group served as ventilated controls.

Arterial blood gas samples were taken prior to lavage, after lavage, and hourly for 4 h. The samples were analysed for arterial oxygen tension (PaO_2) and arterial carbon dioxide tension ($PaCO_2$) on an electrochemical bloodgas analyser (ABL 505, Radiometer, Copenhagen, Denmark).

At the end of the experiment, the animals were killed by an overdose of pentobarbital.

Table 1. Physico-chemical properties of the tested perfluorocarbons. All values are at 25°C.

	APF 140 (Perfluorodecalin)	APF 175A (Perfluorodimethyl-decalin)	APF 215 (Perfluoro-phenantrane)	FC 3280
Surface tension (dynes/cm)	19.3	20.5	21.6	12
Vapor pressure (mmHg)	6	0.67	0.12	61
Density (g/ml)	1.93	1.98	2.01	1.75
O_2 solubility (ml/100 ml PFC)	49	35	37	40

APF-140, APF-175A and APF-215 were provided by Fluoro-Seal Inc, Round Rock, USA. FC-3280 was provided by 3M Chemical Products, Neuss, Germany.

Static pressure-volume curves were recorded using conventional techniques [10]. Total lung capacity (TLC_{35}) was defined as lung volume above collapsed volume at inflation with a distending pressure of 35 cmH_2O. After the pressure-volume recordings BAL was performed five times with saline-$CaCl_2$ 1.5 mmol/litre. The active surfactant component in the BAL fluid was separated from the non-active surfactant component by differential centrifugation followed by subsequent phosphorus analysis, and the ratio between non-active and active components (small aggregate to large aggregate ratio = SA/LA ratio) was calculated, as previously described by Veldhuizen and colleagues [11]. The protein concentration of the BAL fluid was determined using the Bradford method (Bio-Rad protein-assay, Munich, Germany) [12].

Statistical analysis was performed using the Instat statistical package. Inter-group comparisons were analysed with ANOVA and intra-group comparisons were analysed with repeated measures ANOVA. If ANOVA resulted in a $p < 0.05$ a Tukey-Kramer post-test was performed. All data are reported as mean ± SD and $p < 0.05$ was considered statistically significant.

Results

Blood gas values before and immediately after lavage were comparable for all five ventilated groups (Fig.1 and Table 2). All animals survived the study period. In the groups treated with the perfluorocarbons APF 140 and APF 175a, PaO_2 increased significantly after lavage, and remained stable in the APF 175A group but decreased significantly in the APF 140 treated group. In the groups treated with APF 215 and FC 3280, as well as in the ventilated control group, PaO_2 did not improve after lavage.

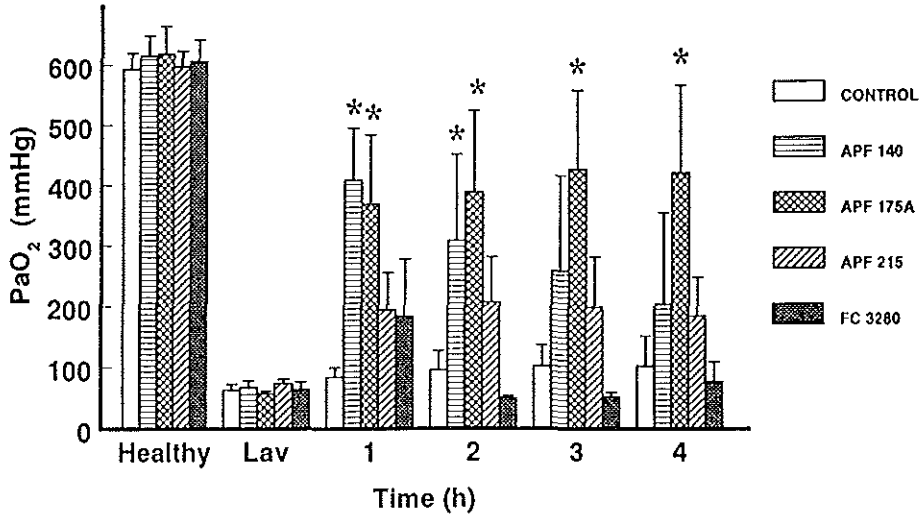

Figure 1. PaO_2 values (mmHg, mean ± SD) for all ventilated groups. Control = ventilated control group. *, $p<0.05$ vs ventilated controls.

Perfluorocarbon was substituted periodically to compensate for the evaporational loss and the substitution doses (ml/kg/h) were 1.6 ± 0.1 (APF 140), 1.1 ± 0.4 (APF 175A), and 3.3 ± 0.5 (FC 3280). In the APF 215 treated animals no substitution was necessary.

$PaCO_2$ data are given in Table 2. After lavage, $PaCO_2$ increased significantly in all groups. Administration of APF 140 and APF 175A resulted in a significant decrease of $PaCO_2$ (Table 2).

