Treatment and Prevention of Acute Respiratory Failure: Experimental Studies

Cover: Microphotograph of an open alveolus

Treatment and Prevention of Acute Respiratory Failure: Experimental Studies
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TREATMENT AND PREVENTION OF ACUTE RESPIRATORY FAILURE: EXPERIMENTAL STUDIES

BEHANDELING EN PREVENTIE VAN ACUUT RESPIRATOIR FALEN: EXPERIMENTELE STUDIES

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Chapter 1

Introduction

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Introduction

The acute respiratory failure (ARF) remains as one of the most common reasons for admission to the intensive care units. It is caused by many factors [1], and its incidence is about 77.6 patients per 100,000/year, with a 90-day mortality of 41% [2]. In all cases of ARF a pathological shortage of surfactant at the alveolar level is observed [3]. This deficit of surfactant increases the alveolar surface tension, promoting end-expiratory instability with alveolar collapse and respiratory dysfunction, which includes hypoxemia and decreased lung compliance [4,5]. It is clear that the more alveolar units are depleted of active surfactant aggregates, the more alveolar units will collapse, and the more severe the respiratory failure will be [6-11]. Based on this pathophysiological process the treatment of the ARF should be based on: preserving the active surfactant aggregates in the remaining functionally alveolar units; re-opening collapsed alveolar units; and restoring the end-expiratory alveolar stability from those surfactant-deficient alveoli [12-14]. Nowadays, it is thought that exogenous surfactant therapy, mechanical ventilation with positive pressure ventilation, and perfluorocarbon therapy might play an important role in modifying the disease process of the ARF [12-17].

PHYSIOLOGY

Endogenous surfactant system

The integrity of the surfactant system of the lung is a prerequisite for normal breathing with the least possible effort [15]. LaPlace, a French mathematician (1749-1827), was the first to draw attention to surface active forces in general, and described the relationship between force, surface tension, and radius of an air-liquid interface of a bubble:

$P = 2\gamma/r$ (P = pressure to stabilise a bubble; γ = surface tension at air-liquid interface; and r = radius of a bubble).

Almost one century later, von Neergaard applied this law to pulmonary alveoli by demonstrating that the pressures required to expand an air-filled lung were almost three times that required to distend a lung filled with fluid [18]. In this way, the surface tension effect at

the air-liquid boundary was eliminated (Fig. 1) From these findings, he concluded that: 1) two-thirds of the retractile forces in the lung are due to surface tension phenomenon which act at the air-liquid interface of the alveoli, and 2) the surface tension at the air-liquid interface must be reduced by the presence of a surface active material with a low surface tension to allow normal breathing [18]. Surfactant is synthesized by the alveolar type II cells and secreted into alveolar spaces and small airways, lowering its surface tension [15]. 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) lying as a layer at the air-liquid interface in the lung [19, 20].

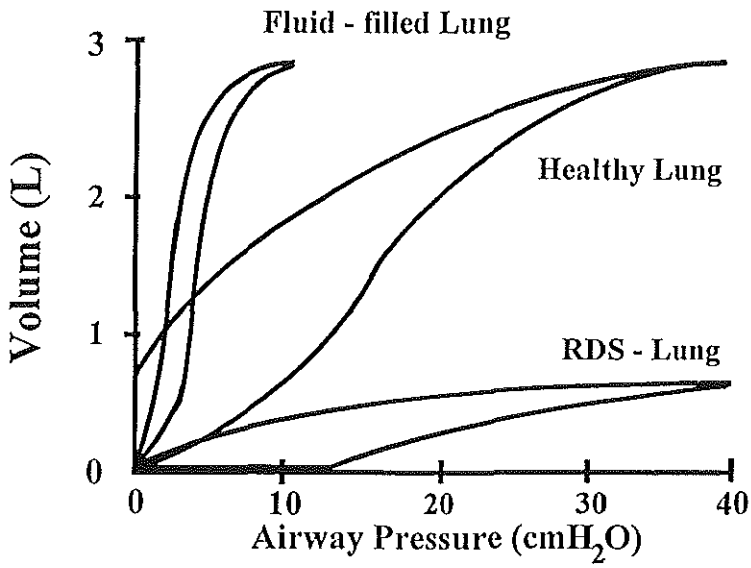

Figure 1. Pressure-volume diagrams of a normal air-filled lung and a ARDS lung. Von Neergaard showed in 1929 that much larger pressures were required to expand an air-filled lung than a lung filled with fluid. In a lung suffering from surfactant deficiency (RDS-Lung) even higher pressures are required to expand the lung, due to the high surface tension at the air-liquid interface in the alveoli caused by a diminished surfactant system.

The normal physiological functions of the pulmonary surfactant system include [21]:

a) Mechanical stabilisation of lung alveoli

The surfactant system acts by decreasing surface tension of the interface between alveoli and air. During deflation of the lung, a static high surface tension would tend to promote alveolar collapse. However, as alveolar size decreases, pulmonary surfactant ensures that surface tension falls approximately to zero. Thus, at small alveolar volumes, surface tension becomes a negligible force and thereby tends to promote alveolar stability [22].

b) Protection against lung edema

Another function of the pulmonary surfactant system is stabilisation of the fluid balance in the lung and protection against lung edema (Fig. 2). In general, alveolar flooding will not occur as long as the suction force in the pulmonary interstitium exceeds the pressure gradient generated by the surface tension in the alveolar air-liquid interface. Since this pressure gradient is inversely related to the radius of the alveolar curvature there is, for each combination of the interstitial reasorptive force and average surface tension, a critical value for surface tension and for alveolar radius, below which alveolar flooding occurs [23].

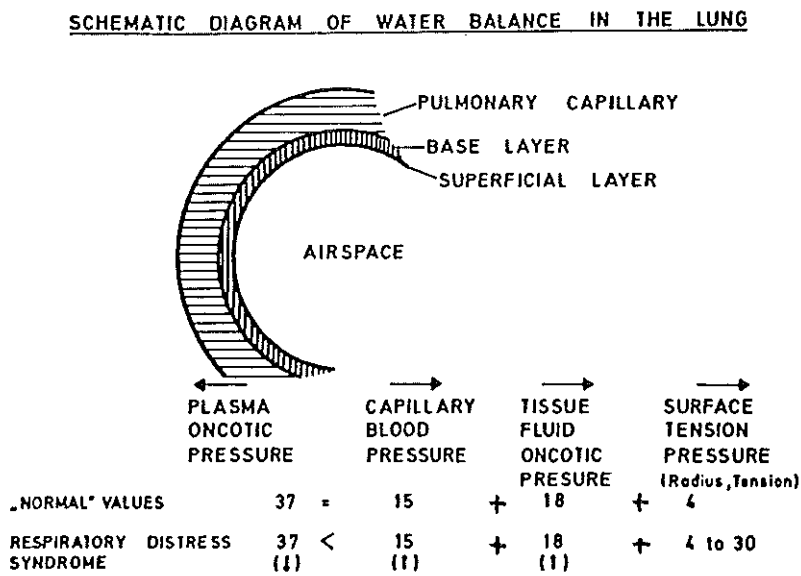

Figure 2. Simplified schematic diagram representing the factors influencing fluid balance in the lung (from reference 33).

c) Surfactant and airways stabilization.

As early as 1970, Macklem et al. [24] called attention to the significance of bronchial surfactant for stabilization of the peripheral airways and hinted that lack of stabilization may cause airway obstruction or collapse of the small bronchi with air trapping. This has been proved in an animal model where the bronchial surfactant was selectively destroyed [25]. It was demonstrated that the pressure to open up the collapsed bronchi is 20 cm H_2O.

Besides its role in mechanical stabilization, bronchial surfactant also has a transport function for mucus and inhaled particles [25]. This has been proven, in vitro, in a study showing that particles on a surface film move in one direction only if the surface film is compressed and dilated, comparable to the compression and expansion during expiration and inspiration [25]. Furthermore, bronchial surfactant also acts as an antiglue factor preventing the development of large adhesive forces between mucus particles, as well as between mucus and the bronchial wall [26].

d) Surfactant and local defence mechanisms

The surfactant system plays a role in the lung's defense against infection [27]. Surfactant, and in particular SP-A, enhances the antibacterial and antiviral defense of alveolar macrophages [27]. It has been shown that the surfactant system may also be involved in protecting the lung against its own mediators (e.g. angiotensin II) and in protecting the cardiocirculatory system against mediators produced by the lung [28, 29].

Disturbance of the surfactant system

Disturbance of the surfactant system can result from different factors [15]. Damage to the alveolar-capillary membrane leads to high-permeability edema with wash-out or dilution of the surfactant and/or inactivation of the surfactant by plasma components, such as fibrin, albumin, globulin and transferrin, hemoglobin and cell membrane lipids [30,31]. These components are known to inhibit pulmonary surfactant function in a dose-dependent way [31]. Furthermore, the pulmonary surfactant my 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 to type II cells [32].

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

1) Decreased pulmonary compliance;
2) Decreased functional residual capacity;
3) Atelectasis and enlargement of the functional right-to-left shunt;
4) Decreased gas exchange and respiratory acidosis;
5) Hypoxemia with anaerobic metabolism and metabolic acidosis;
6) Pulmonary edema with further inactivation of surfactant by plasma constituents [33].

MECHANICAL VENTILATION IN THE TREATMENT OF ARF

Mechanical ventilation has been used for more than 40 years to overcome the hypoxemia and low compliance produced during the ARF. However, it has been shown that mechanical ventilation can damage the lungs when a mode of ventilation, which allowed high inspiratory lung volumes and low levels of PEEP, is applied [6-11, 34-36]. In 1967, Ashbaugh and colleagues discussed the inactivation of the surfactant system by intra-alveolar plasma proteins in patients suffering from acute respiratory distress syndrome [37], and since then several studies have demonstrated qualitative and quantitative changes of surfactant in bronchoalveolar lavage fluid from patients with ARF [38-40]. Gregory and colleagues [41] showed that minimal surface tension, total phospholipids, and surfactant proteins (SP-A and SP-B) were all decreased in the bronchoalveolar lavage fluid obtained from patients suffering of ARF. In addition, this latter group observed that several of these alterations also occur in patients at risk for developing ARF, suggesting that these abnormalities of surfactant occur early in the disease process. Therefore, in experimental animals, and patients suffering from ARF lung damage is produced on the one hand by certain modes of mechanical ventilation, and on the other by the disease process, unless a protective ventilatory strategy is used.

Surfactant changes during mechanical ventilation

Studies have shown that during artificial ventilation several mechanisms are involved in the alterations of the surfactant function: (1) loss of surfactant into the small airways; (2)

conversion of active large into non-active small surfactant aggregates; and (3) inactivation of the alveolar lining layer due to edema fluid.

Mechanical ventilation enhances the release of surfactant from the type II pneumocytes into the alveoli by a metabolically active process [6-10]. This released material is squeezed out of the alveoli into the airways due to a compression of the surfactant film at end-expiration if the surface area of the alveolus becomes smaller than the surface occupied by the surfactant molecules [13]. During the following inflation the lost molecules are replaced by surfactant which is stored within the alveolus (hypophase) and the cells. More surfactant molecules are lost during the next expiration; this is an ongoing cycle (Fig. 3).

Studies by Veldhuizen et al. showed that the pulmonary surfactant can be subdivided into two distinct subfractions: (1) large surface-active aggregates which are the precursor for the (2) small aggregates with poor surface activity [42]. In vivo and in vitro studies have shown that the size of the tidal volume correlates with the magnitude of conversion from large active to small inactive subfractions [43]. Therefore, ventilation with large tidal volume promotes the inactivation of the pulmonary surfactant system.

It has been proven that loss of active molecules of surfactant with an increase in the alveolar surface tension results in a decrease in pericapillary pressure and an increase in the permeability of the alveolo-capillary barrier to small solutes [44-47], indicating that surfactant has a primary role in the regulation of the permeability of the alveolo-capillary barrier to small solutes and proteins. Additionally, mechanical ventilation can disturb the functional integrity of the endothelium and epithelium, which creates an imbalance at the alveolo-capillary membrane. Both increased capillary filtration pressure and altered microvascular protein permeability have been shown to contribute to pulmonary edema after lung overinflation.

Role of pressure and volume in ventilation-induced lung injury

Studies with high peak inspiratory pressure ventilation, in which peak inspiratory lung volume was limited by thorax restriction, have suggested that the end-inspiratory lung volume, and not end-inspiratory pressure, is the main determinant of ventilation-induced lung injury [48,49]. However, the alveolar pressure alone, as measured in such studies, does not provide a measure of alveolar distension. Rather than the absolute airway pressure, the absolute transpulmonary pressure (which is equal to the alveolar pressure minus pleural pressure) is

responsible for injury. Therefore, at a given lung-thoracic compliance, absolute transpulmonary pressure and end-inspiratory lung volume are interchangeable and indistinguishable with respect to their injurious potential.

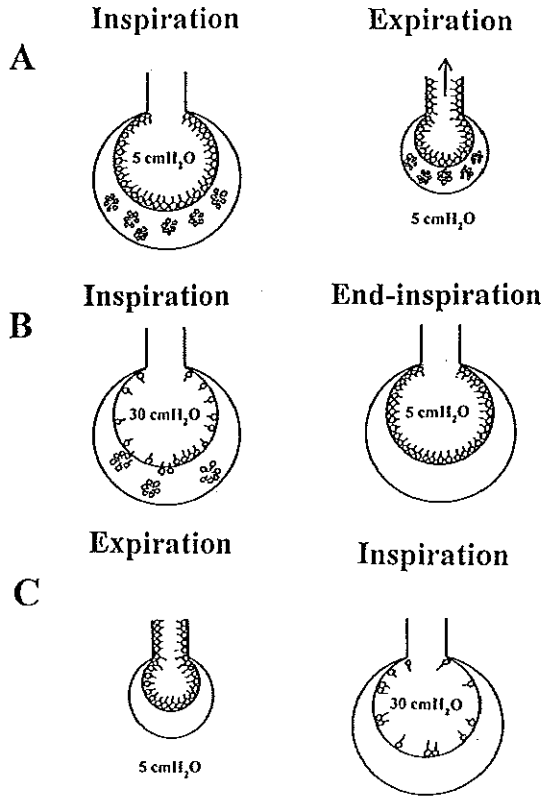

Figure 3. A. Balance between synthesis, release and consumption of surfactant in the healthy lung. The pressure values given represent the intrapulmonary pressure needed to open up the alveolus. At the surface and the hypophase (micelles), there are sufficient molecules of surfactant. These micelles deliver the surfactant necessary to replace the molecules squeezed out during expiration. B) Imbalance between synthesis, release and consumption of surfactant due to artificial ventilation. At the beginning of inspiration, there is an apparent deficiency of surfactant molecules but there is a respreading of molecules stored in the hypophase of the surfactant layer. At the end of inspiration there is, in principle, enough surfactant on the surface. C) With the next expiration, surface active molecules are squeezed out and no surface active molecules are left in the hypophase for respreading, creating the situation where a serious surfactant deficiency follows.

It is known that more than the endothelium or interstitial spaces, the epithelium is rate-limiting for solute and fluid movement between blood and alveolus [50,51]. Effects of overinflation on epithelial permeability have been studied in fluid-filled in situ lobes, to exclude the effect of surface tension. As the epithelium is progressively stretched during static inflation there is a non-reversible opening of water-filled channels between alveolar cells resulting in free diffusion of small solutes and even albumin across the epithelial barrier [52-54]. Such changes were shown to occur only at high distending pressures and have been attributed to peak inspiratory epithelial overstretching which occurs due to inflation in the supra-physiological range only [54-56]. Due to the damage of both the epithelial and endothelial barrier, surfactant components may be lost into the bloodstream [55]. More importantly, protein will accumulate intra-alveolarly which results in dose-dependent inhibition of surfactant [31]. As surfactant is rate-limiting for the transfer of proteins over the alveolo-capillary barrier, loss of surfactant function will lead to further protein infiltration. This may result in a self-triggering mechanism of surfactant inactivation [31, 57-59].

Structural damage of the alveolocapillary barrier due to repeated collapse and re-expansion of alveoli

Pioneering work of Mead and colleagues [60] demonstrated that 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 open and closed alveoli (Fig. 4). An alveolus with surfactant impairment would be predisposed to end-expiratory alveolar collapse and prone to be affected by such "shear forces". Shear forces, rather than end-inspiratory overstretching, may well be the major reason for epithelial disruption, the loss of barrier function of the alveolar epithelium, and considerable increases in regional microvascular transmural pressure.

Important evidence for this mechanism comes from the finding that ventilation at low lung volumes can also augment lung injury in lungs with an impaired surfactant system [61]. A recent study in a model of subtle surfactant perturbation by dioctyl sodium sulphosuccionate showed that surfactant changes make the lung vulnerable to lung parenchymal injury by mechanical ventilation [62]. These studies confirm the earlier work of Nilsson et al. [63] in ventilated newborn premature rabbits with a primary surfactant deficiency. Fetuses treated with

surfactant before receiving mechanical ventilation had less bronchiolar epithelial lesions in comparison with non-surfactant treated controls.

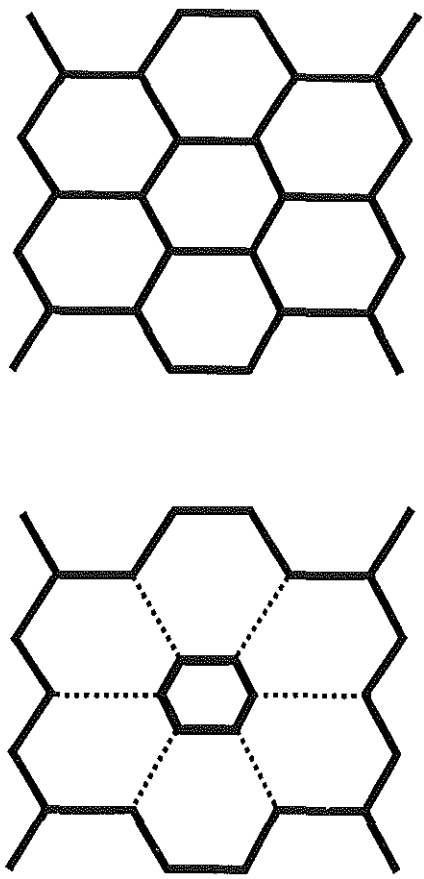

Figure 4. Shear forces are caused between open and closed alveoli due to pulmonary interdependence of alveoli. This figure shows the difference between mechanical ventilation of normal alveoli (upper panel) and mechanical ventilation of the same alveolar unit after surfactant inactivation (lower panel), which results in end-expiratory collapse (adapted from reference 60).

Improvement of gas exchange, lung function, and permeability changes by positive end-expiratory pressure (PEEP) during mechanical ventilation

Initial studies have investigated the effect of increasing levels of PEEP at constant tidal volume ventilation, which resulted in higher end-inspiratory pressures and volumes. Such studies found that increasing levels of PEEP reduced shunt [64-66] and improved oxygenation and lung mechanics which was attributed to reopening of flooded alveoli with redistribution of edema fluid from flooded alveoli into the interstitial spaces [67-69]. Such studies, however, also demonstrated that the use of high PEEP levels did not reduce [64,66,70] or even increase edema formation [65,71]. These findings have been reported in both isolated perfused lungs [64] and in closed-chest healthy animals [66] and closed-chest animals with different forms of lung injury induced by bronchial hydrochloric acid administration [67], alloxan [70] oleic acid [72] or hydrostatic edema due to lobar venous occlusion [71]. Overinflation due to PEEP is probably the explanation for the lack of reduction or even worsening of edema reported with PEEP during such experiments [73]. However, it has now been demonstrated in different animal models that ventilation with PEEP at lower tidal volumes results in less edema than ventilation without PEEP and a higher tidal volume for the same peak or mean airway pressure [34,73,74] and that, more specifically, PEEP prevents alveolar flooding [34,36,75].

Dreyfuss et al. showed in rats ventilated at peak inspiratory pressure of 45 cm H_2O that damage due to mechanical ventilation begins at the endothelial side after 5 min and rapidly progresses to the epithelium after 20 min [35]. A subsequent study showed a reduction of endothelial injury and the preservation of the structure of the alveolar epithelium by use of 10 cm H_2O of PEEP, which was accompanied by a lack of alveolar flooding [49].