Table 2. $PaCO_2$ values in the ventilated groups (mean ± SD, mmHg)

	Ventilated Control	APF 140	APF 175A	APF 215	FC 3280
Before lavage	45 ± 5.1	39 ± 4.1	44 ± 10.8	39 ± 2.3	45 ± 4.5
After lavage	67 ± 11.4	66 ± 5.9	72 ± 12.9	67 ± 10.8	71 ± 13.7
1 h	59 ± 12.7	44 ± 5.1 ‡	53 ± 13.1 ‡	64 ± 12.8 §	49 ± 5.2
2 h	60 ± 14.2 §	41 ± 4.2 ‡	50 ± 13.6 ‡	63 ± 10.9 §	60 ± 7.3 §
3 h	63 ± 15.1	46 ± 4.3 ‡	48 ± 14.2 ‡	69 ± 11.5 §¶	66 ± 3.7 §
4 h	63 ± 18.1	47 ± 2.8 ‡	47 ± 12.4 ‡	70 ± 10.3 §¶	54 ± 12.1

h, hour. § vs. APF 140; ¶ vs. APF 175a; Intra-group comparison: ‡ vs. after lavage

Figure 2 shows the deflation limbs of the pressure-volume curves. Lung volume in the healthy controls exceeded the lung volume in all other groups. TLC_{35} was significantly increased in the APF 175A treated animals compared to the ventilated control group, but not in the other perfluorocarbon treated groups. At deflation below 10 cmH_2O, there was no significant difference in lung volume between the ventilated control group and any of the perfluorocarbon treated animals.

The protein concentration of BAL fluid was significantly increased in the APF 215, FC 3280 and ventilated control groups, but not in groups APF 140 and APF 175a, compared to the healthy controls (Fig. 3). The SA/LA ratio, which is the ratio between non-active and active surfactant components, was increased in all ventilated groups compared to the healthy controls

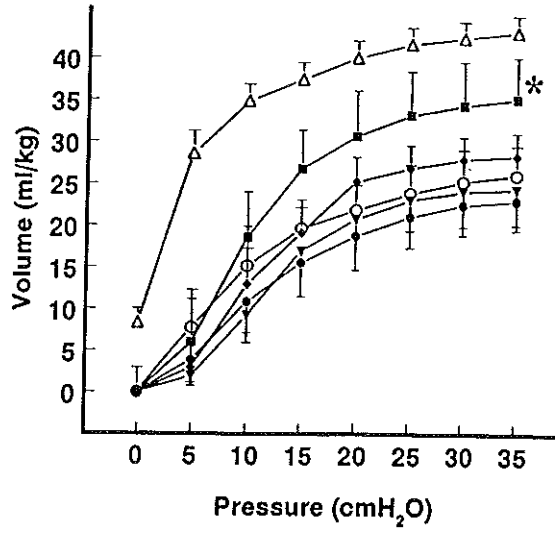

Figure 2. Deflation limbs from the pressure-volume curves, mean ± SD. Volume is lung volume above functional residual capacity. △=healthy controls; ○=ventilated controls; ▼=AFF 140; ■=APF 175A; ◆=APF 215; ●=FC 3280. For all pressures, volume was significantly decreased in all ventilated groups compared to the healthy controls. *, $p<0.05$ vs ventilated controls.

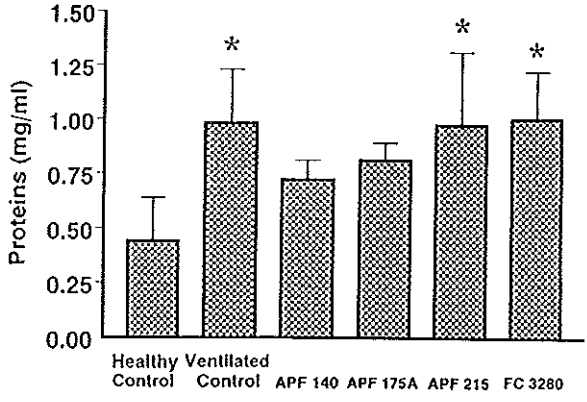

Figure 3. Protein concentration (mean ± SD) of the BAL fluid of the different groups. *, $p<0.05$ vs. healthy control group.

but there were no differences between the ventilated groups. The total amount of phosphorus in the BAL fluid, that was measured to quantify the phospholipid-containing surfactant system, was obviously decreased in all lavaged and ventilated groups compared to the healthy controls but there were no differences between the ventilated groups (Table 3).

Table 3. Data on pulmonary surfactant: Total phosphorus, and small aggregate to large aggregate ratio (SA/LA ratio) in the six study groups (mean ± SD).