Experiments in the same rat model of overinflation have shown a significant conversion of active into non-active surfactant aggregates compared to non-ventilated controls after lung overinflation; 10 cm H_2O PEEP was shown to prevent a significant conversion of large aggregates into small aggregates compared with non-ventilated controls [36]. This latter study suggests that the beneficial effect of PEEP in reducing protein infiltration after overinflation at peak inspiratory pressure of 45 cm H_2O without PEEP in rats is partially attributed to a reduced filtration by surfactant preservation [36].

Two basic mechanisms have been described in literature which explain the surfactant preserving effect of PEEP during mechanical ventilation. Studies by Wyszogrodski et al. have

shown that PEEP prevents a decrease in lung compliance and surface activity of lung extracts indicating a prevention of loss of alveolar surfactant function during lung overinflation [10]. Others have suggested that PEEP prevents alveolar collapse and thus keeps the end-expiratory volume of alveoli at a higher level, thereby preventing excessive loss of surfactant in the small airways by a squeeze-out mechanism during expiration [75-77].

The utilization of PEEP to splint open the airways and alveoli at end-expiration in surfactant-deficient lungs may markedly reduce lung injury. Studies in both saline-lavage isolated perfused rat lungs [61] and saline-lavage intact animals [78,79] have shown that ventilation strategies which keep the alveoli open throughout the respiratory cycle by sufficiently high levels of PEEP induce significantly less morphological injury with better preservation of pulmonary compliance than strategies in which alveolar collapse is allowed at end-expiration. Although healthy lungs do not seem to be damaged when terminal units are repeatedly opened or closed for short periods by negative end-expiratory pressure (which nevertheless reduces compliance and alters gas exchange [62]), it does become clear from what is discussed above, that early surfactant changes, which may be induced by mechanical ventilation itself, predispose lungs for ventilation-induced lung injury by repeated opening and closure of alveolar units [62].

Techniques to protect the lung during mechanical ventilation in ARF

The consequence of a high alveolar surface tension is the end-expiratory alveolar instability and alveolar collapse. It has been shown that in ARF atelectatic lung areas are mainly distributed in the dependent lung regions (vertebral regions), while in the anterior, or non-dependent regions, the lung is mainly composed of open healthy alveoli [80]. Depending on the magnitude of the lung damage, the proportion of alveoli which can consequently be ventilated may be reduced to almost 20-30% of a normal lung. Gattinoni et al. showed that patients with early ARF and collapsed dependent lung regions, have a reduced volume of aerated lung [80]. Volume controlled mechanical ventilation will predominantly ventilate this aerated healthy portion of the lung with overdistension in such regions. If one assumes that 75% of the lung is consolidated and only 25% is ventilated, then even small tidal volume ventilation e.g. 7 ml/kg bodyweight, would result in tidal volumes of 28 ml/kg in such lung

regions with a danger of overdistension and further lung impairment. Use of pressure-controlled time-cycled modes of ventilation in which the alveolar pressure can never exceed the peak inspiratory pressure set on the ventilator is then preferable to reduce dangerous alveolar overdistension in these lung areas [5].

With the intention to protect the lung against VILI, an international consensus conference compiled the following recommendations: The plateau pressure should be limited to 35 cm H_2O, the tidal volume should be as low as 5 ml/kg, permissive hypercapnia was allowed if normocapnia is not achievable at a limited plateau pressure, and the FiO_2 should be minimised. In addition, a re-expansion maneuver should be performed [81].

It was suggested that to prevent overdistension in ARF patients, tidal volumes have to be decreased [82] and that tidal volume reduction would increase oxygen delivery due to better hemodynamics [83,84].

Preliminary reports of reduced tidal volumes by end-inspiratory airway pressure limitation in patients with or at risk of ARF, however, showed no reduction in mortality rate [85-87]. Such findings may be explained by a certain degree of VILI even with small tidal volume ventilation, due to repeated alveolar collapse and re-expansion.

Lachmann et al. proposed that a protective ventilatory strategy based on the law of LaPlace should be used [5,13]. They showed that raising airway pressures higher than 40 cm H_2O resulted in a recruitment of most functional alveolar units. Once opened these units should be kept open by the minimal PEEP level, and gas exchange can be kept in the normal range even at low pressure amplitude between PIP and PEEP. These low pressure amplitudes produce less shear forces, and thus protect against VILI. However, only a few clinical studies have been performed using this ventilatory strategy [5,88]. This strategy produces a ventilatory condition which saves the lung from further damage, allows a reduction of FiO_2, promotes the resorption of interstitial and intrapulmonary edema, and finally reduces the pulmonary artery pressures by overcoming the hypoxic pulmonary vasoconstriction [5].

A similar protective ventilatory strategy can be applied using high frequency oscillatory ventilation (HFOV) at high levels of mean airways pressure, which results in low oscillation pressure amplitude, low tidal volumes and normal values of carbon dioxide ($PaCO_2$) [89,90]. Froese's group showed that HFOV is useful to protect the lung, but only after a re-expansion maneuver; the oscillation pressure amplitude itself is adjusted according to $PaCO_2$ values [89].

The ease of this intervention, makes this strategy a standard of ventilation in some neonatal intensive care units. However, its usefulness has been questioned by multicenter studies, in which no initial re-expansion maneuver was performed, showing no significant differences between HFOV and conventional mechanical ventilation [91].

Additionally, our group showed in an experimental study that using the same ventilatory strategy, a conventional ventilator is as effective as a high frequency oscillatory ventilator in improving gas exchange and lung mechanics [92], and in preserving the exogenous surfactant function (unpublished data).

EXOGENOUS SURFACTANT THERAPY

Re-establishing a physiological surface tension at the air-liquid interface by application of exogenous surfactant during mechanical ventilation will prevent end-expiratory collapse and dangerous shear forces between open and closed alveoli, resulting in improvement of blood oxygenation at lower fractions of inspired oxygen, use of lower airway pressures with reduced barotrauma, and improvement of survival [93].

Clinical experience of surfactant therapy in neonates with respiratory distress syndrome has learned that the response after exogenous surfactant therapy depends not only on the course of the injury, but also on the timing of surfactant therapy, the used dose of exogenous surfactant, the type of surfactant preparation, and the ventilator settings of the mechanical ventilation. In particular the level of PEEP used, and the method of administration of exogenous surfactant (which is important for the distribution of the instilled surfactant) play an important role [93].

The exact amount of exogenous surfactant required in ARF 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 differ from patient to patient, an excess of surfactant should always be given or repeatedly be substituted until blood gas values improve [94]. Experience in neonates has also learned that exogenous surfactant is more effective when administration takes place in the early stages of RDS [94]. Early treatment of ARF may thus require smaller amounts of surfactant and the

outcome results will probably be better.

The currently used technique of delivering exogenous surfactant is the bolus instillation through the endotracheal tube. This method has been used in most animal studies as well as in neonates who suffer from respiratory distress syndrome (RDS) due to primary surfactant deficiency RDS [95]. The advantage of this method of instillation is that it is rapid and able to deliver large quantities of surfactant. From animal studies and in neonates suffering from RDS, it has been demonstrated that natural surfactant preparations are more effective in improving lung function immediately after instillation than the artificial surfactant preparations, due to the lack of surfactant proteins [95, 96]. Also, different studies have demonstrated that surfactant proteins reduce the surfactant inactivation that may be caused by plasma constituents which is of special importance in ARF.

It has been shown that the ventilator pattern strongly influences exogenous surfactant therapy [97,98]. Several studies have demonstrated that surfactant therapy and positive end-expiratory pressure (PEEP) ventilation produces the largest and most sustained therapeutic effect [99,100]. Surfactant administration does not permit immediate withdrawal of PEEP, but it is usually possible to reduce the peak inspiratory pressures as lung function improves. This avoids overdistension of the alveoli and increased perfusion of the lung. It also reduces the number of pneumothoraces [101].

High frequency oscillation studies have shown that ventilation at high end-expiratory lung volumes combined with small volume cycles at high rates best preserves exogenous surfactant and gas exchange in lavaged lungs [100]. However, until now only a few studies have been published on the combined use of surfactant and HFOV in animals or humans [91, 92,102-104]. It was shown that after surfactant therapy HFOV was superior to CMV in improving pulmonary function and reducing lung injury [91,98,102-103]. In these studies, however, HFOV was used in combination with the high-lung volume strategy whereas CMV was not. Froese and colleagues [98] 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 350 mmHg (according to the high-lung volume strategy) in the CMV group after surfactant therapy [98]. This 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 kept stable for 4 hours [104,105]. Froese et al. [98] 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, however, the high-lung volume strategy with CMV was performed by a gradual increase of PIP and PEEP but without an active volume recruitment maneuver as used with HFOV. Furthermore, CMV was used with a constant flow and high tidal volume (20 ml/kg) which is known to increase the conversion from active into non-active surfactant subfractions; this leads to a shortage of "active" surfactant at the alveolar level.

A recent study by our group [99] has shown that exogenous surfactant therapy can also be optimized by conventional pressure controlled mechanical ventilation with small pressure amplitudes and high levels of end-expiratory pressure as it can with high frequency oscillation. These settings resulted in an optimal gas exchange and low levels of protein infiltration with minimal loss of active surfactant subfractions. Therefore, this ventilatory strategy can be directly compared with HFOV on the efficacy of exogenous surfactant therapy.

PARTIAL LIQUID VENTILATION

An alternative technique to maintain end-expiratory stability is by instilling perfluorocarbon fluids (PFC) into the lung. Because PFCs dissolve high amounts of oxygen and carbon dioxide at normospheric pressures, gas exchange over the alveolar air-liquid interface is maintained when conventional mechanical gas ventilation is superimposed. This technique has become known as "partial liquid ventilation" (PLV) [106-110].

The hypothetical mechanism of PLV is explained in Fig. 5; panel A shows the atelectatic ARF lung. After a small dose of PFC (3 ml/kg) a thin film with a low surface tension is formed at the air-liquid interface due to evaporation of the PFC (panel B) and covers the lung units of the whole lung. Due to this film the increased surface tension in the diseased lung is reduced to a low and constant value, which leads to a decrease of inflation pressure; however, this pressure cannot further decrease with additional doses of PFC. Independent of this speculation the dose-dependent improvement in oxygenation results from filling of the

collapsed atelectatic alveoli in the dependent part of the lung by the non-compressible PFC thus preventing them from end-expiratory collapse (panel B vs C) this leads to a continuation of gas exchange even during the expiratory phase of the respiratory cycle. With increasing amounts of PFC in the lung, more collapsed atelectatic alveoli can be opened and prevented from end-expiratory collapse thus eliminating intrapulmonary shunt. This mechanism was recently supported by computed tomographic scans from Quintel et al. [111] who showed that during PLV, PFC is distributed predominantly to the lower lung regions, whereas gas ventilation took place in the upper regions.

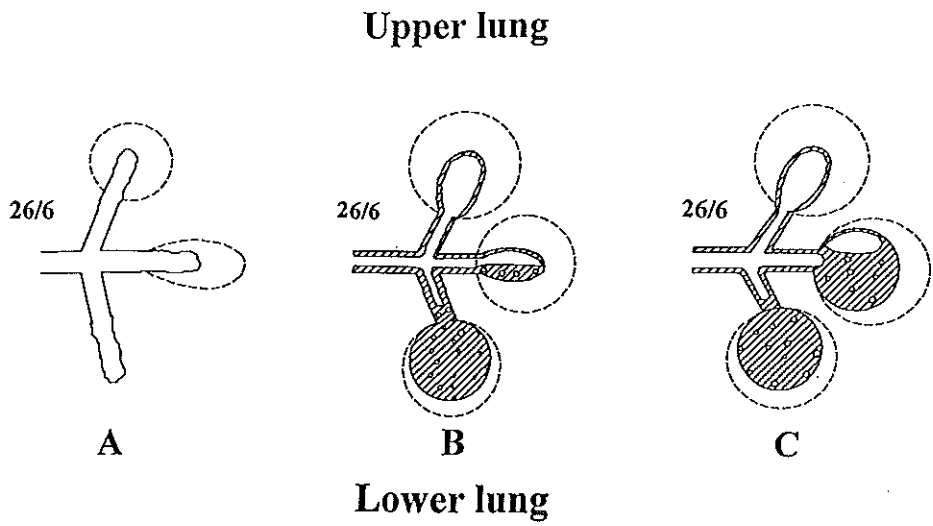

Figure 5. Panel A shows the atelectatic surfactant deficient alveoli at end-inspiration (dashed line) and end-expiration (solid line). Panel B shows what happens when PFC is instilled into the lung. Due to its evaporation a thin layer of PFC is formed at the air-liquid interface and due to its low surface tension, pulmonary compliance is improved. This occurs already at low dose PFC and does not further improve with higher PFC dosing. Some dependent alveoli are prevented from end-expiratory collapse by the non-compressible PFC, and this improves oxygenation. Panel C shows what happens if more PFC is instilled into the lung: more alveoli are recruited at end-expiration. Therefore, there is a dose-dependent improvement in oxygenation with PFC during partial liquid ventilation.

Our group was the first to apply this technique in animals suffering on acute respiratory failure [106-109] which has shown that:

1) Higher doses of PFC lead to higher levels of oxygenation [106]. This is suggested to result from dose–dependent recruitment of collapsed atelectatic alveoli by PFC fluid.

2) Oxygenation deteriorates over time if no additional doses of PFC are applied [107]. This is attributed to evaporation of PFC, which will cause affected alveoli to collapse.

3) Lung mechanics and carbon dioxide elimination improve after an initial low dose of PFC and show no further improvements with subsequent higher doses of PFC [106]. This is attributed to the replacement of the alveolar air-liquid interface with a thin air-PFC interface. Evaporating PFC appears to cover the entire lung surface. As PFCs have a low constant surface tension (which is 18 mN/m), pulmonary compliance is increased after a low-dose PFC and CO_2 elimination is higher. No further improvement is seen after additional PFC dosing.

4) PLV does not impair any cardiovascular parameter; even in animals with a large anterior-posterior thoracic diameter. Mean pulmonary artery pressure decreases when PFC is applied, due to reversal of hypoxic pulmonary vasoconstriction [108].

5) PLV does prevent the progress of histologically assessed lung injury [106-110]. External PEEP has to be applied during PLV to prevent bulk movement of PFC fluids from the alveoli into the airways and to prevent dangerously high airway pressures at the onset of inspiration [106].

6) PLV can be combined with other ventilatory support techniques in ARF [110].

Studies comparing PLV with exogenous surfactant therapy and high levels of PEEP, should be performed. Additionally, due to the physical properties of perfluorocarbons, PLV must be evaluated on the capability to restore the lung function after ventilation-induced lung injury.

References

1. Spragg RG, Smith RM. Biology of acute lung injury. In: Crystal RG, West JB (eds) *The Lung.* Scientific Foundations. Raven press, New York. 1991; 2003-17.
2. Luhr OR, Antonsen K, Karlsson M, et al. Incidence and mortality after acute respiratory failure and acute respiratory distress syndrome in Sweden, Denmark, and Iceland. Am J Respir Crit Care Med 1999; 159: 1849-61.
3. Lewis JF, Jobe AH. Surfactant and the adult respiratory distress syndrome. Am Rev Resp Dis 1993;147:218-33.
4. Verbrugge S, Lachmann B. Mechanisms of ventilation-induced lung injury and its prevention: role of surfactant. Appl Cardiopulm Pathophysiol 1998; 7: 173-98.
5. Lachmann B, Danzmann E, Haendly B, Jonson B. Ventilator settings and gas exchange in respiratory distress syndrome: Applied Physiology in Clinical Respiratory Care. In Prakash O (ed.) Nijhoff, The Hague, 1982. pp 141-76.
6. Mead J, Collier C. Relationship of volume history of lungs to respiratory mechanics in anesthetised dogs. J Appl Physiol 1959; 14: 669-78.
7. Faridy E, Permutt S, Riley R. Effect of ventilation on surface forces in excised dog's lungs. J Appl Physiol 1966; 21: 1453-62.
8. McClenahan J, Urtnowski A. Effect of ventilation on surfactant, and its turnover rate. J Appl Physiol 1967; 23: 215-20.
9. Forrest J. The effect of hyperventilation on pulmonary surface activity. Br J Anaesth 1972; 44: 313-20.
10. Wyszogrodski I, Kyei-Aboagye K, Taeush W, Avery E. Surfactant inactivation by hyperventilation: conservation by end-expiratory pressure. J Appl Physiol 1975; 38: 461-6.
11. Sykes MK. Does mechanical ventilation damage the lung? Acta Anaesthesiol Scand 1991; 35 [suppl]: 126-30.
12. Lachmann B. Open up the lung and keep the lung open. Intensive Care Med 1992; 118: 319-21.
13. Houmes RJ, Bos JAH, Lachmann B. Effects of different ventilator settings on lung mechanics; with special reference to the surfactant system. Appl Cardiopulm Pathophysiol 1994; 5: 117-27.
14. Lachmann B, Gommers D, Govinda N. Rationale and techniques to improve ventilation and gas exchange in acute lung injury. J Jpn Med Soc Biol Interface 1996; 26 (supplement) 115-37.
15. Gommers D, Lachmann B. Surfactant therapy in the adult patient. Current Opinion Crit Care 1995; 1: 57-61.
16. Lachmann B, Gommers D. Is it rational to treat pneumonia with exogenous surfactant? Eur Respir J 1993; 6: 1427-8.
17. Verbrugge SJC, Lachmann B. Partial liquid ventilation. Eur Respir J 1997; 10: 1937-39.
18. Von Neergaard K: Neue Auffassungen über einen Grundbegriff deer Atemmechanik; Die Retraktionskraft deer Lunge, abhängig von deer Oberflächenspannung in den Alveolen, Z Ges Exp Med 1929; 66: 373-94.
19. Van Golde LMG, Batenburg JJ, Robertson B. The pulmonary surfactant system: Biochemical aspects and functional significance. Physiol Rev 1988; 68: 374-455.
20. Johansson J, Curstedt T, Robertson B. The proteins of the surfactant system. Eur Respir J 1994; 7: 372-91.
21. Lachmann B, Danzmann E. Acute respiratory distress syndrome. In: Robertson B, van Golde LMG, Batenburg JJ (eds). Pulmonary surfactant. Amsterdam, Elsevier, 1984; 505-48.
22. Lachmann B, Winsel K, Reutgen H. Deer anti-atelectase faktor deer lunge I. Z Erkr Atm 1972; 137: 267-87.
23. Guyton AC, Moffatt DS, Adair TA. Role of alveolar surface tension in transepithelial movement of fluid. In: Robertson B, van Golde LMG, Batenburg JJ (eds). Pulmonary surfactant . Elsevier, Amsterdam, 1984, pp 171-85.
24. Macklem PT, Proctor DF, Hogg JC. The stability of peripheral airways. Resp Physiol 1970; 8: 191-203.
25. Lachmann B. Possible role of bronchial surfactant. Eur J Resp Dis 1985; 67: 49-61.
26. Reifenrath R. Surfactant action in bronchial mucus. In: Scarpelli EM (eds) Pulmonary Surfactant System. Amsterdam, Elsevier 1983; pp 339-47.