	Total phosphorus (μmol/ml)	SA/LA ratio
Healthy control	0.14 ± 0.06	0.3 ± 0.1
Ventilated control	0.05 ± 0.01	1.5 ± 0.4 *
APF 140	0.05 ± 0.01	1.8 ± 1.0 *
APF 175a	0.05 ± 0.01	2.5 ± 0.8 *
APF 215	0.09 ± 0.03	1.6 ± 1.1 *
FC 3280	0.05 ± 0.01	2.5 ± 0.7 *

* $p<0.05$ vs. Healthy control

Discussion

We could not identify a single factor that predicts efficacy of a perfluorocarbon to improve gas exchange and lung mechanics, or to prevent alveolar protein influx, during partial liquid ventilation. Instillation of APF 175A results in a significant and sustained improvement in gas exchange and total lung capacity, and prevents an increase in alveolar protein influx. Instillation of APF 140 caused a transient increase in oxygenation and prevented an increase in alveolar protein influx, but did not increase total lung capacity. In the other perfluorocarbon treated groups, no differences were found compared with the ventilated controls.

The improvement in gas exchange in the APF 140 and APF 175A treated animals is in accordance with previous reports on partial liquid ventilation in animal models of ALI [1-4, 8,

9]. Recent studies have shown that after intra-tracheal instillation, perfluorocarbons are predominantly distributed to the dependent regions of the lung, leading to recruitment of collapsed alveoli with an increase of end-expiratory lung volume, thereby reducing transpulmonary shunt and thus improving gas exchange [13, 14]. In the present study, in the APF 140 treated animals a decrease in oxygenation was seen after an initial improvement. In previous studies it was noted that, when perfluorocarbons are lost from the lungs by evaporation, alveolar collapse re-occurs leading to a decrease in PaO_2 [3]. However, in the present study evaporational losses were compensated for each hour by refilling the lungs with perfluorocarbon until a meniscus was visible above the tracheostomy catheter. Therefore, the decrease in PaO_2 over the 4 h ventilation period in group APF 140 cannot be explained by evaporation.

The lack of improvement in oxygenation in the APF 215 and FC 3280 treated animals is striking given their physico-chemical profile. Several mechanisms could hamper an improvement in oxygenation, such as insufficient oxygen solubility or an increase in ventilation/perfusion mismatch due to compression of pulmonary vessels by the perfluorocarbons. However, oxygen solubility was comparable in the tested perfluorocarbons, and although APF 215 is the perfluorocarbon with the highest density, it is comparable to the density in APF 175A which showed a sustained improvement in PaO_2 (Fig 1). However, in previous studies in lung lavaged pigs an improvement in oxygenation was found after instillation of FC 3280 [8, 9].

It has been hypothesized that after intra-tracheal instillation of perfluorocarbon, a thin film covering the entire alveolar surface of the non-dependent part of the lung is formed as a result of evaporation of perfluorocarbon from the lower lung parts [3]. Since perfluorocarbons have a low constant surface tension, this results in a reduction of the retractive forces in the surfactant deficient lung, thus allowing large volume changes with only small changes in pressure. This indicates that perfluorocarbons with the lowest surface tension should lead to the highest TLC_{35}. In the present study however, APF 175A, which has almost the highest surface tension of all tested perfluorocarbons, was the only perfluorocarbon with an increase in TLC_{35} compared to the ventilated control group (Fig. 2).

The presence of non-compressable perfluorocarbons in the alveoli prevents them from end-expiratory collapse. There is evidence that repeated alveolar collapse leads to the occurrence

of shear forces, which have been shown to damage the alveolar-capillary membrane and lead to alveolar protein influx [15, 16]. In the APF 140 and 175A treated animals, in which perfluorocarbons prevented alveolar collapse during a large part of the ventilation period as indicated by the sustained improvement in PaO_2, alveolar protein influx was not increased compared to healthy controls (Fig. 3). In the APF 215 and FC 3280 treated groups, as well as in the ventilated control group, repeated alveolar collapse is likely to have occurred given the lack of improvement in PaO_2, which is in accordance with the increase in alveolar protein influx (Fig. 3).

In a previous study, it was found that 3 h of partial liquid ventilation increased the loss of radioactive labeled phospholipids from the lungs of healthy rabbits [17]. In another study, it was found that PLV for 5.5 h in healthy rabbits enhanced surfactant phospholipid production [18]. However, in the present study no significant differences were observed between the ventilated groups in total phosphorus or SA/LA ratio (Table 3). We therefore conclude that 4 h of PLV with all tested perfluorocarbons in this model of surfactant deficiency had no effect on the pulmonary surfactant system, compared with conventional ventilation, but the exact effects of partial liquid ventilation on the pulmonary surfactant system remain to be elucidated.

In conclusion, it proves difficult to predict the efficacy of perfluorocarbons for use in partial liquid ventilation based on their physico-chemical properties. PLV with APF 175A resulted in a sustained improvement in gas exchange, an increase in TLC_{35} and a decrease in alveolar protein influx, suggesting it might be useful for clinical purposes. However, future studies remain necessary to elucidate the exact mechanisms involved.