27. Van Iwaarden F. Surfactant and the pulmonary defense system. In: Robertson B, Van Golde LMG, Batenburg JJ (eds). Pulmonary surfactant. Elsevier, Amsterdam, 1992, pp 215-53.
28. Hein T, Lachmann B, Armbruster S, et al. Pulmonary surfactant inhibits the cardiovascular effects of platelet-activating factor (PAF), 5-hydroxytryptamine (5-HT) and angiotensin II. Am Rev Respir Dis 1987; 135 (Suppl) A 506.
29. So KL, Gommers D, Lachmann B. Bronchoalveolar surfactant system and intratracheal adrenaline. Lancet 1993; 341: 120-21.
30. Seger W, Günther A, Walmrath HD et al. Alveolar surfactant and adult respiratory distress syndrome. Clin Investigator 1993; 71: 177-90.
31. Lachmann B, Eijking EP, So KL, Gommers D. In vivo evaluation of the inhibitory capacity of human plasma on exogenous surfactant function. Intensive Care Med 1994; 20: 6-11.
32. Stinson SF, Ryan DP, Hertwerk MS, Hardy JD et al. Epithelial and surfactant changes in influenza pulmonary lesions. Arch Pathol Lab Med 1976; 100: 147-53.
33. Lachmann B. The role of pulmonary surfactant in the pathogenesis and therapy of ARDS. In: Vincent JL (eds). Update in intensive care and emergency medicine. Springer-Verlag, Berlin, 1987, pp 123-34.
34. Webb HH, Tierney DF. Experimental pulmonary edema due to intermittent positive pressure ventilation with high inflation pressures: protection by positive end-expiratory pressure. Am Rev Respir Dis 1974; 110: 556-65.
35. Dreyfuss D, Basset G, Soler P, Saumon G. Intermittent positive pressure hperventilation with high inflation pressures produces pulmonary microvascular injury in rats. Am Rev Respir Dis 1985; 132: 880-4.
36. Verbrugge SJC, Böhm SH, Gommers D, Zimmerman LJI, Lachmann B. Surfactant impairment after mechanical ventilation with large alveolar surface area changes and effects of positive end-expiratory pressure. Br J Anaesth 1998; 80: 1-5.
37. Ashbaugh DG, Bigelow DB, Petty TL, Levine BE. Acute respiratory distress in adults. Lancet 1967; 2: 319-23.
38. Hallman M, Spragg R, Harrelli JH, Moser KM, Gluck L. Evidence of lung function abnormality in respiratory failure. Study of bronchoalveolar lavage phospholipids, surface activity phospholipase activity, and plasma myoinositol. J Clin Invest 1982; 70: 673-83.
39. Pison U, Seeger W, Buchorn R, et al. Surfactant abnormalities in patients with respiratory failure after multiple trauma. Am Rev Respir Dis 1989; 140: 1033-39.
40. Pison U, Obertacke U, Brand M et al. Altered pulmonary surfactant in uncomplicated and septicemia-complicated courses of acute respiratory failure. J Trauma 1991; 30: 19-26.
41. Gregory TJ, Longmore WJ, Moxley MA et al. Surfactant chemical composition and biophysical activity in acute respiratory distress syndrome. J Clin Invest 1991; 88: 1976-81.
42. Veldhuizen RAW, Marcou J, Yao LJ et al . Alveolar surfactant aggregate conversion in ventilated normal and injured rabbits. Am J Physiol 1996; 270: L 152-8.
43. Ito Y, Veldhuizen RAW, Yao LJ et al. Ventilation strategies affect surfactant aggregate conversion in acute lung injury. Am Rev Respir Crit Care Med 1997; 155: 493-9.
44. Pattle RE. Properties, function and origin of the alveolar lining layer. Nature 1955; 175: 1125-6.
45. Clements JA. Pulmonary edema and permeability of alveolar membranes. Arch Environ Health 1961; 2:280-3.
46. Albert RK, Lakshminarayan S, Hildebrandt J, Kirk W, Butler L. Increased surface tension favors pulmonary edema formation in anaesthetized dogs' lungs. J Clin Invest 1979; 63: 1015-18.
47. Roedenberg CE, Nieman GF, Paskanik AM, Hart KE. Microvascular membrane permeability in high surface tension pulmonary edema. J Appl Physiol 1986; 60: 253-9.
48. Hernandez LA, Peevy KJ, Moise AA, et al. Chest wall restriction limits high airway pressure-induced lung injury in young rabbits. J Appl Physiol 1989; 66: 2364-8.
49. Dreyfuss D, Soler P, Basset G, Saumon G. High inflation pressure pulmonary edema. Respective effects of high airway pressure, high tidal volume, and positive end-expiratory pressure. Am Rev Resp Dis 1988; 137: 1159-64.
50. Gorin AB, Stewart PA. Differential permeability of endothelial and epithelial barriers to albumin flux.

J Appl Physiol 1979; 47: 1315-24.
51. Effros RM, Mason GR, Raj JU. Effects of mechanical ventilation and barotrauma on pulmonary clearance of ^{99m}technetium diethylenetriamine pentaacetate in lambs. Pediatr Res 1990; 27: 100-7.
52. Egan EA. Nelson RM, Olver RE. Lung inflation and alveolar permeability to nonelectrolytes in adult sheep in vivo. J Physiol 1976; 260: 409-24.
53. Ramanathan R, Mason GR, Raj JU. Effects of mechanical ventilation and barotrauma on pulmonary clearance of ^{99m}technetium diethylenetriamine pentaacetate in lambs. Pediatr Res 1990; 27: 70-4.
54. Kim KJ, Crandall ED. Effects of lung inflation on alveolar epithelial solute and water transport properties. J Appl Physiol 1982; 52: 1498-505.
55. Evander E, Wollmer P, Jonson B. Pulmonary clearance of inhaled ^{99}Tc-DPTA: Effects of ventilation pattern. Clin Physiol 1990; 10: 189-99.
56. O'Brodovich H, Coates G, Marrin M. Effects of inspiratory resistance and PEEP on ^{99m}Tc-DPTA clearance. J Appl Physiol 1986; 60: 1461-5.
57. Said SI, Avery ME, Davis RK, Banerjee CM, El-Cohary M. Pulmonary surface activity in induced pulmonary edema. J Clin Invest 1965; 44: 458-64.
58. Seeger W, Stör G, Wolf HRD, Neuhof H. Alteration of surfactant function due to protein leakage: special interaction with the fibrin monomer. J Appl Physiol 1985; 58: 326-38.
59. Kobayashi T, Nitta K, Ganzuka M, Inui S, Grossmann G, Robertson B. Inactivation of exogenous surfactant by pulmonary edema fluid. Pediatr Res 1991; 29: 353-6.
60. Mead J, Takishima T, Leith D. Stress distribution in lungs: a model of pulmonary elasticity. J Appl Physiol 1970; 28: 596-608.
61. Muscerede JG, Mullen JBM, Gan K, Slutsky AS. Tidal ventilation at low airway pressures can augment lung injury. Am J Respir Crit Care Med 1994; 149: 1327-34.
62. Taskar V, John E, Evander P, et al. Surfactant dysfunction makes lungs vulnerable to repetitive collapse and reexpansion. Am J Respir Crit Care Med 1997; 155: 313-20.
63. Nilsson R, Grossman G, Robertson B. Pathogenesis of neonatal lung lesions induced by artificial ventilation: evidence against the role of barotrauma. Respiration 1980; 40: 218-25.
64. Caldini P, Leith JD. Brennan MJ. Effect of continuous positive-pressure ventilation (CPPV) on edema formation in dog lung. J Appl Physiol 1975; 39: 672-9.
65. Toung T, Saharia P, Permutt S, Zuidema GD, Cameron JL. Aspiration pneumonia; beneficial and harmful effects of positive end-expiratory pressure. Surgery 1997; 82: 279-83.
66. Hopewell PC, Murray JF. Effects of continuous positive pressure ventilation in experimental pulmonary edema. J Appl Physiol 1976; 40: 568-74.
67. Permutt S. Mechanical influences on water accumulation in the lungs. In: Fishman AP and Renkin EM eds. Pulmonary edema: Clinical Physiological Series. American Physiological Society, Bethesda, 1979, pp 175-93.
68. Peré PD, Warriner EM, Baile EM, Hogg JC. Redistribution of pulmonary extravascular water with positive end-expiratory pressure in canine pulmonary edema. Am Rev Resp Dis 1983; 127: 590-3.
69. Malo J, Ali J, Wood LDH. How does positive end-expiratory pressure reduce intrapulmonary shunt in canine pulmonary edema? J Appl Physiol 1984; 57: 1002-10.
70. Hopewell PC. Failure of positive end-expiratory pressure to decrease lung water content in alloxan-induced pulmonary edema. Am Rev Resp Dis 1979; 120: 813-19.
71. Demling RH, Staub NC, Edmunds Jr LH. Effect of end-expiratory airway pressure on accumulation of extravascular lung water. J Appl Physiol 1975; 38: 907-12.
72. Luce JM, Huan TW, Robertson HT, et al. The effects of prophylactic expiratory positive airway pressure on the resolution of oleic-acid induced lung injury in dogs. Ann Surg 1983; 197: 327-36.
73. Bshouty Z, Ali J, Younes M. Effect of tidal volume and PEEP on rate of edema formation in situ perfused canine lobes. J Appl Physiol 1988; 64: 1900-7.
74. Houmes RJM, Bos HAH, Lachmann B. Effect of different ventilator settings on lung mechanics with special reference to the surfactant system. Appl Cardiopulm Pathophysiol 1994; 5: 117-27.
75. Dreyfuss D, Saumon G. Ventilator induced lung injury. Lessons from experimental studies. Am Rev Respir Dis 1998; 157: 294-323.
76. Veldhuizen RAW, Marcou J, Yao LJ, McGraig L, Ito Y, Lewis JF. Alveolar surfactant aggregate conversion in ventilated normal and injured rabbits. Am J Physiol 1996; 270: 152-8.

77. Verbrugge SJC, Vazquez de Anda G, Gommers D, Neggers SJCMM, Šorm V, Böhm SH, Lachmann B. Exogenous surfactant preserves lung function and reduces alveolar Evans blue dye influx in a rat model of ventilation-induced lung injury. Anesthesiology 1998; 89: 467-74.
78. Argiras EP, Blakeley CR, Dunnill MS, Otremski S, Sykes MK. High PEEP decreases hyline membrane formation in surfactant deficient lungs. Br J Anaesth 1987; 59: 1278-85.
79. Sandhar BK, Niblett DJ, Argiras EP, Dunnill MS, Sykes MK. Effects of positive end-expiratory pressure on hyaline membrane formation in a rabbit model of the neonatal respiratory distress syndrome. Intensive Care Med 1998; 14: 538-46.
80. Gattinoni L, Pesenti A, Torresin A et al. Adult respiratory distress syndrome profiles by computed tomography. J Thorac Imag 1986; 1: 25-30
81. Slutsky A. Consensus conference on mechanical ventilation January 28-30, at North-brook, Illinois, USA, Part 1. Intensive Care Med 1994; 29: 64-79.
82. Roupie E, Dambrosio M, Servillo G et al. Titration of tidal volume and induced hypercapnia in acute respiratory distress syndrome. Am J Resp Crit Care Med 1995; 152: 121-8.
83. Leatherman JW, Lari RL, Iber C Ney AL. Tidal volume reduction in ARDS: effect on cardiac output and arterial oxygenation. Chest 1991; 99: 1227-31.
84. Kiiski R, Takala A, Kari A, Milic-Emili J. Effect of tidal volume on gas exchange and oxygen transport in the adult respiratory distress syndrome. Am Rev Resp Dis 1992; 146: 1131-5.
85. Brochard L, Roudot-Thoroval F. Tidal volume reduction in acute respiratory distress syndrome: a multicenter randomized study. Am J Resp Crit Care Med 1997; 155: A 505.
86. Brower R, Shanholtz C, Shade D, et al. Randomized controlled trial of small tidal volume ventilation in ARDS. Am J Resp Crit Care Med 1997; 155: A 93.
87. Stewart TE, Meade MO, Cook DJ, et al. Evaluation of a ventilation strategy to prevent barotrauma in patients at high risk for acute respiratory distress syndrome. N Engl J Med 1998; 338: 355-61.
88. Amato MBP, Barbas CSV, Medeiros DM, et al. Beneficial effects of the "Open Lung Approach" with low distending pressures in acute respiratory distress syndrome. Am J Respir Crit Care med 1995; 152: 1835-46.
89. Froese A, Bryan Ch. High frequency ventilation. Am Rev Respir Dis 1987; 135: 1363-74.
90. Froese A. High frequency oscillatory ventilation for adult respiratory distress syndrome: let's get it right this time. Crit Care Med 1997; 25: 906-8.
91. Ogawa Y, Miyasaka K, Kawano T, et al. A multicenter randomized trial of high frequency oscillatory ventilation as compared with conventional mechanical ventilation in preterm infants with respiratory failure. Early Hum Develop 1992; 32: 1-10.
92. Vazquez de Anda GF, Hartog A, Verbrugge SJC, et al. The open lung concept: pressure controlled ventilation is as effective as high frequency oscillatory ventilation in improving gas exchange and lung mechanics in surfactant-deficient animals. Intensive Care Med 1999; 25: 990-6.
93. Gommers D, Lachmann B. Surfactant therapy: does it have a role in adults? Clinical Intensive Care 1993; 4: 284-95.
94. Jobe AH. Pulmonary surfactant therapy. N Engl J Med. 1993; 328: 861-8.
95. Lewis JF, Tabor B, Ikegami, et al. Lung function and surfactant distribution in saline-lavaged sheep given instilled vs. nebulized surfactant. J Appl Physiol. 1993; 74: 1256-64.
96. Gommers D, van't Veen A, Verbrugge SJC, Lachmann B. Comparison of eight different surfactant preparations on improvement of blood gases in lung-lavaged rats. Appl Cardiopulm Pathophysiol 1998; 7: 95-102
97. Verbrugge SJC, Šorm V, Lachmann B. Mechanisms of acute respiratory distress syndrome: Role of surfactant changes and mechanical ventilation. J Physiol Pharmacol 197; 48: 537-57.
98. Froese AB, McCulloch PR, Sugiura M, et al. Optimizing alveolar expansion prolongs the effectiveness of exogenous surfactant therapy in the adult rabbit. Am Rev Respir Dis 1993; 148: 569-77.
99. Verbrugge SJC, Gommers D, Lachmann B. Conventional ventilation modes with small pressure amplitudes and high end-expiratory pressure levels optimize surfactant therapy. Critical Care Med (in press).
100. Hallman M, Merritt TA, Kari A, et al. Factors affecting surfactant responsiveness. Ann Med 1991; 23:

693-8.

101. Gerstmann DR, Minton SD, Stoddard RA, et al. The PROVO multicenter early high-frequency oscillatory ventilation trial: improved pulmonary and clinical outcome in respiratory distress syndrome. Pediatrics 1996; 98: 1044-57.
102. Nilsson R, Berggren P, Curstedt T, et al. Surfactant and ventilation by high frequency oscillation in premature newborn rabbits: effect on survival, lung aeration, and bronchiolar epithelial lesions. Pediatr Res 1985; 19: 143-7.
103. Jackson JC, et al & Hodson WA: Reduction in lung injury after combined surfactant and high-frequency ventilation. Am J Respir Crit Care Med 1994; 150: 534-9.
104. Gommers D, Hartog A, Schnabel R, De Jaegere A, Lachmann B. High-frequency oscillatory ventilation is not superior to conventional mechanical ventilation in surfactant-treated rabbits with lung injury. Eur Respir J 1999; 14: 738-744.
105. Gommers D, Vilstrup C, Bos JAH, et al. Exogenous surfactant therapy increases static lung compliance, and cannot be assessed by measurements of dynamic compliance alone. Crit Care Med 1993; 21: 567-574.
106. Tütüncü AS, Faithfull S, Lachmann B. Intratracheal perfluorocarbon administration combined with mechanical ventilation in experimental respiratory distress syndrome: dose-dependent improvement of gas exchange. Crit Care Med 1993; 21: 962-969.
107. Tütüncü AS, Akpir K, Mulder P, Erdmann W, Lachmann B. Intratracheal perfluorocarbon administration as an aid in the ventilatory management of respiratory distress syndrome. Anesthesiology 1993; 79: 1083-93.
108. Houmes RJM, Verbrugge SJC, Hendrik ER, Lachmann B. Hemodynamic effects of partial liquid ventilation with perfluorocarbon in acute lung injury. Intensive Care Med 1995; 21: 966-72.
109. Houmes RJM, Hartog A, Verbrugge SJC, Böhm SH, Lachmann B. Combining partial liquid ventilation with nitric oxide to improve gas exchange in acute lung injury. Intensive Care Med 1997: 23: 162-8.
110. Hirschl RB, Parent RB, Tooley R, et al. Liquid ventilation improves pulmonary function, gas exchange and lung injury in a model of respiratory failure. Ann Surg 1995; 21: 79-88.
111. Quintel M, Hirschl R, Roth H, et al. Computer tomographic assessment of perfluorocarbon and gas distribution during partial liquid ventilation for acute respiratory failure. Am J Respir Crit Care Med 1998; 158: 249-55.

Chapter 2

The open lung concept: pressure controlled ventilation is as effective as high frequency oscillatory ventilation in improving gas exchange and lung mechanics in surfactant-deficient animals

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Summary

Objective: To demonstrate in experimental animals with respiratory insufficiency that under well-defined conditions, commercially available ventilators allow settings which are as effective as high frequency oscillatory ventilators (HFOV), with respect to the levels of gas exchange, protein infiltration, and lung stability.

Design: Prospective, randomized, animal study.

Setting: Experimental laboratory of a University.

Subjects: Eighteen adult male Sprague-Dawley rats.

Interventions: Lung injury was induced by repeated whole-lung lavage. Thereafter, the animals were assigned to pressure control ventilation (PCV) plus the Open Lung Concept (OLC) or HFOV plus OLC ($HFOV_{OLC}$). In both groups, an opening maneuver was performed by increasing airway pressures to improve the arterial oxygen tension/fractional inspired oxygen (PaO_2/FiO_2) ≥ 500 mmHg; thereafter, airway pressures were reduced to minimal values, which kept PaO_2/FiO_2 ≥ 500 mmHg. Pressure amplitude was adjusted to keep CO_2 as close as possible in the normal range.

Measurements and Results: Airway pressure, blood gas tension, and arterial blood pressure were recorded every 30 min. At the end of the 3-h study period, a pressure-volume curve was recorded and a broncho-alveolar lavage was performed to determine protein content. After the recruitment maneuver, the resulting mean airway pressure to keep a PaO_2/FiO_2 ≥ 500 mmHg was 25 ± 1.3 cm H_2O during PCV_{OLC} and 25 ± 0.5 cm H_2O during $HFOV_{OLC}$. Arterial oxygenation in both groups was above ≥ 500 mmHg and arterial carbon dioxide tension was kept close to the normal range. No differences in mean arterial pressure, lung mechanics and protein influx were found between the two groups.

Conclusion: This study shows that in surfactant-deficient animals, PCV, in combination with a recruitment maneuver, opens atelectatic lung areas and keeps them open as effectively as HFOV.

Introduction

It is becoming increasingly clear that besides inspiratory epithelial overstreching [1], the repeated collapse and reexpansion of alveoli, which leads to the development of shear forces, contributes to a great extent to ventilation-induced lung injury (VILI) [2]. It has been suggested that collapsed alveoli should be recruited before starting long-term mechanical ventilation, and high inspiratory lung volumes should be avoided by using small pressure amplitudes [the Open Lung Concept (OLC)] [3].

More than 25 years ago, high frequency oscillatory ventilation (HFOV) was introduced as a new ventilatory technique for treating the neonatal respiratory distress syndrome (RDS) [4]. The small pressure amplitudes applied during HFOV were expected to reduce VILI, but it has been demonstrated that HFOV only leads to less lung damage when it is applied to re-expanded lungs (i.e., open lungs) by use of a relatively high mean airway pressure (MAwP) [5, 6]. This is called the high-lung volume strategy; the results of recent pilot studies in neonates with RDS applying this strategy are encouraging [7-10].

The idea has become established that, due to the larger pressure swings, conventional mechanical ventilation (CMV) recruits alveoli at inspiration but can not prevent them from collapse at end-expiration and that only an increase in positive end-expiratory pressure (PEEP) during CMV would reduce the amount of alveolar derecruitment at the cost of higher peak inspiratory pressures [11,12]. Studies comparing CMV and HFOV with respect to gas exchange seem to support this idea [5,6, 13-15]. These studies showed that, although the lung can be opened during CMV with relatively high peak inspiratory pressures, the lung could not be kept open during the ventilation period. The required high level of PEEP and high tidal volumes to keep the lung open and provide adequate gas exchange in these studies resulted in barotrauma and circulatory impairment [5, 6]. However, earlier studies with CMV using a pressure-controlled time-cycle mode (PCV) applying small pressure amplitudes combined with high levels of PEEP and high inspiratory pressure for a short time, have shown that PCV can effectively recruit alveoli and keep them open during the entire respiratory cycle [16, 17].