Speculation

Most published experimental and clinical data on partial liquid ventilation were obtained with Perflubron. Although Perflubron was not tested in the present study, our group earlier investigated Perflubron in lung-lavaged adult rabbits. The results that we obtained on PaO_2 with APF 175A are comparable with the results that were obtained with Perflubron. In the latter study, Tütüncü et al. administered Perflubron in a dose of 12 ml/kg to lung-lavaged rabbits, who were subsequently ventilated with a PEEP of 6 cmH_2O (Fig. 4) [2]. In the Perflubron treated animals a decrease in PaO_2 was observed 4 h after lavage, which was attributed to evaporational

loss of Perflubron, since no substitution doses were administered in that study. The data from the present study show that for improving oxygenation, APF 175a could be a useful alternative for Perflubron.

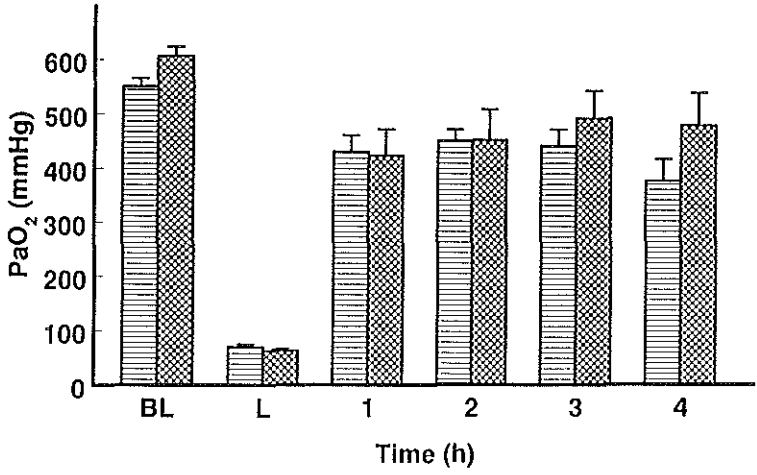

Figure 4. PaO_2 values (mmHg, mean ± SD) from APF 175A treated animals from the present study, and Perflubron treated animals. Striped bar=lung lavaged rabbits treated with Perflubron (12 ml/kg); hatched bar=lung lavaged rats treated with APF 175 A ((15 ml/kg) (from reference [3]).

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Summary and conclusions

The central role of the pulmonary surfactant system in the development and progression of the acute respiratory distress syndrome (ARDS) is outlined in *Chapter 1*. In ARDS, a lack of active surfactant leads to an increase in retractive forces in the lung resulting in alveolar collapse.

Mechanical ventilation is mandatory in this situation; however, if alveoli are not stabilized during expiration repeated alveolar collapse will occur, and open lung units will be overdistended when large tidal volumes are used in partially collapsed lungs. The pulmonary surfactant system is vulnerable to damage by mechanical ventilation, especially in conditions where its function is already compromised, such as in ARDS. An additional decrease in surfactant function caused by mechanical ventilation can lead to progression of the syndrome; that is why ARDS might be, in part, a product of our therapy - rather than the progression of the underlying disease. A physiologic rationale for ventilation strategies that aim to prevent loss of surfactant function is discussed, as well as the possibilities for substitution of the lost active surfactant.

Chapter 2 presents a study which investigated whether prevention of end-expiratory alveolar collapse by applying a high enough positive end-expiratory pressure (PEEP) during initiation of ARDS could attenuate the decrease in pulmonary function. During surfactant depletion by repeated lung lavage in rats, end-expiratory alveolar collapse was prevented in one group by mechanical ventilation with a PEEP of 15 cmH_2O (open lung group), and compared with a group ventilated with a PEEP of 8 cmH_2O which did not prevent alveolar collapse (ventilated control group). After surfactant depletion, ventilation was continued for 4 hours. It was shown that application of the high PEEP prevents a decrease in gas exchange, attenuates the deterioration in lung mechanics, and attenuates an increase in protein leakage, compared with the ventilated control group. The attenuation of the decrease in lung mechanics in the open lung group was attributed to the reduction in inhibition of the remaining pulmonary surfactant by plasma proteins.