Therefore, in the present study in experimental animals with respiratory insufficiency, we

investigated whether under well-defined conditions, commercially available ventilators allow settings which are as effective as HFOV, with respect to the levels of gas exchange, protein infiltration, and lung stability.

Materials and Methods

The study protocol was approved by the institutional Animal Investigation Committee. Care and handling of the animals were in accordance with European Community guidelines (86/609/EC). The study was performed in 18 adult male Sprague-Dawley rats (body weight 280-350 g). Anesthesia was induced with 2% enflurane and 65% nitrous oxide in oxygen. Immediately after induction of anesthesia, 6 animals were killed, the thorax was opened, and a static pressure-volume curves (P/V curves) were recorded and a broncho-alveolar lavage (BAL) was performed. These animals served as a healthy non-ventilated control group (Healthy). In the remaining animals, a polyethylene catheter (0.8 mm outer diameter) was inserted into the right carotid artery for drawing arterial blood samples, and for continuous monitoring of arterial pressure to adjust hemodynamic support. Before tracheotomy, the animals received 30 mg/kg pentobarbital sodium, intraperitoneally (Nembutal®; Algin, Maassluis, The Netherlands). After tracheotomy, muscle relaxation was induced with pancuronium bromide 0.6 mg/kg, intramuscularly (Pavulon®; Organon Teknika, Boxtel, The Netherlands) immediately followed by connection to the ventilator and to a pressure transducer (Siemens Sirecust 1280, Siemens, Danvers, Mass, USA) for continuous arterial pressure monitoring. The animals were mechanically ventilated with a Servo Ventilator 300 (Siemens-Elema, Solna, Sweden) in a pressure-controlled time-cycled mode, at a fractional inspired oxygen concentration (FIO_2) of 1.0, frequency of 30 breaths per minute (bpm), peak inspiratory pressure (PIP) of 12 cmH_2O, PEEP of 2 cm H_2O, inspiratory/expiratory I/E ratio of 1:2. Anesthesia was maintained with pentobarbital sodium (Nembutal®; 30 mg/kg); neuromuscular block was maintained with pancuronium bromide, i.m. (Pavulon®; 0.6 mg/kg). Body temperature was kept within the normal range by means of a heating pad. Initially, PIP was increased to 20 cm H_2O for 30 seconds to open up atelectatic regions in the lungs due to the surgical procedure. After this procedure to open up the lungs, the ventilator settings were reset to the previous ones and a 0.15 ml blood sample was taken and replaced by heparinized (10 IU/ml) saline (0.9% NaCl). Arterial oxygen tension (PaO_2) and carbon dioxide tension ($PaCO_2$) were measured by conventional methods (ABL 505, Radiometer Copenhagen, Denmark). Next, respiratory failure was induced by repeated

whole-lung lavage as described by Lachmann et al. [18]. Each lavage was performed with saline (32 ml/kg body weight) heated to 37°C. Just before the first lavage, PIP and PEEP were elevated to 26 and 6 cm H_2O, respectively. Lung lavage was repeated five to seven times with 5-min intervals to achieve a $PaO_2/FIO_2 \leq 85$ mmHg. Within 10 min after the last lavage, the animals were randomized to one of the following groups (n=6 per group). In the first group, PCV_{OLC}, a procedure to open up the lungs (defined as $PaO_2/FIO_2 \geq 500$ mmHg), at the following ventilator settings: PIP 40 cm H_2O, static PEEP 12 cm H_2O, I/E ratio 4:1, FIO_2 1.0, respiratory frequency 150 bpm. After 1 to 2 min at these settings, a blood sample was drawn to verify that PaO_2/FIO_2 was ≥ 500 mmHg. After this recruitment procedure, total PEEP (PEEPt= static PEEP plus intrisic PEEP) was decreased in approximately in 2 to 3 min steps to the minimal level which kept $PaO_2/FIO_2 \geq 500$ mmHg. Then the pressure amplitude was set to keep $PaCO_2$ as close as possible to the normal range and was not changed thereafter [19,20]. The second group, $HFOV_{OLC}$, was ventilated with HFOV (type OHF-1, Dufour, Villeneuve d'Ascq, France); an opening maneuver was performed by setting the ventilator to oscillation mode without sigh, respiratory rate at 10 Hz, oscillatory pressure amplitude of 28 cm H_2O, FIO_2 1.0. The MAwP was initiated at 28 cm H_2O. After about 1-2 min at these ventilator settings, a blood gas sample was drawn to verify that PaO_2/FiO_2 was ≥ 500 mmHg. Thereafter, the level of MAwP was decreased in 2 to 3 minute steps, to the minimal level which kept $PaO_2/FIO_2 \geq 500$ mmHg. Then, the oscillatory pressure amplitude was set to maintain $PaCO_2$ as close as possible to normal range and was not changed thereafter.

Airway pressures were continuously monitored with a tip catheter pressure transducer (Raychem EO 2A 121, USA), using a water column as a reference pressure, connected with a Y-piece to the tracheal tube, and recorded (Siemens Sirecust 1280, Siemens, Danvers, Mass, USA). Additionally, intrinsic PEEP was determined by subtracting set PEEP from total PEEP in the PCV_{OLC} group and in the $HFOV_{OLC}$ total PEEP was defined as the lowest pressure within the oscillatory pressure amplitude. The highest pressure within the oscillatory pressure amplitude was defined as PIP.

After surfactant depletion and performance of the recruitment procedure, airway pressures were

determined and blood gas samples were taken at 15, 30, 60, 90, 120, 150 and 180 min. At the same time points, arterial pressure was recorded. Hemodynamic support was provided by infusion of 1 ml saline 0.9% (to a maximum of 3 ml per h) when mean arterial pressure (MAP) decreased below 100 mmHg.

After 180 min, all animals were killed with an overdose of pentobarbital sodium injected through the penile vein. Then static P/V curves were recorded 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 (Validyne model DP 45-32, Validyne Engineering, Northridge, Calif., USA), and pressures were recorded on a polygraph (Grass model 7B, Grass Instrument, Quincy, Mass, USA). Using a syringe filled with nitrogen (N_2) the lungs were first inflated (within 10 s) to an airway pressure of 35 cm H_2O, which was maintained for 5 s, followed by deflation to an airway pressure of 0 cmH_2O. Then the lungs were re-inflated in steps of 0.5 ml until an airway pressure of 35 cmH_2O was reached. Each inflation step took 1-2 s followed by a 5-s pause to allow pressure equilibration. After this, in the same way, the lungs were then deflated until an airway pressure of 0 cmH_2O was reached. The volume of N_2 left in the syringe was recorded. Maximal compliance (C_{max}) was calculated from the steepest part of the deflation limb [21]. Total lung capacity (TLC_{35}) was defined as lung volume at inflation with a distending pressure of 35 cm H_2O.

The Gruenwald index, which characterizes the surfactant system in situ, was calculated from the pressure-volume curve, defined as $(2V_5+V_{10})/2V_{max}$, where V_5, V_{10} and V_{max} are the lung volumes at transpulmonary pressures of 5, 10, and 35 cm H_2O from the deflation limb, respectively [22].

After P/V recordings, BAL was performed five times with saline-$CaCl_2$ 1.5 mmol/L. Thereafter, cell debris was removed from BAL by centrifugation at 400 g for 10 min, and protein concentration was measured using the Bradford method (Biorad protein assay, Munich, Germany) [23].

Statistical analysis was performed using the Instat 2.0 biostatistics package (Graph Pad Software, San Diego, Calif., USA). Analysis of variance was performed to compare intragroup and intergroup differences at every time point; if $p<0.05$, a Tukey post-hoc test was performed. All data are reported as mean ± standard deviation (SD).

Results

Blood gas values before and immediately after lavage were comparable for both groups (Fig. 1, Table 1). None of the animals died during the 3-h study period. Carbon dioxide values decreased significantly from 64±9.0 mmHg after lung-lavage to 32±8.6 mmHg 15 min after the recruitment procedure ($p< 0.01$) and from 54±5.7 to 32±7.5 mmHg ($p< 0.01$) in PCV_{OLC} and $HFOV_{OLC}$, respectively, and remained comparable during the entire observation period (Table 1).

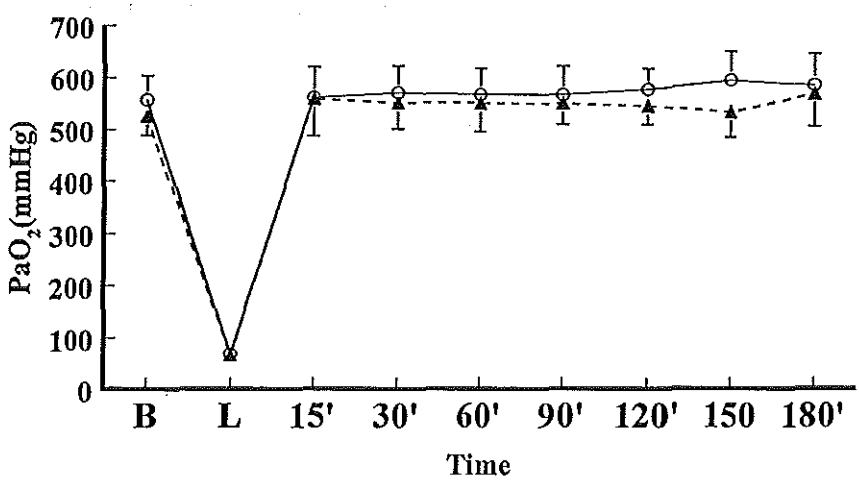

Figure 1. PaO_2 values (mean ± SD) over the whole study period. B= before lavage, L= after lavage. Pressure controlled time-cycled ventilation with open lungs (continuous line) and high frequency oscillatory ventilation with open lungs (dashed line). No statistical differences within or between the two groups over time were found after the Open Lung Concept was applied.

Figure 2 shows the mean airway pressures recorded from the tip catheter pressure transducer 3 h after the recruitment procedure. PIP and PEEPt values were significantly lower in the PCV_{OLC} group than in the $HFOV_{OLC}$ group. However, the driving pressure amplitude was significantly higher

in the PCV_{OLC} group (18.8±2.2 cm H_2O) compared with the $HFOV_{OLC}$ group (14.0±1.6 cm H_2O, $p<0.05$). The MAwP were not significantly different between the two groups (25±1.3 cm H_2O in PCV_{OLC} and 26±0.5 cm H_2O in $HFOV_{OLC}$). The total PEEP in PCV_{OLC} consisted of 10±0.3 cm H_2O static PEEP and 3±2.4 cm H_2O intrinsic PEEP.

In PCV_{OLC}, mean values of MAP (Table 1) 15 min after the recruitment maneuver were kept above 100 mmHg, and no intergroup differences were found during the 3-h study period. However, intragroup differences were observed in the $HFOV_{OLC}$ group where the mean values of MAP were significantly lower at the end of the study period. Fluid replacement after the recruitment maneuver was required in 2 animals in the PCV_{OLC} group and in 3 animals in the $HFOV_{OLC}$ group. There was no statistical difference in the rate of saline infusion during the 3-h study period between groups, with 0.5 ml/h in PCV_{OLC} and 0.8 ml/h in $HFOV_{OLC}$.

Table 1. Data on arterial carbon dioxide tension ($PaCO_2$) and mean arterial pressure (MAP) over time in the groups with pressure-controlled ventilation with open lungs (PCV_{OLC}) and high frequency oscillatory ventilation with open lungs ($HFOV_{OLC}$). Values are mean ± SD

	Time (min)	PCV_{OLC}	$HFOV_{OLC}$
$PaCO_2$ (mmHg)	**Basal**	40± 6.4	37± 6.2
	Lavage	64± 9.0	54± 5.7
	15′	32± 8.6*	32± 7.5*
	30′	37± 8.3*	37± 6.4*
	60′	33± 6.6*	35± 6.0*
	90′	35± 8.4*	37± 5.5*
	120′	36± 10.0*	37± 4.5*
	150′	38± 13.0*	34± 3.2*
	180′	34± 10.0*	32± 4.3*
MAP (mmHg)	**Basal**	134± 22.5	144± 14.7
	Lavage	90± 21.0	100± 9.0
	15′	115± 13.0	121± 14.3
	30′	122± 12.1	122± 23.2
	60′	126± 10.0	114± 19.0
	90′	123± 14.5	114± 11.9
	120′	125± 13.0	108± 14.0
	150′	122± 12.4	108± 18.9
	180′	118± 14.4	101± 17.0

* vs after lavage $p \leq 0.05$

Figure 3 shows the P/V curves from the Healthy, PCV_{OLC}, and $HFOV_{OLC}$ groups. No statistical differences were found in TLC_{35} between groups (43±2 ml/kg in the Healthy control group, 41±6 ml/kg in PCV_{OLC}, and 42±2 ml/kg in $HFOV_{OLC}$). As expected, in the Healthy control group C_{max} was significantly higher (4.0±0.2 ml/cm H_2O per kg) than in the surfactant-depleted lungs ventilated either with PCV_{OLC} or $HFOV_{OLC}$ (2.4±0.6 and 2.5±0.2 ml/cm H_2O per kg, respectively). Obviously, the Gruenwald index was also significantly higher in the Healthy control group than in PCV_{OLC} (1.06±0.20 vs 0.67±0.13; $p<0.01$) and $HFOV_{OLC}$ (1.06±0.20 vs 0.53±0.15, $p < 0.001$).

The protein concentration of BAL fluid was not significantly different between the three groups: 0.44±0.20 in the Healthy control group; 0.55±0.23 in PCV_{OLC}, and 0.59±0.28 in $HFOV_{OLC}$.

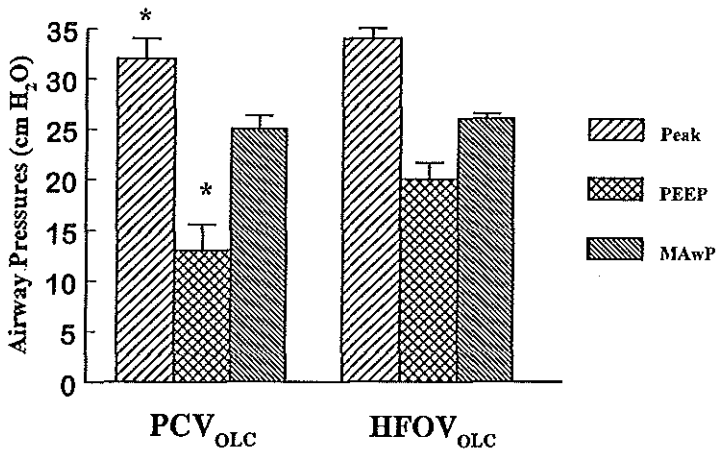

Figure 2. Airway pressures (mean±SD) recorded with the tip catheter pressure transducer 3 hours after the recruitment maneuver. In pressure controlled time-cycled ventilation with open lungs (PCV_{OLC}) there was a significantly (* $p< 0.05$) lower peak pressure (Peak) and lower positive end-expiratory pressure (PEEP) compared with high frequency oscillatory ventilation with open lungs ($HFOV_{OLC}$), at the same mean airway pressure (MAwP).

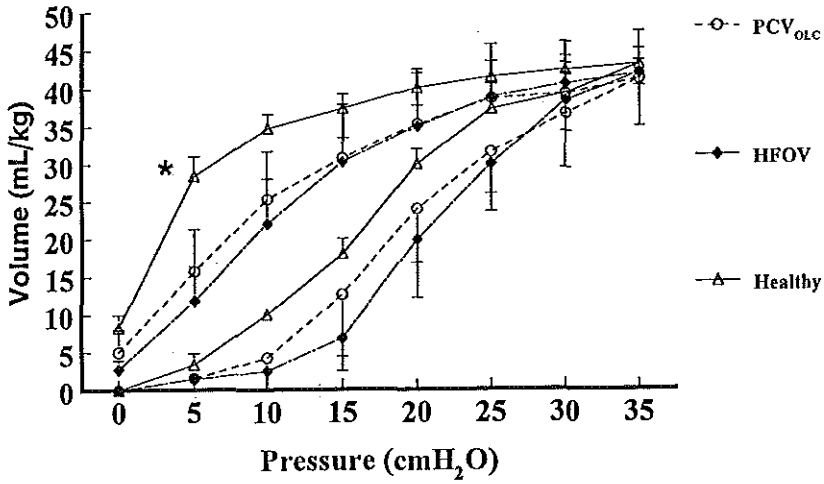

Figure 3. Pressure-volume curves (mean ± SD). At total lung capacity, no statistical differences were found between the three groups. In the Healthy non-ventilated controls (Healthy) C_{max} was significantly higher than those in the pressure controlled time-cycled ventilation with open lungs (PCV_{OLC}) and high frequency oscillatory ventilation with open lungs ($HFOV_{OLC}$) (* $p<0.001$).

Discussion

This study shows in experimental animals with respiratory insufficiency that under well-defined conditions, commercially available ventilators allow settings which are as effective as HFOV with respect to the level of gas exchange, protein infiltration, and lung stability.

In the present study we used the lung lavage model, which has proved to be a consistent and convenient model of acute lung injury [18]. It has been postulated that, in the acute phase, this model reflects more a primary surfactant deficiency, as seen in neonatal RDS [16, 17]. Despite the fact that the lung injury in this study is not exactly representative of the pathology seen in humans with RDS, this model is ideal for testing various therapeutic interventions for RDS [16, 17].

It has been demonstrated that arterial oxygenation increases with increasing functional residual capacity as alveoli re-expand and shunt flow decreases [5, 6, 20, 24]. Therefore, in the present study we used arterial oxygenation as a parameter to characterize the state of alveoli recruitment. Previous studies in rabbits with acute lung injury have shown that HFOV applied with the "high-lung volume strategy" is able to reach oxygenation levels above 350 mmHg and normocapnia [5, 6, 12]. A prerequisite for this latter strategy is that, high pressures have to be applied, for a short period, to reaerate collapsed lung regions, which means that after reaeration oscillation takes place on the deflation limb of the P/V curve. Under this condition, carbon dioxide elimination is controlled by the oscillation pressure amplitude. However, according to Froese and Bryan's studies [6, 11, 12], the small swing in pressures and low tidal volumes produced by HFOV in the past, could not be produced with CMV. In contrast to these latter studies, our study demonstrates that it is also possible to reach high levels of arterial oxygenation and normocapnia by applying small driving pressure amplitudes in the PCV_{OLC} mode. Pressure readings from the tip catheter pressure transducer showed that mean airway pressures were comparable in both OLC groups, with comparable good oxygenation. Hypercapnia was not observed and $PaCO_2$ levels were close to normal values during the 3-h study period in both groups. However, the driving pressure amplitude was almost 5 cmH_2O higher in the PCV_{OLC} group. Whether the latter observation has any clinical impact on VILI cannot be answered from this study. If one considers that protein influx is a sensitive parameter for VILI [25], then at least the higher driving pressure amplitude had no additional negative effect on the protein influx over the study period.

It is known that high MAwP can decrease venous return of the systemic circulation by impairment of the pulmonary circulation due to overdistention of alveoli, which results in compression of the pulmonary capillaries [17, 26]. When applying the OLC, hemodynamic compromise should be minimized by setting the MAwP finally at a level that just compensates for the increased tendency of the alveoli to collapse. However, if this still leads to hemodynamic compromise it should be compensated for by proper fluid management and hemodynamic support by inotropics [3, 19, 27, 28]. In our study, we observed a decrease in blood pressure only during the recruitment maneuver, which returned to normal levels within 1-2 min after reaching the airway pressures which kept the lungs open.

After the recruitment maneuver, the MAwP in both OLC groups was the same, which resulted in mean MAP values above 100 mmHg over the whole study period in both groups, and that is why the demand for fluids in both groups was not significantly different. These results agree with clinical trials which assessed the beneficial effects of open lungs in patients with adult ARDS [27 - 30].

There was no difference between the groups in the amount of protein recovered in the BAL, nor when compared with healthy, normal nonventilated control animals. The epithelium rather than the endothelium is rate limiting for the transfer of protein across the alveoli capillary barrier [25]. Although peak inspiratory epithelial overstretching has been considered the main contributing factor for epithelial injury and intra-alveolar protein infiltration [1, 31, 32] it is realized more and more that repeated alveolar collapse and reexpansion leads to shear stress with epithelial and endothelial damage, resulting in alveolar protein accumulation [33, 34]. In a surfactant-deficient model of acute lung injury, application of OLC decreases protein leakage [35]. It is known that counterbalancing the increased collapse tendency of the surfactant deficient alveoli with appropriate airway pressures favors the shift of fluid from the alveoli to the interstitium by decreasing the pressure gradient across the alveolar-capillary membrane [36]. In addition, ventilating the alveoli with the smallest possible pressure amplitude will prevent epithelial overstretching. These two mechanisms may explain the comparable protein values in both groups compared with the healthy control group.

All changes in the P/V curves after surfactant depletion (e.g., decreased C_{max} and Gruenwald index, and increased opening pressure) confirm earlier results in this animal model [5, 6, 12, 16, 17] on the one hand and, on the other hand, demonstrate that the two modes of mechanical ventilation did not influence lung mechanics during the 3-h observation period.

In summary, this study shows that in surfactant-deficient animals, PCV in combination with a recruitment maneuver results in the same level of oxygenation, carbon dioxide elimination, protein infiltration and lung mechanics as HFOV. Moreover, mean values of MAP were kept above 100 mmHg in both modes of ventilation during the 3-h study period. These data indicate that the preferred use of high frequency oscillators for certain clinical conditions over pressure-controlled ventilators needs to be re-considered. Rather than using special modes of mechanical ventilation in RDS, one should apply a general concept of ventilation which provides an open lung over the entire respiratory cycle, with the least possible hemodynamic compromise.

References

1. Dreyfuss D, Soler P, Basset G, Saumon G (1988) High inflation pressure pulmonary edema: respective effects of high airway pressure, high tidal volume, and positive end-expiratory pressure. Am Rev Respir Dis 137: 1159-1164
2. Taskar V, John J, Evander E, Robertson B, Jonson B (1997) Surfactant dysfunction makes lungs vulnerable to repetitive collapse and re-expansion. Am J Respir Crit Care Med 155: 313-320
3. Lachmann B (1992) Open up the lung and keep the lung open. Intensive Care Med 18: 319-321
4. Marchak BE, Thompson WK, Duffy P, Miyaki T, Bryan MH, Bryan C, Froese A (1981) Treatment of RDS by high frequency oscillatory ventilation: a preliminary report. J Pediatr 99: 287-292
5. Hamilton PP, Onayemi A, Smyth J, Gillan J, Cuty E, Froese A, Bryan C (1983) Comparison of conventional and high-frequency ventilation: oxygenation and lung pathology. J App Physiol 55: 131-138
6. McCulloch P, Forkert PG, Froese A (1987) Lung volume maintenance prevents lung injury during high frequency oscillatory ventilation in surfactant-deficient rabbits. Am Rev Respir Dis 137: 1185-1192
7. Ogawa Y, Miyasaka K, Kawano T, Imura S, Inukai K, Okuyama K, Oguchi K, Togari H, Nishida H, Mishina J (1992) A multicenter randomised trial of high frequency oscillatory ventilation as compared with conventional mechanical ventilation in preterm infants with respiratory failure. Early Hum Develop 32:1-10
8. Clark RH, Yoder BA, Sell MS (1994) Prospective, randomized comparison of high-frequency oscillation and conventional ventilation in candidates for extracorporeal membrane oxygenation. J Pediatr 124:447-454
9. Arnold JH, Hanson JH, Toro-Figuero LO, Gutierrez J, Berens RJ, Anglin DL (1994) Prospective, randomized comparison of high-frequency oscillatory ventilation and pressure controlled ventilation in pediatric respiratory failure. Crit Care Med 22:1530-1539
10. Gerstmann Dr, Minton SD, Stoddard RA, Meredith KS, Monaco F, Bertrand JM, Battisti O, Langhendries JP, Francois A, Clark RH (1996) The PROVO multicenter early high frequency oscillatory ventilation trial: improved pulmonary and clinical outcome in respiratory distress syndrome. Pediatrics 98: 1044-1057
11. Froese A, Bryan C (1987) High frequency ventilation. Am Rev Respir Dis 135: 1363-1374
12. Kolton M, Cattran CH, Kent G, Volgyesi G, Froese A, Bryan C (1982) Oxygenation during high-frequency ventilation compared with conventional mechanical ventilation in two models of lung injury. Anesth Analg 61: 323-332
13. Mook PH, Proctor HJ, Yee HV, Ennema JJ, Wildevuur CR (1984) High-frequency ventilation in rabbits with respiratory insufficiency. J Surg Res 36: 614-619
14. Bond M, McAloon J, Froese A (1994) Sustained inflations improve respiratory compliance during high-frequency oscillatory ventilation but not during large tidal volume positive-pressure ventilation in rabbits. Crit Care Med 22: 1269-1277
15. Thompson W, Marchack E, Froese AB, Bryan C (1982) High-frequency oscillation compared with standard ventilation in pulmonary injury model. J Appl Physiol Respir Envr Exercise Physiol 52: 543-548
16. Lachmann B, Jonson B, Lindroth M, Robertson B (1982) Modes of artificial ventilation in severe respiratory distress syndrome: Lung function and morphology in rabbits after wash-out of alveolar surfactant. Crit Care Med 10: 724-732
17. Lachmann B, Danzmann E, Haendly B, Jonson B (1982) Ventilator settings and gas exchange in respiratory distress syndrome. In: Prakash O (ed). Applied physiology in clinical respiratory care. Nijhoff, The Hague, pp 141-176
18. Lachmann B, Robertson B, Vogel J (1980) In vivo lung lavage as an experimental model of respiratory distress syndrome. Acta Anaesthesiol Scand 24: 231-236
19. Böhm SH, Vazquez de Anda GF, Lachmann B (1998) "The Open Lung Concept". In Vincent JL (ed). Yearbook of intensive care and emergency medicine. Springer, Berlin Heidelberg, New York, pp 430-440
20. Vazquez de Anda GF, Lachmann B (1998) Protecting the lung during mechanical ventilation with The Open Lung Concept. Acta Anaesthesiol Scand 112; 63-66
21. Gommers D, Vilstrup C, Bos JAH, Larsson A, Werner O, Hannapel E, Lachmann B (1993) Exogenous

surfactant therapy increases static lung compliance and, cannot be assessed by measurements of dynamic compliance alone. Crit Care Med 21: 567-574

22. Gruenwald P (1963) A numerical index of the stability of lung expansion. J Appl Physiol 18: 665-667
23. Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Ann Biochem 72: 248-254
24. Suzuki H, Papazoglou K, Bryan A (1992) Relationship between PaO_2 and lung volume during high frequency oscillatory ventilation. Acta Paediatr Jpn 34: 494-500
25. Verbrugge S, Böhm S, Gommers D, Zimmerman L, Lachmann B (1998) Surfactant impairment after mechanical ventilation with large alveolar surface area changes and effects of positive end-expiratory pressure. Br J Anaesth 80:360-364
26. Nelle M, Yillow E, Linderkamp O (1997) Effects of high-frequency oscillatory ventilation on circulation in neonates with pulmonary interstitial emphysema or RDS. Intensive Care Med 23: 271-676
27. Amato M, Barbas C, Medeiros D, Schettino G, Lorenzi Filho G, Kairalla R, Deheinzelin D, Morais E, Fernandes T,Takagaki T, Carvalho C (1995) Beneficial effects of the "open lung approach" with low distending pressures in acute respiratory distress syndrome. Am J Respir Crit Care Med 152:1835-1846
28. Carvalho C, Barbas C, Medeiros D, Magaldi R, Lorenzi Filho G, Kairalla R, Deheinzelin D, Munhoy C, Kaufmann M, Ferreira M, Takagaki T, Amato MBP (1997) Temporal hemodynamic effects of permissive hypercapnia associated with ideal PEEP in ARDS. Am J Respir Crit Care Med 156: 1458-1466
29. Kesecioglu J, Tibboel D, Lachmann B (1994) Advantages and rationale for pressure control ventilation. In: Vincent JL, ed. Yearbook of intensive care and emergency medicine. Springer Berlin, Heidelberg, New York, pp 524-533
30. Amato MBP, Barbas CSV, Medeiros DM, Magaldi R, Schettino G, Lorenzi-Filho G, Kairalla R, Deheinzelin D, Munoz C, Oliveira R, Takagaki T, Carvalho C (1998) Effect of a protective-ventilation strategy on mortality in the respiratory distress syndrome. N Engl J Med 338: 347-354
31. Gorin AB, Stewart PA (1979) Differential permeability of endothelial and epithelial barriers to albumin flux. J Appl Physiol 47: 1315-1324
32. Dreyfuss D, Basset G, Soler P, Saumon G (1985) Intermittent positive pressure hyperventilation with high inflation pressures produces pulmonary microvascular injury in rats. Am Rev Respir Dis 132: 880-884
33. Dreyfuss D, Saumon G (1993) Role of tidal volume, FRC, and end inspiratory volume in the development of pulmonary edema following mechanical ventilation. Am Rev Respir Dis 148: 1194-1203
34. Verbrugge S, Vazquez de Anda G, Gommers D, Neggers S, Šorm V, Böhm S, Lachmann B (1998) Exogenous surfactant preserves lung function and reduces alveolar Evans blue dye influx in a rat model of ventilation-induced lung injury. Anesthesiology 89: 467-474
35. Hartog A, Vazquez de Anda GF, Gommers D, Kaisers U, Verbrugge S, Schnabel R, Lachmann B (1999) Comparison of exogenous surfactant therapy, mechanical ventilation with high end-expiratory pressure and partial liquid ventilation in a model of acute lung injury. Br J Anaesth 82: 81-86
36. Permutt S (1979) Mechanical influences on water accumulation in the lungs. In: Pulmonary edema. (Clinical physiology series). Fishman AP, Renkin EM (eds) American Physiology Society. Bethesda,. pp 175-193

Chapter 3

Mechanical ventilation with high PEEP and small driving pressure amplitude is as effective as high-frequency oscillatory ventilation to preserve the function of exogenous surfactant in lung-lavaged rats

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Submitted for publication

Summary

Objective: To demonstrate that under well-defined conditions, pressure controlled ventilators (PCV) allow settings which are as good as high frequency oscillatory ventilators (HFOV) to preserve the function of exogenous surfactant in lung-lavaged rats.

Design: Experimental, comparative study.

Setting: Research laboratory of a large university.

Subjects: Sixteen adult male Sprague-Dawley rats (280-310 g).

Interventions: Lung injury was induced by repeated lavage. After last lavage, all animals received exogenous surfactant, and were then randomly assigned to two groups (n=8 per group). The first group received PCV with small pressure amplitudes and 'high' positive end-expiratory pressure. The second group received HFOV. In both groups, an opening maneuver was performed by increasing airway pressure to improve $Pao_2/Fio_2 \geq 500$ torr.

Measurements and Main Results: Blood gases were measured every 30 min for 3 hours. Airway pressures were measured with a tip catheter pressure transducer. At the end of the study period, a pressure-volume curve was recorded and a broncho-alveolar lavage was performed to determine protein content and surfactant composition. The results showed that arterial oxygenation in both groups could be kept above 500 torr during the 3-hour study period by using a mean airway pressure of 13±3 cm H_2O in PCV and 13±2 cm H_2O in HFOV. Further, there was no differences in the Gruenwald index, protein influx, or ratio of small to large aggregates between both study groups.

Conclusion: PCV with sufficient level of positive end-expiratory pressure and small driving pressure amplitudes is as effective as HFOV to maintain optimal gas exchange, to improve lung mechanics, and to prevent protein influx and conversion of large into small aggregates after exogenous surfactant therapy in lung-lavaged rats.

Introduction

In surfactant-deficient animals, it has been demonstrated that high-frequency oscillatory ventilation (HFOV), which combines small volume cycles at high rates, is as injurious to the lungs as conventional mechanical ventilation (CMV) unless HFOV is used with the so-called "high-lung volume strategy" [1,2]. With this latter strategy, all alveoli are recruited by using a pressure greater than the opening pressure and kept open by use of a relatively 'high' mean airway pressure. This results in a so-called 'open lung' that prevents lung damage and is characterized by a $Pao_2/Fio_2 \geq 500$ torr [66.7 kPa] [3]. First HFOV studies in neonates with respiratory distress syndrome (RDS) applying this strategy showed better clinical improvement with reduction in lung injury compared with CMV [4,5]. In all these clinical and experimental HFOV studies, in the control group that received CMV, surprisingly, no lung recruitment strategy was used [1-5].

Froese and colleagues [6] were the only investigators who compared HFOV to CMV at low and high-lung volume. In lung-lavaged rabbits, it was shown that after surfactant treatment HFOV with small volume cycles at high rates combined with a 'high-lung volume' strategy resulted in a constant improvement of Pao_2 with a lower alveolar protein influx and a higher amount of active surfactant than CMV at high-lung volume where Pao_2 decreased over time. Such differences were explained by differences in volume cycles which were ten-fold higher during CMV than during HFOV. In surfactant-deficient animals, several studies have shown that the level of positive end-expiratory pressure (PEEP) has a major impact on the effect of exogenous surfactant therapy on arterial oxygenation [7-9]. Recently, we have shown in lung-lavaged rats that during pressure-controlled ventilation (PCV), ventilator settings that combine small driving pressure amplitudes (10-14 cm H_2O) with high levels of PEEP lead to a sustained improvement of Pao_2 to prelavage values, low alveolar protein influx and best preserve the large aggregate, the surface active component of exogenous surfactant [9]. Therefore, the purpose of the present study was to demonstrate that under well-defined conditions, PCV allows settings which are as effective as HFOV to preserve the function of exogenous surfactant in lung-lavaged rats.

Material and methods

This study was approved by the local Animal Committee of Erasmus University Rotterdam, and the care and handling of the animals conformed with European Community

guidelines (86/609/EC). The study was performed in 16 adult male Sprague-Dawley rats (body weight 280-310 g). After induction of anaesthesia with 2% enflurane and 65% nitrous oxide in oxygen, a polyethylene catheter (0.8 mm outer diameter) was inserted into the right carotid artery for drawing arterial blood samples. Before tracheotomy, the animals received 30 mg/kg pentobarbital sodium, i.p. (Nembutal®; Algin B.V., Maassluis, The Netherlands). After tracheotomy, muscle relaxation was induced with pancuronium bromide 0.6 mg/kg, i.m. (Pavulon®; Organon Teknika B.V., Boxtel, The Netherlands) immediately followed by connection to the ventilator. The animals were mechanically ventilated with a Servo Ventilator 300 (Siemens-Elema AB, Solna, Sweden) in a pressure controlled 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 cmH_2O, PEEP of 2 cm H_2O, I/E ratio of 1:2. Anesthesia was maintained with pentobarbital sodium (Nembutal®; 30 mg/kg); neuromuscular block was maintained with pancuronium bromide, i.m. (Pavulon®; 0.6 mg/kg). Body temperature was kept within normal range by means of a heating pad. Initially, PIP was increased to 20 cm H_2O for 30 seconds to open up atelectatic regions in the lungs due to the surgical procedure. During this maneuver all other ventilator settings were unchanged. After this procedure the PIP was reset to the previous one, and a 0.15 ml blood sample was taken and replaced by heparinized (10 IU/ml) saline (0.9% NaCl). Pao_2 and $Paco_2$ were measured by conventional methods (ABL 505, Radiometer Copenhagen, Denmark).

Acute lung injury was induced by repeated whole-lung lavage as described by Lachmann et al. [10]. Each lavage was performed with saline (32 mL/kg) heated to 37 °C. Just before the first lavage, PIP and PEEP were elevated to 26 and 6 cm H_2O, respectively. Lung lavage was repeated 5-7 times at 5 min intervals to achieve a $Pao_2 \leq 85$ torr [11.3 kPa]. Within 10 min after the last lavage, all animals received exogenous surfactant at a dose of 100 mg/kg intratracheally, for which the animals were disconnected from the ventilator. The surfactant suspension, at concentration of 40 mg/mL, was administered as a bolus followed by a bolus of air (28 mL/kg) directly into the endotracheal tube via a syringe, and was immediately followed by re-connection to the ventilator. The surfactant used was isolated from minced pig lungs that were processed as previously described [11]. Within 5 min after surfactant application, the animals were randomized to one of the two groups (n=8 per group). In one group, PCV with the Servo 300 was continued and a procedure to open up the lungs (defined as $Pao_2/Fio_2 \geq 500$ torr) was performed at the following

ventilator settings: PIP 30 cm H_2O, static PEEP 15 cm H_2O, I/E ratio 1:1, Fio_2 1.0, respiratory frequency 150 bpm. After 1 to 2 minutes at these settings, a blood sample was drawn to verify that Pao_2/Fio_2 was ≥ 500 torr. After this recruitment procedure, PEEP was decreased in approximately 2 to 3 minute steps to the point of derecruitment which was defined as the point where Pao_2/Fio_2 decreased below 500 mmHg. A new procedure to re-open up the lungs was performed and PEEP was immediately decreased to the minimal level which kept Pao_2/Fio_2 ≥ 500 torr. Then the driving pressure amplitude was set to keep $Paco_2$ as close as possible to the normal range and was not changed thereafter [12-14]. The second group was connected to a high-frequency oscillator (type OHF-1, S.A. Dufour, Villeneuve d'Ascq, France), a procedure to open up the lungs was performed by setting the ventilator to oscillation mode without sigh, respiratory rate at 10 Hz, oscillatory pressure amplitude of 20 cm H_2O, Fio_2 1.0. The MAwP was initiated at 25 cm H_2O. After about 1-2 minutes at these ventilator settings, a blood gas sample was drawn to verify that Pao_2/Fio_2 was ≥ 500 torr. Thereafter, the level of MAwP was decreased in 2 to 3 minute steps to the point of derecruitment which was defined as the point where Pao_2/Fio_2 decreased below 500 mmHg. A new procedure re-open up the lungs was performed and the MAwP was immediately decreased to the minimal level which kept PaO_2/FIO_2 ≥ 500 torr. Then, the oscillatory pressure amplitude was set to maintain the $Paco_2$ as close as possible to normal range and was not changed thereafter.

Airway pressures were continuously monitored with a tip catheter pressure transducer (Raychem EO 2A 121, USA), using a water column as a reference pressure, connected with a Y-piece to the tracheal tube, and recorded (Siemens Sirecust 1280, Siemens, Danvers, Massachusetts, USA). Additionally, PEEP in HFOV was defined as the lowest pressure within the oscillatory pressure amplitude and the highest pressure within the oscillatory pressure amplitude was defined as PIP.

After surfactant administration and performance of the recruitment procedure, airway pressures were determined and blood gas samples were taken at 30, 60, 90, 120, 150 and 180 minutes after the recruitment procedure.

After 180 min all animals were killed with an overdose of pentobarbital sodium injected through the penile vein. Then static P/V curves were recorded 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 (Validyne model DP 45-32, Validyne Engineering, Northridge, CA, USA), and pressures were recorded on a polygraph (Grass model 7B, Grass Instrument, Quincy,

MA., USA). Using a syringe filled with nitrogen (N_2) the lungs were first inflated (within 10 sec) to an airway pressure of 35 cm H_2O, which was maintained for 5 sec, followed by deflation to an airway pressure of 0 cmH_2O. Then the lungs were re-inflated in steps of 0.5 ml until an airway pressure of 35 cmH_2O was reached. Each inflation step took 1-2 sec followed by a 5-sec pause to allow pressure equilibration. After this, in the same way, the lungs were then deflated until an airway pressure of 0 cm H_2O was reached. The volume of N_2 left in the syringe was recorded. Total lung capacity (TLC_{35}) was defined as lung volume at inflation with a distending pressure of 35 cm H_2O.

The Gruenwald index which characterizes the surfactant system *in situ*, was calculated from the pressure-volume curve, defined as $(2V_5+V_{10})/2V_{max}$, where V_5, V_{10} and V_{max} are the lung volumes at transpulmonary pressures of 5, 10 and 35 cm H_2O from the deflation limb, respectively [15].