Mechanical ventilation with high PEEP, partial liquid ventilation and exogenous surfactant therapy have been shown to improve oxygenation in animal models of acute lung injury, and are therefore currently under investigation in clinical studies. In *Chapter 3*, these new

treatment strategies were compared for their effect on gas exchange, lung mechanics, lung injury, protein leakage, and the surfactant system. Following induction of acute respiratory failure by repeated lung lavage during ventilation with a PEEP of 6 cmH_2O, the rats were either ventilated with a high PEEP that kept the lungs open, or received exogenous surfactant (120 mg/kg) followed by ventilation with unchanged ventilator settings, or received perfluorocarbon (15 ml/kg) followed by ventilation with unchanged ventilator settings (partial liquid ventilation). These three groups were compared with a control group that was ventilated with a low PEEP. Although all three strategies improved oxygenation, only ventilation with high PEEP and exogenous surfactant therapy prevented transfer of proteins into the alveoli, whereas partial liquid ventilation did not. The conversion of active into non-active surfactant aggregates was increased in the partial liquid ventilation group and the group ventilated with high PEEP, but obviously not in the surfactant-treated group. Lung injury score was reduced in the partial liquid ventilation group and the surfactant group compared with the groups that were ventilated only. It was concluded that although all three strategies improved oxygenation to pre-lavage values, their impact on pulmonary function differed markedly. Only exogenous surfactant therapy led to an improvement in all measured parameters.

Using the same model as in *Chapter 3*, the difference in protein leakage into the alveoli between the animals that were ventilated only with high or low PEEP in *Chapter 3* was further evaluated in *Chapter 4*. Because surfactant function is known to be inhibited by plasma proteins, it was investigated whether the efficacy of exogenous surfactant in improving pulmonary function, when administered after 4 hours of mechanical ventilation, could be enhanced by application of high PEEP compared to a ventilated control group. Two groups of rats underwent whole lung lavages to induce acute respiratory failure, followed by mechanical ventilation with either a high or low PEEP for 4 hours. Then, both groups received an equal dose of exogenous surfactant (120 mg/kg), after which ventilation with unchanged settings was continued for only 15 min in order to study the acute effects of surfactant administration. In the group that was ventilated with high PEEP prior to surfactant administration, protein leakage was lower and lung mechanics were better after surfactant administration than in the group that was ventilated with conventional ventilator settings. These results indicate that in the clinical setting, where patients with ARDS are usually ventilated before exogenous surfactant therapy, it is important to apply

a ventilation strategy that does not increase protein leakage.

High frequency oscillatory ventilation (HFOV) applies a high mean airway pressure and small pressure amplitude, and has previously been propagated as a safe ventilation technique to recruit and stabilize collapsed alveoli when used as a "high lung volume strategy". *Chapter 5* presents a study in which exogenous surfactant therapy, which allows alveolar recruitment and stabilization at lower airway pressures, was combined with either HFOV or conventional mechanical ventilation (CMV), in lung-lavaged rabbits. Two additional groups were studied that received HFOV or CMV only. Oxygenation increased in both HFOV groups and also in the CMV/surfactant group, but when mean airway pressures were decreased after 4 hours of ventilation PaO_2 decreased to post-lavage values in the HFOV group that did not receive surfactant, but not in either of the surfactant-treated groups. In the surfactant-treated animals less lung injury was found than in the animals that were ventilated only. It is concluded that HFOV recruits and stabilizes alveoli with a high mean airway pressure, whereas exogenous surfactant therapy allows reduction in mean airway pressure without the occurance of alveolar collapse and aggravation of lung injury.

Apart from enhancing gas exhange in ARDS by improving ventilation, there are indications that it might be beneficial to alter pulmonary perfusion by nitric oxide (NO) inhalation. NO is a gas that can be administred during mechanical ventilation and results in a selective pulmonary vasodilatation (i.e. only in aerated lung areas), thus reducing intrapulmonary shunt. In clinical trials a considerable percentage of patients fails to respond to NO inhalation, and it has been suggested that alveolar recruitment and stabilization prior to NO inhalation might be beneficial. Three strategies that prevent end-expiratory alveolar collapse are exogenous surfactant therapy, ventilation with an increased PEEP, and partial liquid ventilation. In the study described in *Chapter 6*, NO inhalation was started in lung-lavaged rabbits after initiation of either ventilation with a PEEP of 10 cmH_2O or administration of exogenous surfactant (25 mg/kg). After the initial improvement in oxygenation that was obtained by exogenous surfactant or ventilation with a PEEP of 10 cmH_2O alone, an additional increase in PaO_2 during NO inhalation was seen only in the surfactant-treated group. In the control group that was ventilated only with a PEEP of 6 cmH_2O, NO inhalation did not improve oxygenation.

In *Chapter 7* the combination of NO inhalation and alveolar recruitment and

stabilization by perfluorocarbon instillation is described. Lung lavaged pigs were submitted to partial liquid ventilation by administration of four incremental doses of 5 ml/kg of perfluorocarbon per dose. After each dose of perfluorocarbon, NO inhalation was started and the effect on oxygenation was recorded. After each dose of perfluorocarbon there was an increase in oxygenation, as well as an additional increase of PaO_2 by NO inhalation. Also, at each dose of perfluorocarbon, the maximal increase in oxygenation that was obtained with NO inhalation was similar to the increase obtained with administration of the subsequent dose of perfluorocarbon. It is concluded that alveolar recruitment and prevention of end-expiratory alveolar collapse prior to NO inhalation improves effective delivery of NO and thereby enhances its efficacy improve oxygenation. The lack of response to inhaled NO in the high PEEP group was attributed to mechanical compression of the pulmonary vessels, which did not allow pulmonary vasodilatation.