After P/V recordings, a broncho-alveolar lavage (BAL) (30 mL/kg) was performed five times with saline-$CaCl_2$ 1.5 mmol/L. The active surfactant component in the BAL fluid was separated from the non-active surfactant component by differential centrifugation [16] followed by subsequent phosphorus analysis, and the ratio of non-active to active (small to large aggregate) surfactant was calculated. The protein concentration of the supernatant of BAL fluid was determined using the Bradford method (Biorad protein assay, Munich, Germany) [17].

Statistical analysis was performed using the Instat 2.0 biostatistics package (GraphPad software, San Diego, CA, USA). Intra-group comparisons were analyzed with repeated measures ANOVA. If a difference was found, a post-hoc test was performed (Tukey-Kramer). Inter-group comparisons were analyzed with a t-test at 90% of confidence interval. Statistical significance was accepted at p-values <0.05. All data are expressed as mean $\pm$SD.

Results

Blood gases before and directly after lavage were comparable in all animals (Fig. 1 and Table 1). None of the animals developed a pneumothorax and all animals survived the 3-h study period.

In the PCV group, PIP and PEEP were decreased from 30 to 21$\pm$ 0.9 cm H_2O and from 15 to 9$\pm$1 cm H_2O, respectively, within 5 min while mean Pao_2 values maintained above 500 torr

[66.7 kPa] (Fig. 1). In the HFOV group, the initial mean airway pressure was 25 cm H_2O and was decreased to 13±2 cm H_2O within 10 min while Pao_2 remained stable. The corresponding airway pressures measured with the tip catheter pressure transducer 3 hours after the recruitment procedure were comparable in both groups and no differences were observed. In PCV a PIP of 21±0.9 cm H_2O, a PEEP 9±1.3 cm H_2O and MAwP of 13±3 cm H_2O were recorded, while in HFOV a PIP of 20±2 cm H_2O, a PEEP of 9±1 cm H_2O and MAwP of 13±2 cm H_2O were recorded.

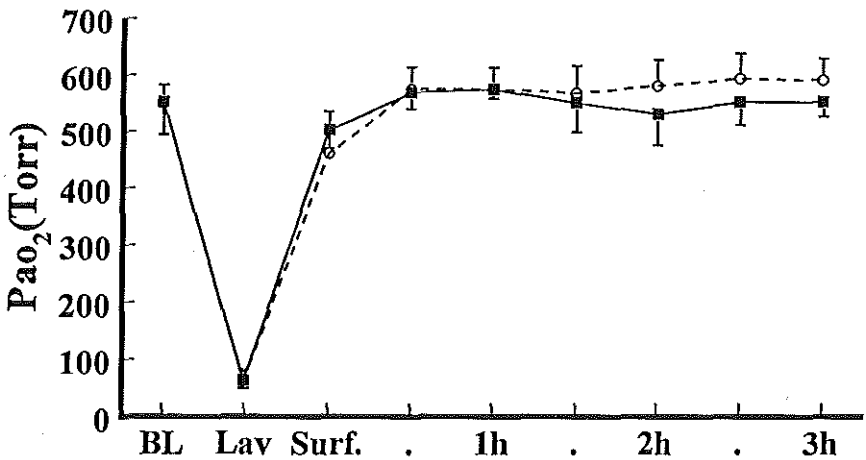

Figure 1. Change in mean arterial oxygenation (Pao_2) (mean ± SD) of both study groups before lung lavage (BL), after lavage (Lav), after exogenous surfactant treatment (surf.) and during the subsequent 3-h observation period. *Broken line*, animals (n=8) that received exogenous surfactant followed by HFOV; *Solid line*, animals (n=8) that received exogenous surfactant followed by PCV.

After surfactant administration and before randomization, mean Pao_2 values were above 450 torr [59.9 kPa] in both groups. After the recruitment maneuver the mean PaO_2 values were above 500 torr [66.6 kPa] and did not differ between the PCV and HFOV group during the entire

study period (Fig. 1). In both study groups mean $Paco_2$ values increased significantly after the lavage procedure and decreased after surfactant application and stayed within normal range (Table 1).

Data on TLC_{35}, the Gruenwald index, total protein concentration, total phosphorus concentration of non-active surfactant or small aggregates (SA), total phosphorus concentration of active surfactant or large aggregates (LA), small to large aggregate ratio, and recovery of BAL fluid are given in Table 2. There was no significant difference between these parameters in both study groups.

Table 1. Data on $Paco_2$ of both study groups (PCV and HFOV). Values are given as mean ± SD.

		BL	Lav	Surf.	1 h	2 h	3 h
Group							
PCV	**torr**	39±9	55±11	40±3	44±4	38±7	41±9
	Kpa	5.1±1.1	7.3 ±4.5	5.3±1.4	5.8±0.5	5.0±0.9	5.4±1.1
HFOV	**torr**	32±4	51±9	41±7	38±6	39±9	33±12
	Kpa	4.3±0.5	6.7±1.1	5.4±0.9	5.0±0.8	5.1±1.1	4.4±1.6

BL, before lavage. Lav, the repeated saline lavage in order to induce the lung injury. Surf., surfactant (100 mg/kg). PCV, pressure controlled ventilation. HFOV, high frequency oscillatory ventilation.

Table 2. Data on total lung capacity (TLC_{35}), Gruenwald index, total protein concentration (conc), total phosphorus small aggregates (SA), total phosphorus large aggregates (LA) and ratio of small to large aggregates (SA/LA) and recovery of broncho-alveolar lavage of both study groups (PCV and HFOV). Values are given as mean ± SD.

	PCV	HFOV
TLC_{35}	40 ± 4.4	40 ± 9.5
Gruenwald Index	0.88 ± 0.09	0.91 ± 0.08
Total protein conc (mg/mL)	0.35 ± 0.10	0.41 ± 0.13
SA (mmol)	3.6 ± 1.1	0.41 ± 0.13
LA (mmol)	11.2 ± 4.1	11.2 ± 4.2
SA/LA ratio	0.44 ± 0.15	0.43 ± 0.20
Recovery BAL (%)	94 ± 1.0	94 ± 2.0

PCV, pressure control ventilation. HFOV, high frequency oscillatory ventilation

Discussion

In the present study we used the lung lavage model which has proved to be a consistent and convenient model of acute lung injury [10]. It has been postulated that, in the acute phase, this model reflects more a primary surfactant deficiency, as seen in neonatal RDS [18,19]. Despite the fact that the lung injury in this study is not exactly representative of the pathology as seen in humans with RDS, this model is used for testing various therapeutic interventions for RDS such as exogenous surfactant therapy or different forms of mechanical ventilation [18,19]. In the same animal model, our group has previously demonstrated [9] that after exogenous surfactant treatment, PEEP levels of 2 cm H_2O and PIP of 26 cm H_2O (pressure amplitudes of 24 cm H_2O) resulted in oxygenation levels comparable with the post-lavage values (85 ± 20 mmHg), and the highest level of the small/large surfactant aggregates ratio, and the highest amount of proteins in the BAL fluid. A group ventilated with 6 cm H_2O of PEEP and PIP of 26 cm H_2O (pressure amplitudes of 20 cm H_2O) resulted in oxygenation levels comparable with pre-lavage values, but still with a high level of small/large surfactant aggregates ratio, and a higher amount of protein

in the BAL fluid when compared with animals which were ventilated with high levels of PEEP and small pressure amplitudes. In contrast, two groups ventilated with 10 cm H_2O of PEEP and PIP of 20 cm H_2O (pressure amplitude of 10 cm H_2O) resulted in oxygenation levels comparable with pre-lavage values, a lower amount of proteins in the BAL fluid, and a lower level of small/large surfactant aggregates ratio. Additionally, it was shown that effective carbon dioxide removal could be achieved by applying a ventilation mode that creates auto-PEEP. From this earlier study, it has been suggested that under well-defined conditions, PCV allows settings which are as effective as HFOV to preserve the function of exogenous surfactant in lung-lavaged rats [9].

The results of this study demonstrate that in lung-lavaged rats PCV with sufficient PEEP and small driving pressure amplitudes is as effective as HFOV to maintain optimal gas exchange, to improve lung mechanics, and to prevent protein influx and conversion of active into non-active surfactant components under conditions in which the entire lung is fully recruited (open lungs). These results are in contrast with the results of Froese et al. [6] who showed that HFOV at high-lung volume was superior to CMV at low and high-lung volume in improving lung function and preserving exogenous surfactant efficacy. In their study, it was shown that after surfactant therapy HFOV at high-lung volume resulted in a sustained improvement of Pao_2 to prelavage values whereas Pao_2 decreased over time during CMV at high-lung volume. Whereas HFOV was applied to expanded lungs during the entire observation period, in the CMV group a recruitment maneuver was not performed; in this latter group, despite the gradual increase of peak inspiratory pressure over time, the required opening pressure was never reached. It is, however, known that a critical opening pressure has to be reached before previously collapsed alveoli can be opened [12]. Once they are open, they remain open until the pressure drops below a critical level, then immediate collapse occurs. Reopening requires higher recruiting pressures. Therefore, alveoli should first be actively opened and then lower pressures are needed to keep the lung open and to achieve an adequate gas exchange [12, 13]. This is shown in the present study in which Pao_2 could be kept stable at prelavage values during the entire observation period with PCV at a peak inspiratory pressure of only 20.5±0.9 cm H_2O and a PEEP of 9.1±1.3 cm H_2O resulting in the same mean airway pressure as with HFOV after an initial opening procedure.

It becomes clear that the ventilator pattern strongly influences exogenous surfactant therapy [20]. Several studies have demonstrated that surfactant therapy and high end-expiratory

lung volumes produce the largest and most sustained therapeutic effect [6,9]. Administration of exogenous surfactant leads to alveolar expansion with stabilization during expiration [11]. However, surfactant administration does not permit immediate withdrawal of PEEP [9]. It has been suggested that PEEP also contributes to the clearance of the excess fluid in which the surfactant is suspended [21]. In contrast, peak inspiratory pressure can normally be reduced shortly after surfactant administration as lung function improves, this avoids overdistension of the alveoli, increases perfusion of the lung, reduces the number of pneumothoraces, and reduces the pressure swings [21]. Recent studies in vivo by Veldhuizen et al. [22] in rabbits showed that the conversion of active into non-active surfactant subfractions was not dependent on the respiratory rate but was dependent on tidal volume and time. The results of our study showed that the ratio of non-active to active surfactant components was comparable between PCV and HFOV, indicating that the alveolar volumes are comparable. However, it is believed that during PCV tidal volumes have to be ten times higher than during HFOV [3]. Data from a pilot study (unpublished data) showed that for rats ventilated in the PCV mode the used pressure amplitudes at a respiratory rate of 150 bpm resulted in a tidal volume of about 4.8 mL/kg bodyweight, and for rats ventilated with HFOV at 10 Hz the tidal volume was about 3.5 mL/kg (measurements were performed with a bodybox). In the present study, however, we used much smaller pressure amplitudes than normally used with PCV, and found that also these small pressure amplitudes in combination with higher frequencies were able to obtain normocapnia. In addition, we found no difference in protein influx between PCV and HFOV groups and the values were comparable with normal values of healthy rats. These results confirmed previous results in lung-lavaged rats in which application of the 'open lung concept' with PCV resulted in decreased protein influx compared to PCV where Pao_2 values were kept between 450-500 torr (59.9-66.6 kPa) [23]. Thus, the optimal ventilator mode should produce minimal pressure swings during the respiratory cycle and keep the lung volume at end-expiration equal to or just above functional residual capacity level to preserve the function and composition of exogenous surfactant, to achieve optimal gas exchange, and to prevent damage to the lung [9, 12].

We conclude that PCV with sufficient level of PEEP and small driving pressure amplitudes is as effective as HFOV, both applied to fully aereated lungs, to maintain optimal gas exchange, to improve lung mechanics, and to prevent protein influx and conversion of large into small

aggregates after exogenous surfactant therapy in lung-lavaged rats. This indicates that achieving and maintaining alveolar expansion is more important than the type of mechanical ventilation.

References

1. Hamilton PP, Onayemi A, Smyth JA, et al: Comparison of conventional mechanical and high-frequency ventilation: oxygenation and lung pathology. *J Appl Physiol* 1983; 55:131-138
2. McCulloch PR, Forkert PG, Froese AB: Lung volume maintenance prevents lung injury during high frequency oscillatory ventilation in surfactant-deficient rabbits. *Am Rev Respir Dis* 1988; 137:1185-1192
3. Froese AB, Bryan AC: High frequency ventilation. *Am Rev Respir Dis* 1987; 135:1363-1374
4. Ogawa Y, Miyasaka K, Kawano T, et al: A multicenter randomized trial of high frequency oscillatory ventilation as compared with conventional mechanical ventilation in preterm infants with respiratory failure. *Early Human Dev* 1992; 32:1-10.
5. Gerstmann DR, Minton SD, Stoddard RA, et al: The provo multicenter early high-frequency oscillatory ventilation trial: improved pulmonary and clinical outcome in respiratory distress syndrome. *Pediatrics* 1996; 98:1044-1057
6. Froese AB, McCulloch PR, Sugiura M, et al: Optimizing alveolar expansion prolongs the effectiveness of exogenous surfactant therapy in the adult rabbit. *Am Rev Respir Dis* 1993; 148:569-577
7. Kobayashi T, Kataoka H, Ueda T, et al: Effects of surfactant supplement and end-expiratory pressure in lung-lavaged rabbits. *J Appl Physiol* 1984; 57:995-1001
8. Ito Y, Manwell SEE, Kerr CL, et al: Effects of ventilation strategies on the efficacy of exogenous surfactant therapy in a rabbit model of acute lung injury. *Am J Respir Crit Care Med* 1998; 157:149-155
9. Verbrugge SJC, Gommers D, Lachmann B: Conventional ventilation modes with small pressure amplitudes and high end-expiratory pressure levels optimize surfactant therapy. *Crit Care Med* (In press)
10. Lachmann B, Robertson B, Vogel J: In vivo lung lavage as an experimental model of respiratory distress syndrome. *Acta Anaesthesiol Scand* 1980; 24:231-236
11. Gommers D, Vilstrup C, Bos JAH, et al: Exogenous surfactant therapy increases static lung compliance, and cannot be assessed by measurements of dynamic compliance alone. *Crit Care Med* 1993; 21:567-574
12. Lachmann B. Open up the lung and keep the lung open. *Intensive Care Med* 1992; 18:319-321
13. Böhm SH, Vazquez de Anda GF, Lachmann B: "The Open Lung Concept". In Vincent JL (ed). Yearbook of intensive care and emergency medicine. Springer-Verlag, Berlin Heidelberg 1998, pp 430-440
14. Vazquez de Anda GF, Lachmann B: Protecting the lung during mechanical ventilation with The Open Lung Concept. *Acta Anaesthesiol Scand* 1998; 112; 63-66
15. Gruenwald P: A numerical index stability of lung expansion. *J Appl Physiol* 1963; 88:359-367
16. Rouser G, Fleischer S, Yamamoto A: Two dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. *Lipids* 1970; 5:494-496
17. Bradford MM: A rapid and sensitive method of quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt Biochem* 1976; 72:248- 254
18. Lachmann B, Jonson B, Lindroth M, et al: Modes of artificial ventilation in severe respiratory distress syndrome: Lung function and morphology in rabbits after wash-out of alveolar surfactant. *Crit Care Med* 1982; 10: 724-732
19. Lachmann B, Danzmann E, Haendly B, et al: Ventilator settings and gas exchange in respiratory distress syndrome. In: Prakash O (ed). Applied physiology in clinical respiratory care. Nijhoff, The Hague, 1982. pp 141-176
20. Verbrugge SJC, Šorm V, Lachmann B: Mechanisms of acute respiratory distress syndrome: Role of surfactant changes and mechanical ventilation. *J Physiol Pharmacol* 1997; 48: 537-557
21. Hallman M, Merritt TA, Kari A, et al: Factors affecting surfactant responsiveness. *Ann Med* 1991; 23:693-698
22. Veldhuizen RAW, Marcou J, Yao L-J, et al: Alveolar surfactant aggregate conversion in ventilated normal and injured lungs. *Am J Physiol* 1996; 270:L152-L158
23. Hartog A, Vazquez de Anda G, Gommers D, et al: Comparison of exogenous surfactant therapy, mechanical ventilation with high end-expiratory pressure and partial liquid ventilation in a model of acute lung injury. *Br J Anaesth* 1999,82:81-86

Chapter 4

Comparison of exogenous surfactant therapy, mechanical ventilation with high end-expiratory pressure and partial liquid ventilation in a 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 value <13 kPa. Animals were then allocated randomly (n=12 in each group) to receive exogenous surfactant therapy, ventilation with high PEEP (18 cm H_2O), partial liquid ventilation, or ventilation with low PEEP (8 cm H_2O) (ventilated controls). Blood-gas values were measured hourly. At the end of the 4-h study period, in six animals per group pressure-volume curves were constructed and bronchoalveolar 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 to non-active surfactant components increased significantly. In the three treatment groups, PaO_2 increased rapidly to >50 kPa and remained over the next 4 h. The protein concentration of 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 groups, 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 improve PaO_2 to >50 kPa, the impact on protein transfer into the alveoli, surfactant system, and lung injury differed 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 concentrations 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 improves 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 results are promising, they have not been compared directly [11-15]. In this study, we compared these three techniques for their efficacy in 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.

Materials and 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 obtaining arterial blood samples. Before tracheostomy, the animals received pentobarbital (pentobarbitone) 60 $mgkg^{-1}$ i.p. (Nembutal®,

Algin BV, Maassluis, the Netherlands). After tracheostomy, neuromuscular block was produced with pancuronium 1 $mg\,kg^{-1}$, i.m. (Pavulon®, Organon Teknika, Boxtel, the Netherlands) followed immediately by connection to a ventilator. The animals underwent mechanical 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 30 bpm, peak inspiratory pressure (PIP) 12 cm H_2O, positive end-expiratory pressure (PEEP) 2 cm H_2O, and inspiratory/expiratory (I/E) ratio 1:2. Anaesthesia was maintained with pentobarbital 40 $mg\,kg^{-1}\,h^{-1}$, i.p. neuromuscular block was maintained with pancuronium 1 $mg\,kg^{-1}\,h^{-1}$, i.m. Body temperature was maintained within normal range using a 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 $ml\,kg^{-1}$) 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 after 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 2-3 min, PIP was decreased to 35 cm H_2O and PEEP to 18 cm H_2O, and arterial blood-gas values obtained. Ventilator setting remained unchanged for the rest of the study. The second group received an intra-tracheal bolus dose of perfluorocarbon 15 $ml\,kg^{-1}$ (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 $g\,ml^{-1}$, a vapour pressure 0.09 kPa, surface tension 20.5 dynes cm^{-1} and an oxygen solubility 35 ml O_2 per 100 ml perfluorocarbon per atmosphere of oxygen pressure (all values at 25°C). During the study, evaporation 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 $mg\,kg^{-1}$. 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 $mg\,ml^{-1}$, and administered intra-tracheally, after disconnection from the ventilator. The surfactant suspension was administered as a bolus followed by a bolus of air (12 $ml\,kg^{-1}$), 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 cm H_2O (PIP/PEEP of 28/8 cm H_2O) to prevent critical hypoxia and remained unchanged throughout the 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 variables 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 cm H_2O. After pressure-volume recordings, BAL was performed five times with saline-$CaCl_2$ 1.5 mmol $litre^{-1}$. The active surfactant component in 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 (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 by repeated measures ANOVA. If ANOVA resulted in $P < 0.05$ 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 in all groups (Fig.1 and 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 study in the surfactant-treated group and the group ventilated with high PEEP (Fig. 1). In the partial liquid ventilation group, administration of a bolus dose of perfluorocarbon 15 ml kg^{-1} resulted in a significant improvement in PaO_2 but pre-lavage values were not reached (Fig. 1). Perfluorocarbon was substituted periodically to compensate for the evaporation loss and the substitution dose of perfluorocarbon was 1.1 (0.4) ml kg^{-1} h^{-1}.

Table 1. Pa_{CO2} (mean (SD)) values (kPa) in the four treatment groups, before lavage (Healthy), immediately after lavage (Lav.) and 1, 2, 3 and 4 h after lavage. * P< 0.05 compared with surfactant group; † P< 0.05 vs control group; ‡ P< 0.05 vs Lav. Control = ventilated controls with low PEEP; H-PEEP = high PEEP; PLV = partial liquid ventilation; SURF = exogenous surfactant therapy.