In *Chapter 8*, the mechanisms involved in the improvement in pulmonary function by partial liquid ventilation are investigated. Four perfluorocarbons with different physico-chemical profiles were compared in lung-lavaged rats to study their efficacy in improving gas exchange and lung mechanics, and their effect on protein leakage and the surfactant system. Although the different preparations showed a range of responses, it was not possible to predict the efficacy of a perfluorocarbon to improve pulmonary function during partial liquid ventilation based on the physico-chemical properties alone.

In conclusion, the studies described in this thesis contribute to the general knowledge on strategies that are currently under investigation for use in patients with acute lung injury/ARDS. It is shown that recruitment of collapsed alveoli and prevention of repeated end-expiratory alveolar collapse is beneficial in improving pulmonary function, whereas the strategy used to achieve this is of secondary importance. The best results are obtained with exogenous surfactant therapy, which allows ventilation with lower airway pressures. Without surfactant, an open lung strategy with higher airway pressures can be applied that prevents further deterioration of pulmonary function, especially when instituted in an early phase of respiratory failure. Furthermore, prior recruitment and stabilization of collapsed lung units can improve the efficacy of subsequent treatment with exogenous surfactant or inhaled nitric oxide. Future studies are necessary to determine the exact role of these different treatment strategies in clinical

use for patients with ARDS.

Samenvatting en conclusies

In *Hoofdstuk 1* wordt de centrale rol van het pulmonale surfactant systeem bij het ontstaan en beloop van het acute respiratory distress syndrome (ARDS) beschreven. Bij ARDS leidt een tekort aan actief surfactant tot een toename van de retractive krachten in de long, hetgeen resulteert in het collaberen van alveoli. In deze situatie is kunstmatige beademing absoluut noodzakelijk. Echter, indien de alveoli in de uitademingsfase niet gestabiliseerd worden, zullen ze iedere keer collaberen. Daarbij kunnen de geopende longdelen overrekt worden indien grote slagvolumes worden toegepast op de gedeeltelijk gecollabeerde longen. Het is bekend dat het pulmonale surfactant systeem kwetsbaar is voor schade die kan ontstaan tijdens kunstmatige beademing, vooral in situaties waar de surfactant functie al is aangedaan, zoals bij ARDS. Een verdere verslechtering van de surfactant functie kan dan de progressie van dit syndroom versnellen. Om die reden is het waarschijnlijk dat ARDS, in ieder geval gedeeltelijk, een gevolg is van onze behandeling, in plaats van alleen een voortschrijding van het onderliggende ziekteproces. De fysiologische achtergronden van beademingsstrategieën die als doel hebben dit verdere verlies van surfactant-functie te voorkomen en mogelijkheden om verloren surfactant te vervangen, worden in dit hoofdstuk besproken.

In *Hoofdstuk 2* wordt een studie beschreven waarin werd onderzocht of preventie van eind-expiratoire alveolaire collaps tijdens inductie van ARDS door toepassing van een voldoende hoge positieve eind-expiratoire druk (PEEP) de verslechtering van de longfunctie kan verminderen. In deze studie ondergingen ratten herhaalde long lavages om een surfactant deficiëntie te induceren en daarmee een acute respiratoire insufficientie. In deze studie werd eind-expiratoire alveolaire collaps tijdens kunstmatige beademing in één groep voorkómen door toepassing van een PEEP van 15 cmH_2O (open long groep). De resultaten werden vergeleken met die van een groep die werd beademd met een PEEP van 8 cmH_2O, hetgeen niet voldoende is om alveolaire collaps te voorkomen (beademde controle groep). Na surfactant depletie werd beademing gecontinueerd gedurende vier uur. Toepassing van een hoge PEEP voorkwam verslechtering van de gasuitwisseling, verminderde de achteruitgang van longmechanica en verminderde de toename van eiwitlekkage, in vergelijking met de beademde controlegroep. De verminderde achteruitgang van longmechanica in de open long groep was te wijten aan de

afgenomen inactivatie van het resterende longsurfactant door plasma eiwitten.