Group	Healthy	Lav.	1 h	2 h	3 h	4 h
Control	5.8 (0.9)	9.0 (1.4)	7.5(1.7)*‡	7.7 (1.7)*‡	7.8 (1.8)*‡	8.2 (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)‡

$PaCO_2$ 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 with 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 threated with surfactant. TLC_{35} was significantly decreased in the ventilated control group, but not in the three tratment groups, compared with 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).

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

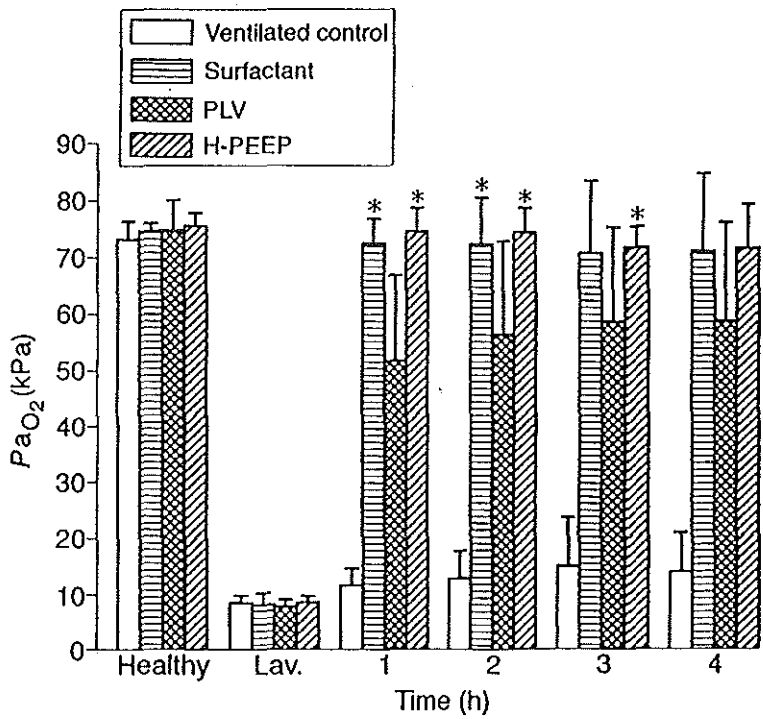

Figure 1. Mean (SD) PaO_2 values in ventilated control, surfactant-treated, partial liquid ventilation (PLV) and high (H) PEEP groups. * $p < 0.05$ vs PLV group.

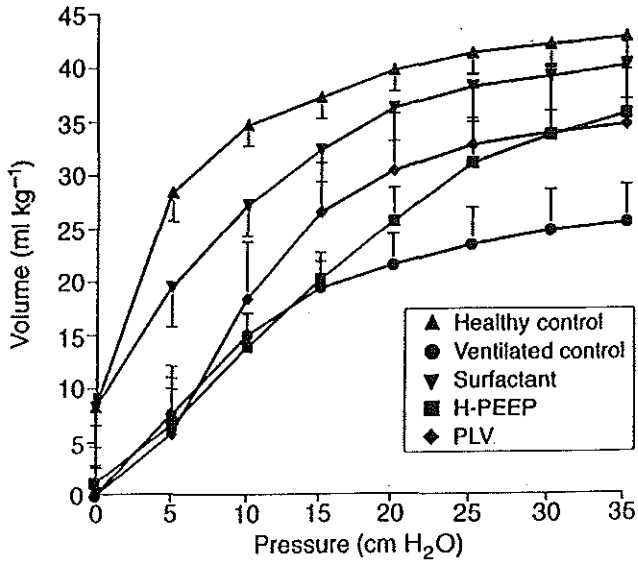

Figure 2. Deflation limbs from the pressure-volume curves, (mean (SD)) in the healthy control, ventilated control, surfactant-treated, partial liquid ventilation (PLV) and high (H) PEEP groups. Volume is lung volume above FRC. At deflation less than 15 cm H_2O, lung volume in the healthy controls exceeded the 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.

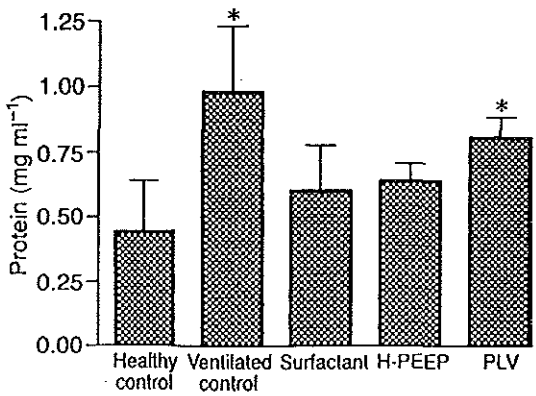

Figure 3. Mean (SD) protein concentration of BAL fluid in the healthy control, ventilated control, surfactant-treated, partial liquid ventilation (PLV) and high (H) PEEP groups. * $P < 0.05$ *vs* healthy control group.

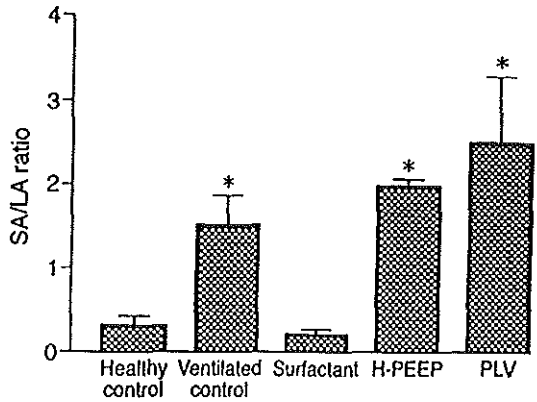

Figure 4. Ratio between non-active and active surfactant components (mean (SD)) in the healthy control, ventilated control, surfactant-treated, partial liquid ventilation (PLV) and high (H) PEEP groups (SA/LA= small to large aggregates). * $P < 0.05$ *vs* healthy control group.

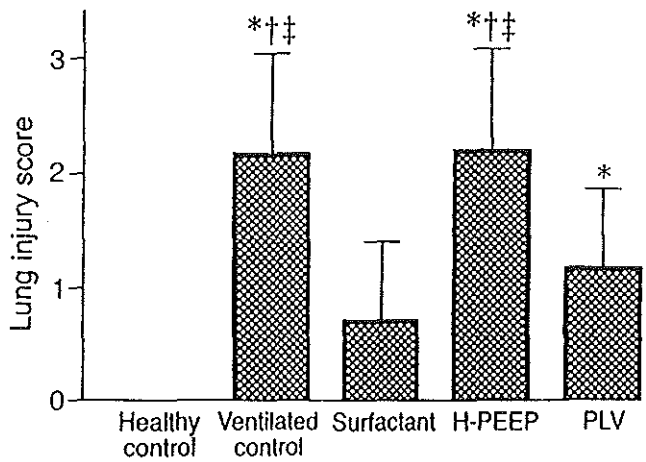

Figure 5. Lung injury score (mean (SD)) in the healthy control, ventilated control, surfactant-treated, partial liquid ventilation (PLV) and high (H) PEEP groups. * $P < 0.05$ *vs* healthy control group; † $P < 0.05$ *vs* surfactant group; ‡ $P < 0.05$ *vs* PLV group.

Table 2. Total phosphorus recovered from bronchoalveolar lavage fluid (mean (SD)) (h). * P<0.05 *vs* healthy controls (Healthy); † P<0.05 *vs* to 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^{-1})
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)†

Discussion

We have shown that although exogenous surfactant therapy, ventilation with high PEEP and partial liquid ventilation all increased PaO_2 to greater than 50 kPa, their 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 surfactant groups compared with the groups that were ventilated only.

The sustained improvement in PaO_2 compared to the pre-lavage value in the group ventilated with high PEEP indicates that applied PIP and PEEP were sufficient to open the lungs and 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 these 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 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 alveoli was increased after partial liquid ventilation for 4-h (Fig. 2). The mechanism responsible for this is not known. It is hypothesized that improvement in 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 end-expiratory collapse. In the non-dependent part of the lung, a thin film of perfluorocarbon 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 perfluorocarbons, the retractive forces in the non-dependent part of the lung are reduced, resulting in large volume changes at small increments in pressure, making these 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 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 surfactant groups underwent ventilation with the same PIP and PEEP pressures, but protein concentration of 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 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 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 at 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 have been 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 and 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 lung injury in this study was not representative of the pathology seen 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 was there improvement in all variables. Some studies have reported physiologic and pathological benefits of partial liquid ventilation or ventilation with high PEEP in combination with exogenous surfactant, but whether any of these hybrid techniques has advantages over the use of exogenous surfactant alone has yet to be confirmed.

References

1. Lewis JF, Jobe AH. Surfactant and the Adult Respiratory Distress Syndrome. *Am Rev Respir Dis* 1993;147:218-33
2. Houmes RJM, Bos JAH, Lachmann B. Effect of different ventilator settings on lung mechanics: with special reference to the surfactant system. *Appl Cardiopulm Pathophysiol* 1994;5:117-27
3. Muscedere G, Mullen JBM, Gan K, Slutsky AS. Tidal ventilation at low airway pressures can augment lung injury. *Am J Respir Crit Care Med* 1994; 149: 1327-34
4. Lachmann B, Jonson B, Lindroth M, Robertson B. Modes of artificial ventilation in severe respiratory distress syndrome: Lung function and morphology in rabbits after wash-out of alveolar surfactant. *Crit Care Med* 1982; 10: 724-32
5. Lachmann B, Danzmann E, Haendly B, Jonson B. Ventilator settings and gas exchange in respiratory distress syndrome. In: Prakash O, ed. *Applied Physiology in Clinical Respiratory Care.* The Hague: Nijhoff, 1982; 141-76
6. Tütüncü AS, Faithfull NS, Lachmann B. Intratracheal perfluorocarbon administration combined with mechanical ventilation in experimental respiratory distress syndrome: Dose-dependent improvement of gas exchange. *Crit Care Med* 1993; 21: 962-9
7. Tütüncü AS, Akpir K, Mulder P, Erdmann W, Lachmann B. Intratracheal perfluorocarbon administration as an aid in the ventilatory management of respiratory distress syndrome. *Anesthesiology* 1993; 79: 1083-93
8. Lachmann B, Fujiwara T, Chida S, et al. Surfactant replacement therapy in the experimental acute respiratory distress syndrome (ARDS). In: Cosmi EV, Scarpelli EM (eds) Pulmonary surfactant system. Amsterdam, Elsevier, 1983; 231-5
9. Häfner D, Beume R, Kilian U, Kraznai G, Lachmann B. Dose-response comparisons of five lung surfactant factor (LSF) preparations in an animal model of adult respiratory distress syndrome (ARDS). *Br J Pharmacol* 1995; 115: 451-8
10. Hirschl RB, Tooley R, Parent A, Johnson K, Bartlett RH. Improvement of gas exchange, pulmonary function, and lung injury with partial liquid ventilation. *Chest* 1995; 108: 500-8
11. Kesecioglu J, Tibboel D, Lachmann B. Advantages and rationale for pressure control ventilation. In: Vincent JL ed. *Yearbook of Intensive Care and Emergency Medicine* Berlin-Heidelberg-New York: Springer-Verlag, 1994;524-33
12. Amato MBP, Barbas CSV, Medeiros DM, et al. Beneficial effects of the "open lung approach" with low distending pressures in acute respiratory distress syndrome *Am J Respir Crit Care Med* 1995; 152: 1836-45
13. Hirschl RB, Pranikoff T, Gauger P, et al. Liquid ventilation in adults, children, and full-term neonates. *Lancet* 1995; 346: 1201-2
14. Gauger PG, Pranikoff T, Schreiner RJ, Moler FW, Hirschl RB. Initial experience with partial liquid ventilation in pediatric patients with acute respiratory distress syndrome. *Crit Care Med* 1996; 24: 16-22
15. Gregory TJ, Steinberg KP, Spragg R, et al. Bovine surfactant therapy for patients with acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1997; 155: 1309-15
16. Lachmann B, Robertson B, Vogel J. In vivo lung lavage as an experimental model of the respiratory distress syndrome. *Acta Anaesthesiol Scand* 1980; 24: 231-6
17. Gommers D, Vilstrup C, Bos JAH, et al. Exogenous surfactant therapy increases static lung compliance, and cannot be assessed by measurements of dynamic compliance alone. *Crit Care Med* 1993; 21:567-74
18. Kaisers U, Max M, Schnabel R, et al. Partial liquid ventilation with FC 3280 in experimental lung injury: Dose-dependent improvement of gas exchange and lung mechanics. *Appl Cardiopulm Pathophysiol* 1996; 6: 163-70
19. Veldhuizen RAW, Inchley K, Hearn SA, Lewis JF, Possmayer F. Degradation of surfactant associated protein B (SP-B) during in vitro conversion of large into small surfactant aggregates. *Biochem J* 1993; 295: 141-7
20. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Ann Biochem* 1976;72:248-54
21. Lachmann B, Eijking EP, So KL, Gommers D. In vivo evaluation of the inhibitory capacity of human plasma on exogenous surfactant function. *Intensive Care Med* 1994; 20: 6-11
22. Amato MBP, Barbas CSV, Medeiros DM, et al. Effect of a protective-ventilation strategy on mortality in the acute respiratory distress syndrome. *N Engl J Med* 1998; 338: 347-54
23. Tütüncü AS, Faithfull NS, Lachmann B. Comparison of ventilatory support with intratracheal perfluorocarbon

administration and conventional mechanical ventilation in animals with acute respiratory failure. *Am Rev Respir Dis* 1993; 148: 782-5

24. Egan EA, Nelson RM, Olver RE. Lung overinflation and alveolar permeability to non-electrolytes in the adult sheep in vivo. *J Physiol* 1976; 260: 409-24
25. Dreyfuss D, Saumon G. Ventilator-induced lung injury: Lessons from experimental studies. *Am J Respir Crit Care Med* 1998; 157:294-323
26. Cox PN, Morris K, Frndova H, Babyn P, Bryan AC. Relative distribution of gas and perfluorocarbon (PFC) during partial liquid ventilation (PLV). *Pediatr Res* 1996;39:45A
27. Mrozek JD, Smith KM, Bing DR, Meyers PA, Simonton SC, Connett JE, Mammel MC. Exogenous surfactant and partial liquid ventilation. *Am J Respir Crit Care Med* 1997; 156: 1058-65
28. Smith KM, Bing DR, Meyers PA, et al. Partial liquid ventilation: a comparisson using conventional and high frequency techniques in an animal model of acute respiratory failure. *Crit Care Med* 1997; 25: 1179-86
29. Smith TM, Steinhorn DM, Thusu K, Fuhrman BP, Dandona P. A liquid perfluorochemical decreases the in vitro production of reactive oxygen species by alveolar macrophages. *Crit Care Med* 1995; 23: 1533-39
30. Virmani R, Fink LM, Gunter K, English D. Effect of perfluorochemical blood substitutes on human neutrophil function. *Transfusion* 1984; 24: 343-7
31. Burger R, Bryan AC. Pulmonary hypertension after postlavage lung injury in rabbits: possible role of polymorphonuclear leukocytes. *J Appl Physiol* 1991;71:1990-5

Chapter 5

Treatment of ventilation-induced lung injury with exogenous surfactant

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Submitted for publication

Summary

Pulmonary surfactant plays a role in ventilation-induced lung injury (VILI). Therefore, we investigated whether exogenous surfactant might restore gas exchange and lung mechanics in an established model of VILI. From 24 adult rats, 6 animals were killed immediately after induction of anesthesia and were used as healthy controls. In 18 rats, VILI was induced by increasing peak inspiratory pressure (PIP) to 45 cm H_2O without positive end-expiratory pressure (PEEP) for 20 min. Thereafter, animals were randomly divided into three groups of six animals each: One group was killed immediately after VILI (non-ventilated controls). In the other two groups, ventilator settings were changed to PIP of 30 cm H_2O and PEEP of 10 cm H_2O, and respiratory rate of 40 bpm. One group received surfactant and the other group received no treatment. Blood gas tension and arterial blood pressures were recorded every 30 min for two hours. Then, a pressure-volume curve was recorded, a broncho-alveolar lavage was performed to determine protein content, minimal surface tension and surfactant composition. Oxygenation, lung mechanics, surfactant function and composition were significantly improved in the surfactant-treated group compared to the ventilated and non-ventilated control groups. We conclude that exogenous surfactant can be used to treat VILI.

Introduction

It is known that modes of mechanical ventilation which allow alveolar end-expiratory collapse and/or end-inspiratory alveolar overstretching lead to decreases in lung compliance (1-4) and gas exchange (5), and result in atelectasis, pulmonary edema, pneumonitis and fibrosis (6, 7). Development of intra-alveolar edema in healthy rats subjected to intermittent positive pressure ventilation at high inflation pressures, without positive end-expiratory pressure (PEEP), was first demonstrated by Webb and Tierney and was later confirmed by Dreyfuss and colleagues who suggested that high inspiratory lung volumes induce endothelial and epithelial overstretching leading to microvascular injury (8, 9). However, it is increasingly realized that impairment of the surfactant system plays a key role in the mechanism of ventilation-induced lung injury (VILI) in the above-mentioned model (5, 10-12); further on it has been shown that surfactant function is impaired by pulmonary edema constituents (13-15). Loss of surfactant function will increase the surface tension at the air-liquid interphase of the alveolar walls (1, 3), which will lead, amongst others, to alveolar collapse and to an increased suction force on the pulmonary interstitium resulting also in alveolar edema (5, 8-12). Continuous re-expansion and collapse during the ventilatory cycles causes epithelial and endothelial damage mainly due to shear forces (5, 10). In addition, we have shown that exogenous surfactant administration preceding mechanical ventilation with high peak inspiratory lung volumes without PEEP, could partially prevent VILI which is characterized by e.g. impaired gas exchange and lung mechanics (11). In this study we wanted to investigate whether exogenous surfactant is able to restore gas exchange and lung mechanics in VILI.

Material and methods

Animal Preparation

This study was approved by the local Animal Committee at the Erasmus University Rotterdam, and the care and handling of the animals conformed with European Community guidelines (86/609/EC).

The study was performed in 24 adult male Sprague-Dawley rats (body weight 280-350 g). Anesthesia was induced with 2% enflurane and 65% nitrous oxide in oxygen, a polyethylene catheter was inserted into a carotid artery for drawing arterial blood samples and continuous

monitoring of arterial blood pressure. Immediately after, in a separate group of 6 animals a blood gas sample was taken and arterial blood pressure was measured, then the animals were killed, the thorax was opened, and a static pressure-volume curve (P-V curve) was recorded and a bronchoalveolar lavage (BAL) was performed. These animals served as a non-VILI, non-ventilated control group (Healthy). In the remaining animals, before tracheostomy, the animals received 30 mg/kg pentobarbital sodium, i.p. (Nembutal®, Algin BV, Maassluis, the Netherlands). After tracheostomy, muscle relaxation was induced by pancuronium bromide 0.6 mg/kg, i.m. (Pavulon®, Organon Teknika, Boxtel, the Netherlands) immediately followed by connection to a ventilator and a pressure transducer for continuous monitoring of arterial blood pressure. 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, postive end-expiratory pressure (PEEP) of 2 cm H_2O, and inspiratory/expiratory (I/E) ratio of 1:2. Anesthesia was maintained with pentobarbital sodium 30 mg/kg/h, i.p.; muscle relaxation was maintained with pancuronium bromide 0.6 mg/kg/h, i.m. Body temperature was kept within normal range by means of a heating pad.

Experimental Design

In order to produce VILI, PIP was increased to 45 cm H_2O and PEEP was decreased to zero for 20 min, other settings were not changed. Thereafter, PIP was decreased to 26 cm H_2O and PEEP was increased to 6 cm H_2O for 5 min, in order to increase arterial CO_2 tension. These ventilator settings were chosen based on a pilot study (unpublished data) in which we observed that when animals were ventilated at 45/0 cm H_2O (PIP/PEEP, respectively) for 20 minutes and then ventilated at 30/10 cm H_2O, the animals died from severe hypocapnia. Then, the animals were disconnected from the ventilator and the lungs were emptied of edema fluid and a randomization was performed.