Kunstmatige beademing met hoge PEEP, partiële vloeistof beademing en exogene surfactant therapie, verbeteren alle de oxygenatie in diermodellen met acuut longfalen en worden momenteel geëvalueerd in klinische studies. In *Hoofdstuk 3* worden deze nieuwe behandelingsstrategieën met elkaar vergeleken voor wat betreft het effect op gasuitwisseling, longmechanica, longschade, eiwitlekkage en surfactant systeem. In deze studie werd bij ratten acuut longfalen geïnduceerd door herhaalde long lavages tijdens beademing met een PEEP van 6 cmH_2O. Hierna werden de dieren óf beademd met een hoge PEEP die de longen open hield, óf werden ze behandeld met exogeen surfactant (120 mg/kg), óf met perfluorocarbon (15 ml/kg) (partiële vloeistof beademing). Deze drie groepen werden vergeleken met een controlegroep die werd beademd met een lage PEEP. Hoewel bij alle drie de strategieën de oxygenatie verbeterde, werd eiwitlekkage in de alveoli alleen voorkomen door beademing met een hoge PEEP en exogene surfactant therapie. Partiële vloeistof beademing deed dit niet. De conversie van actieve in niet-actieve surfactant aggregaten bleek verhoogd te zijn in de partiële vloeistof beademingsgroep en in de groep die werd beademd met een hoge PEEP, maar niet in de groep die met exogeen surfactant was behandeld. In de partiële vloeistof beademingsgroep en in de surfactant groep werd minder longschade gevonden, vergeleken met de groepen die alleen werden beademd. Wij concludeerden dat door de drie strategieën de oxygenatie verbeterde, maar dat de invloed op de longfunctie nogal verschillend was. Alleen behandeling met exogeen surfactant resulteerde in een verbetering van alle gemeten parameters.

Het verschil in eiwitlekkage naar de alveoli tussen de groepen die alleen werden beademd met een hoge dan wel een lage PEEP, dat werd gevonden in de voorgaande studie, werd verder onderzocht in *Hoofdstuk 4*. Het is bekend dat surfactant functie wordt geremd door plasma eiwitten. Daarom werd onderzocht of de effectiviteit waarmee exogeen surfactant de longfunctie verbetert, kan worden vergroot door het surfactant toe te dienen na een periode van kunstmatige beademing met een hoge PEEP. In twee groepen ratten werd acuut respiratoir falen geïnduceerd door long lavages, waarna één groep werd beademd met een hoge PEEP en een tweede groep met een lage PEEP. Na vier uur werden beide groepen behandeld met een gelijke dosis exogeen surfactant (120 mg/kg), waarna de beademing werd voortgezet gedurende 15 minuten om de acute effecten van surfactant toediening te onderzoeken. In de groep die vóór de

surfactant toediening werd beademd met een hoge PEEP, werd na surfactant toediening minder eiwitlekkage en betere longmechanica gevonden dan in de groep die met een lage PEEP werd beademd. Deze resultaten impliceren dat in de klinische situatie, waarin ARDS-patiënten meestal voorafgaand aan exogene surfactant toediening worden beademd, het van belang is dat een beademingsstrategie wordt gebruikt die niet leidt tot een toename van eiwitlekkage.

Tijdens high frequency oscillatory ventilation (HFOV) wordt een hoge gemiddelde beademingsdruk en een kleine drukamplitude gebruikt. Deze strategie is in het verleden aangeprezen als een veilige beademingstechniek om gecollabeerde alveoli te rekruteren en te stabiliseren indien HFOV wordt gebruikt als "hoog long volume strategie". In *Hoofdstuk 5* wordt een studie beschreven waarin bij gelaveerde konijnen HFOV of conventionele mechanische ventilatie (CMV) werd gecombineerd met toediening van exogeen surfactant. Hierdoor is rekrutering en stabilisatie van alveoli met lagere beademingsdrukken mogelijk. Twee extra groepen werden alleen beademd met HFOV of CMV. De oxygenatie verbeterde in beide HFOV groepen en in de CMV/surfactant groep. Zodra na vier uur beademing de gemiddelde beademingsdruk werd verlaagd, daalde de PaO_2 naar dezelfde waarde als die van direct post-lavage in de groep die alleen met HFOV werd beademd. Dit gebeurde niet in beide surfactant groepen. In de groepen die met surfactant werden behandeld, werd minder longschade gevonden dan bij de dieren die alleen beademd werden. Wij concludeerden dat HFOV alveoli rekruteert en stabiliseert door toepassing van een hoge gemiddelde beademingsdruk, terwijl exogene surfactant toediening een verlaging van de gemiddelde beademingsdruk toelaat, zonder dat de alveoli opnieuw collaberen en longschade verergerd wordt.

Naast het verbeteren van de gasuitwisseling tijdens ARDS door het optimaliseren van de ventilatie, zijn er aanwijzingen dat het gunstig kan zijn om de pulmonale perfusie te veranderen met stikstof monoxide (NO) inhalatie. NO is een gas dat toegediend kan worden tijdens kunstmatige beademing, hetgeen resulteert in een selectieve pulmonale vasodilatatie (i.e. alleen in de geventileerde longdelen) en daardoor een afname van de intrapulmonale shunt. In klinische studies wordt vaak waargenomen dat een aanzienlijk percentage patiënten niet reageert op NO inhalatie. Hierbij wordt gesuggereerd dat rekrutering en stabilisatie van alveoli voorafgaand aan NO inhalatie gunstig zou zijn. Drie strategieën die eind-expiratoire alveolaire collaps voorkómen, zijn exogene surfactant therapie, beademing met een hoge PEEP en partiële

vloeistof beademing. In de studie beschreven in *Hoofdstuk 6* werd gestart met NO inhalatie in een groep gelaveerde konijnen beademd met een PEEP van 10 cmH_2O en in een groep waaraan exogeen surfactant werd toegediend (25 mg/kg). Dit resulteerde in beide groepen in enige alveolaire rekrutering, zonder dat de longen volledig werden geopend. Na de initiële toename van de oxygenatie die werd verkregen door exogeen surfactant of beademing met een PEEP van 10 cmH_2O, werd alleen in de groep die met surfactant werd behandeld een additionele toename van de PaO_2 door NO inhalatie gezien. In de controlegroep, die alleen werd beademd met een PEEP van 6 cmH_2O, verbeterde NO de oxygenatie niet. In *Hoofdstuk 7* wordt de combinatie van NO inhalatie met alveolaire rekrutering en stabilisatie door toediening van perfluorocarbonen beschreven. Bij varkens werd na long lavage begonnen met partiële vloeistof beademing door toediening van vier opeenvolgende doses perfluorocarbon van 5 ml/kg per dosis. Na iedere dosis perfluorocarbon werd gestart met NO inhalatie, waarna het effect op de oxygenatie werd geregistreerd. De oxygenatie verbeterde na iedere dosis perfluorocarbon en na NO inhalatie. De maximale toename van de oxygenatie door NO inhalatie was gelijk aan de toename in oxygenatie door toediening van de volgende dosis perfluorocarbon. Rekrutering van gecollabeerde alveoli en preventie van eind-expiratoire collaps lijken de effectieve afgifte van geïnhaleerd NO te verbeteren, waardoor het effect op de oxygenatie groter is. In de met een hoge PEEP beademde groep werd geen effect gezien van geïnhaleerd NO. Dit wordt geweten aan mechanische compressie van de pulmonale vaten, waardoor vasodilatatie niet meer mogelijk is.

In *Hoofdstuk 8* werden tenslotte vier perfluorocarbonen met verschillende fysisch-chemische eigenschappen vergeleken in een diermodel wat betreft effectiviteit in het verbeteren van gasuitwisseling en longmechanica èn het effect op eiwitlekkage en surfactant systeem. Hoewel meerdere verschillen tussen de preparaten werden gevonden, bleek het niet mogelijk om op basis van fysisch-chemische eigenschappen alléén de effectiviteit te voorspellen waarmee perfluorocarbonen de longfunctie verbeteren tijdens partiële vloeistof beademing.

De studies beschreven in dit proefschrift dragen bij aan de algemene kennis omtrent beademingsstrategieën die momenteel worden geëvalueerd voor klinische toepassing bij patiënten met acuut longfalen/ARDS. Aangetoond werd dat rekrutering van gecollabeerde alveoli en het voorkomen van herhaaldelijk collaberen aan het eind van de expiratiefase gunstig is voor de longfunctie, terwijl de strategie die hiervoor wordt gebruikt van ondergeschikt belang

is. De beste resultaten worden behaald met exogene surfactant therapie, waarbij lagere beademingsdrukken gebruikt kunnen worden. Zonder surfactant kan een open long beademingsstrategie worden toegepast die een verdere achteruitgang van de longfunctie voorkomt, met name indien wordt begonnen in een vroege fase van longfalen. Tevens kan het rekruteren en stabiliseren van gecollabeerde longdelen voorafgaand aan verdere behandeling met exogeen surfactant en stikstof monoxide inhalatie de effectiviteit van deze toepassingen verbeteren. Toekomstige studies zijn nodig om de exacte plaats van deze strategieën in de behandeling van ARDS patiënten te bepalen.

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Curriculum vitae

De auteur van dit proefschrift werd geboren in Tegelen op 10 maart 1969. Aan het Bisschoppelijk College Broekhin te Roermond behaalde hij diverse diploma's, waaronder het vwo-diploma in 1990. Aansluitend werd begonnen met de studie Geneeskunde aan de Erasmus Universiteit Rotterdam, alwaar hij in 1996 het doctoraalexamen en in 1999 het artsexamen behaalde. Gedurende zijn studie werkte hij op de afdeling experimentele anesthesiologie en voltooide hij de studies beschreven in dit proefschrift. Sinds 1999 is hij in dienst van het Academisch Ziekenhuis Rotterdam, alwaar hij wordt opgeleid tot anesthesioloog (opleider a.i.: Dr. J. Klein).

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