Experimental Groups

The animals were randomized to one of three groups (n=6). The first group (Surfactant) received a bolus of exogenous surfactant (100 mg/kg) intratracheally. The surfactant used was isolated from minced pig lungs, that were processed as previously described (16). The surfactant suspension, at a concentration of 40 mg/mL, was administered as a bolus followed by a bolus of air 28 ml/kg, directly into the endotracheal tube via a syringe, and was immediately followed by

re-connection to the ventilator. Mechanical ventilation was continued at a PIP of 30 cm H_2O, PEEP of 10 cm H_2O, I/E ratio of 1:2, FiO_2 1.0, and respiratory rate of 40 bpm for two hours. These ventilator settings, were chosen based on results of a preliminary study which showed that applied ventilation pressures of 26/6 cm H_2O (PIP, PEEP, respectively) and 28/8 cm H_2O were too low to keep animals alive for an observation period of 2 hours. The second group (Ventilated) did not receive exogenous surfactant but received a sham bolus of air 28 mL/kg intra-tracheally and was mechanically ventilated at the same settings as the Surfactant group. The third group of animals (Non-Ventilated) were killed after the 5 minute ventilation period of 26/6 with an overdose of pentobarbital and were used as a non-treated, non-ventilated control group.

Gas Exchange and Hemodynamics

Arterial blood gas samples were taken in all groups before, after VILI, and at 5 min after the 26/6 period, and in the Surfactant and Ventilated control groups at 5 min after the 30/10 period, and every 30 min for 2 h. The samples were analyzed for arterial oxygen tension (PaO_2) and arterial carbon dioxide tension ($PaCO_2$) by conventional methods (ABL 505, Radiometer, Copenhagen, Denmark). At the same time points, arterial pressure was recorded. Hemodynamic support was provided by infusion of 1 ml of saline 0.9% (to a maximum of 2 ml per hour) when mean arterial pressure (MAP) decreased below 60 mmHg.

Pressure-Volume Curves

At 120 min after exogenous surfactant therapy all animals were killed with an overdose of pentobarbital sodium injected through the penile vein. Then static P-V curves were recorded. After the thorax and diaphragm were opened, the tracheostomy catheter was connected to a pressure transducer (Validyne model DP 45-32, Validyne Engineering Co., Northridge, CA, USA) with a syringe attached to it, and pressures were recorded on a polygraph (Grass model 7B, Grass Instrument Co., Quincy, MA., USA). Using a syringe filled with nitrogen (N_2) the lungs were first inflated (within 10 sec) to an airway pressure of 35 cm H_2O, which was maintained for 5 sec, followed by deflation to an airway pressure of 0 cm H_2O. Then the lungs were re-inflated in steps of 0.5 ml until an airway pressure of 35 cm H_2O was reached. Each inflation step took 1-2 sec followed by a 5-sec pause to allow pressure equilibration. After this, in the same way, the lungs were then deflated until an airway pressure of 0 cm H_2O was reached. The volume of N_2 left in the syringe was recorded. Maximal compliance (C_{max}) was calculated from the steepest part of the

deflation limb (16). Total lung capacity (TLC_{35}) was defined as lung volume at inflation with a distending pressure of 35 cm H_2O (17).

Gruenwald Index

The Gruenwald index which characterizes the surfactant system *in situ* (18), was calculated from the P-V curve, defined as $(2V_5+V_{10})/2V_{max}$, where V_5, V_{10} and V_{max} are the lung volumes at transpulmonary pressures of 5, 10 and 35 cm H_2O from the deflation limb, respectively.

Functional Residual Capacity (FRC)

After P-V recordings, the lungs were removed *en bloc* and weighed, and lung volume at an airway pressure of 5 cm H_2O (V_5) was determined by fluid displacement. A positive pressure of 5 cm H_2O was chosen to compensate for the loss of transpulmonary pressure in the open chest (19). The total lung volume at this distending pressure was considered close to FRC.

Bronchoalveolar lavage

After the FRC measurement a BAL (30 ml/kg) was performed five times with saline-$CaCl_2$ 1.5 mmol/litre (crude lavage). Thereafter, cell debris were removed from BAL by centrifugation at 400 *g* for 10 min. 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 of non-active to active (small to large aggregate) surfactant was calculated (20). Finally, the protein concentration of the BAL fluid was determined using the Bradford method (Bio-Rad protein-assay, Munich, Germany) (21).

Minimal Surface Tension

Minimal surface tension of the crude lavage was determined by means of a modified Wilhelmy balance (E. Biegler GmbH, Mauerbach, Austria). In this method, a tight-fitting teflon barrier reduces the surface area of a teflon trough from 100-20% at a cycle speed of 0.33/min. Saline is used as subphase and is kept at 37°C. The force on a platinum slide (1x1 cm), dipped into the subphase, is measured by a force transducer and expressed as surface tension. Further, maximal surface tension is measured at 100% surface area and minimal surface tension at 80% surface compression and expressed as milli Newton/meter (mN/m). Surface tension characteristics of a BAL sample are measured after application on the surface of the saline-filled trough. In this study 300 µl of BAL fluid was applied to the surface of the trough; minimal surface tension was measured after 3 cycles (22).

Statistical data analysis

Statistical analysis was performed using the Instat 2.0 biostatistics package (GraphPad software, San Diego, CA, USA). Intragroup comparisons were analyzed with repeated measures ANOVA. Intergroup comparisons for protein concentration in the supernatant of BAL, total phosphorous of small aggregates, total phosphorous of large aggregates, non-active/active total phosphorous ratio, minimal surface tension of the crude lavage, C_{max}, TLC_{35}, Gruenwald index and V_5 were analysed by means of an ANOVA. If a $p < 0.05$ was found, a post-hoc test was performed (Tukey-Kramer). A t-test analysis was performed for intergroup comparisons during the 2-h study period, for PaO_2, PCO_2 and MAP in the Surfactant and Ventilated control groups. Statistical significance was accepted at p-values <0.05. All data are expressed as mean ± standard deviation.

Results

Figure 1 shows the PaO_2 levels during the whole study period. After the ventilator settings were set at 26/6 cm H_2O for 5 minutes the PaO_2 decreased below 100 torr in all animals. The Surfactant group showed a significant increase in PaO_2 values to pre-VILI levels ($p< 0.001$), and were maintained during the 2-h study period. In the Ventilated control group, mean PaO_2 values remained below 200 torr during the 2-h study period: the difference between the values in the Ventilated group and the Surfactant group was significant throughout the study ($p< 0.001$).

Table 1 shows that the $PaCO_2$ and MAP levels were comparable in both ventilated groups during the whole study period.

Table 2 shows data from BAL fluid and lung mechanics. Protein concentration was significantly higher in the three VILI groups when compared with Healthy controls. Additionally, protein concentration was significantly lower in the Surfactant group than in the Non-Ventilated control group, but not significantly different from the Ventilated control group. The ratio of small to large aggregates in BAL fluid was significantly lower in the Surfactant group compared to the Non-Ventilated and the Ventilated control groups, and not different when compared with the Healthy group. The minimal surface tension of the crude lavage fluid in the Surfactant group was significantly lower than in the Non-Ventilated and the Ventilated control groups. In the Surfactant group the Gruenwald index, TLC_{35}, and C_{max} were comparable with healthy values, and significantly higher than in the Non-ventilated and Ventilated control groups. However, V_5 values

were significantly lower in the Surfactant group than in the Healthy control group, but significantly higher than in the Non-ventilated and Ventilated control groups.

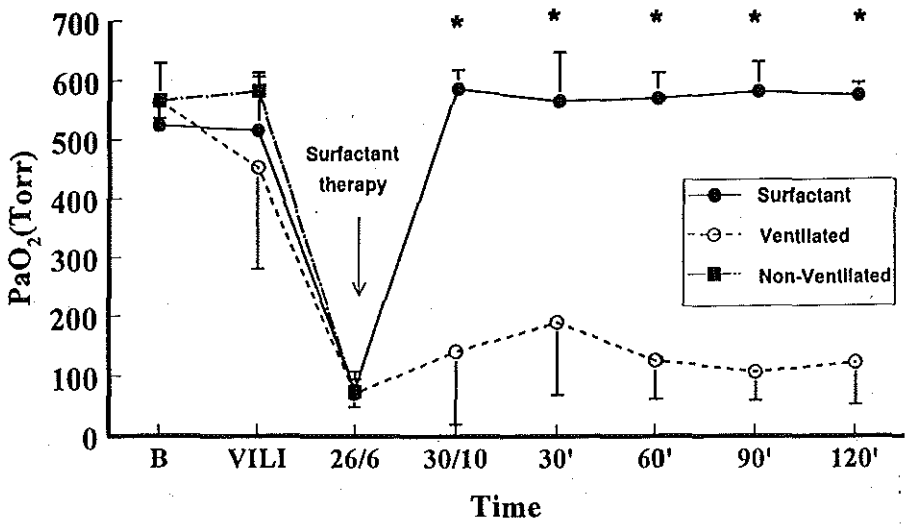

Figure 1. Arterial oxygen tension (mean ± standard deviation) during the whole study period. B= basal, VILI= ventilation with Peak inspiratory pressure (PIP) of 45 cm H_2O without PEEP after 20 min, 26/6= after 5 min at PIP 26 cm H_2O, 6 cm H_2O PEEP, 30/10= 5 min PIP 30 cm H_2O, 10 cm H_2O PEEP. * indicates significant difference between the Surfactant group and the Ventilated control group.

Figure 2 shows the deflation limbs from the P-V curves. The Surfactant group had TLC_{35}, and C_{max} values comparable with the Healthy group, and significantly higher than both the Non-ventilated and the Ventilated control groups.

Table 1. Data on arterial carbon dioxide tension ($PaCO_2$) and mean arterial pressure (MAP) over time in the healthy control group (Healthy), non-Treated, non-Ventilated control group (Non-Ventilated), Ventilated control group (Ventilated), and treated with surfactant group (Surfactant). Values are mean ± standard deviation.

	Time	Healthy	Non-Ventilated	Ventilated	Surfactant
$PaCO_2$ (torr)	**Basal**	39±5	38±8	38±6	41±6
	VILI		14±3*	21±11*	14±2*
	5' 26/6		33±10	36±8	34±6
	5' 30/10			39±5	47±9
	30'			38±7	40±7
	60'			43±8	41±5
	90'			46±10	40±3
	120'			49±13	39±6
MAP (torr)	**Basal**	140±10	151±8	134±12	157±23
	VILI		77±26*	83±29*	89±31*
	5' 26/6		74±32*	68±43*	76±28*
	5' 30/10			106±20*	122±23
	30'			107±20	104±16
	60'			73±23*†	101±14
	90'			86±23	89±22
	120'			88±28	119±10

* vs Baseline $p<0.05$, † vs Surfactant $p<0.05$

Table 2. Amount of recovered broncho-alveolar lavage (BAL) fluid, protein concentration, total phosphorus of small aggregates (SA) and total phosphorus of large aggregates (LA), non-active/active total phosphorus ratio (SA/LA ratio), minimal surface tension (min surf) of crude BAL fluid, Gruenwald Index, total lung volume at a transpulmonary pressure of 5 cm H_2O (V_5), lung volume above FRC at pressure 35 cm H_2O (TLC_{35}) and maximum compliance (C_{max}). Values are mean ± standard deviation

	Healthy	Non-Ventilated	Ventilated	Surfactant
Recovery BAL fluid (%)	90±1	90±1	90±1	90±1
Protein concentration BAL (mg/ml)	0.3±0.1	0.9±0.03*†	0.7±0.2†	0.6±0.1†
SA (mmol)	0.53±0.1*	1.2±0.1*	1.2±0.2*	2.6±0.6
LA (mmol)	1.8±0.2*	1.0±0.2*	1.3±0.5*	11±2
SA/LA ratio	0.39±0.05	1.3±0.22*†	1.0±0.33*†	0.22±0.08
Min surf (mN/m)	22.8±2.5*	32.2±2.6*†	29.5±1.1*†	17.3±2.2
Gruenwald Index	1.0±0.01	0.20±0.08*†	0.37±0.2*†	0.96±0.06
V_5 (ml/kg)	24.3±5.6	3.5±0.5*†	5.5±0.5*†	13.0±1.0†
TLC_{35} (ml/kg)	41±3.6	32±8*†	32±5*†	42±3
C_{max} (ml/kg)	4±0.5	1.8±0.7*†	1.6±0.3*†	3.2±0.7

* vs Surfactant $p < 0.05$

† *vs Healthy* $p < 0.05$

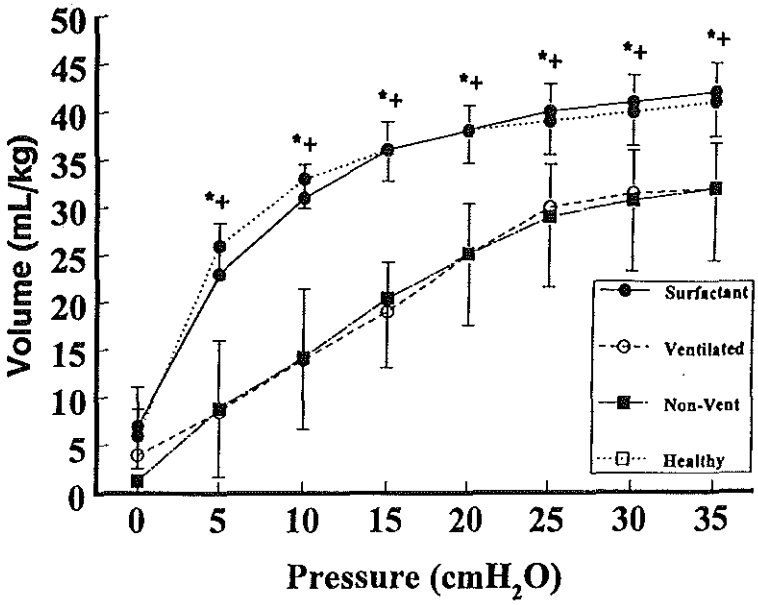

Figure 2. Deflation limbs from the pressure-volume curves, (mean ± standard deviation). * indicates significant difference between the Surfactant group and the two control groups, and + indicates significant difference between the Healthy group and the two control groups).

Discussion

This study shows that exogenous surfactant given to rats suffering from VILI restored the gas exchange at the used ventilator settings to basal values, and improved lung mechanics.

In the present study, 20 minutes of ventilation with high peak-inspiratory pressures without PEEP resulted in pulmonary edema and hypoxemia, and in impairment of the surfactant system. The latter is characterized by a decrease in pulmonary compliance, V_S, and Gruenwald Index. The exact mechanism by which the lung damage is produced by artificial ventilation is not yet entirely clear, but the role of surfactant changes is increasingly realised (1-5, 11, 12, 23, 24). Two primary mechanisms of surfactant inactivation by mechanical ventilation have been described. In the first mechanism mechanical ventilation enhances surfactant release from the pneumocytes type II into the alveolus (1-4). This material is subsequently lost into the small airways as a result of compression of the surfactant film when the surface of the alveolus becomes smaller than the surface occupied by the surfactant molecules, so that surface active material moves into the

airways (1, 4, 24). The second mechanism describing the surfactant changes associated with mechanical ventilation is based on the observation that the alveolar surface area changes associated with mechanical ventilation result in the conversion of large surface-active surfactant aggregates into small nonsurface–active surfactant aggregates (5, 25, 26). These two mechanisms will lead to alveolar collapse and protein infiltration (5, 8-12) in which the latter leads to further inactivation of surfactant (13-15). These mechanisms produce self-perpetuating changes which require higher ventilator pressures which may finally be responsible for more parenchymal damage (5, 8-12).

In the current study we used an exogenous surfactant to replace the surfactant lost and/or inactivated during VILI, trying to re-establish the physiological surface tension at the air-liquid interface. The exogenous surfactant used contains 1-2% of the surfactant proteins B and C which are a pre-requisite for a rapid adsorption at the air-liquid interface. In the Surfactant group a significant increase in arterial oxygen tension levels, comparable with basal values, was seen within 5 minutes and was sustained during the 2-h study period. At the end of the study period TLC_{35}, C_{max}, and the Gruenwald Index, were significantly higher in the Surfactant group compared with the Ventilated and Non-Ventilated controls groups, and not significantly different from the healthy control group. Additionally, in the Surfactant group a low minimal surface tension of the BAL fluid was observed. It is known that one of the most important functions of the pulmonary surfactant system is the mechanical stabilisation of the lung alveoli during end-expiration. This is achieved by decreasing the surface tension in parallel with the decrease in alveolar radius (27). Conversely, a high surface tension will promote alveolar collapse during deflation of the lung (1-4, 8-10). Based on our results, we assume that the alveolar surface tension was restored by exogenous surfactant in the Surfactant group, providing, together with PEEP, open alveoli resulting in almost normal arterial oxygen tension. In contrast, the Ventilated control group showed an impaired gas exchange and decreased alveolar stability, probably caused by the demonstrated high surface tension in the BAL fluid of this group.

Another important function of pulmonary surfactant is the stabilization of fluid balance in the lung and preventing pulmonary edema (27-29). Therefore, loss of surfactant function will lead to alveolar edema which dilutes and inactivates the pulmonary surfactant. The protein level from the crude BAL fluid was significantly higher in all exposed to VILI groups compared with the Healthy group. Therefore the application of exogenous surfactant had no additional effect on the resolution of this edema during the 2-h study period. More studies have to be performed to

determine if there are any changes in lung water, microvascular permeability, and histological parameters of edema when exogenous surfactant is used after VILI.

The model of VILI used in this study might resemble a clinical situation, especially when high inspiratory lung volumes are applied. It is known that mechanical ventilation may damage the lung in the presence or absence of pre-existing lung disease and produces a similar pattern of injury as that observed during ARDS (23); mechanical ventilation can induce lung parenchymal damage especially in the surfactant deficient parts of the ARDS lungs and may further induce surfactant changes in those parts of the ARDS lung which still have an adequately functioning surfactant system (12). The possible clinical relevance of our study is that exogenous surfactant can be used not only to prevent VILI, but also as a treatment after VILI, restoring the surfactant function in those alveolar units already damaged and preventing damage of the intact alveolar units.

In conclusion, our results show that exogenous surfactant can be used as a treatment for VILI, restoring lung function and lung mechanics.

References

1. Faridy E., S. Permutt, and R Riley. 1966. Effect of ventilation on surface forces in excised dog's lungs. *J Appl Physiol* 21: 1453-1462.
2. McClenahan J., A. Urtnowski. 1967. Effect of ventilation on surfactant, and its turnover rate. *J Appl Physiol* 23: 215-220.
3. Forrest J. 1972. The effect of hyperventilation on pulmonary surface activity. *Br J Anaesth* 44: 313-320.
4. Wyszogrodski I., K. Kyei-Aboagye, W. Taeush, and E. Avery. 1975. Surfactant inactivation by hyperventilation: conservation by end-expiratory pressure. *J Appl Physiol.* 38: 461-466.
5. Verbrugge S., S. Böhm, D. Gommers, L. Zimmerman, and B. Lachmann. 1998. Surfactant impairment after mechanical ventilation with large alveolar surface area changes and effects of positive end-expiratory pressure. *Br J Anaesth.* 80: 360-364.
6. Lachmann B., E. Danzmann, B. Haendly, and B. Jonson. 1982. Ventilator settings and gas exchange in respiratory distress syndrome: Applied Physiology in Clinical Respiratory Care. In Prakash O (ed.) Nijhoff, The Hague,. pp 141-176.
7. Sykes MK. 1991. Does mechanical ventilation damage the lung? *Acta Anaesthesiol Scand.* 35 [suppl]: 126-130.
8. Webb H., and D. Tierney. 1974. Experimental pulmonary edema due to high intermittent positive pressure ventilation with high inflation pressures. Protection by positive end-expiratory pressure. *Am Rev Respir Dis.* 110: 556-565.
9. Dreyfuss D., G. Basset, P. Soler, and G. Saumon. 1985. Intermittent positive pressure hyperventilation with high inflation pressures produces pulmonary microvascular injury in rats. *Am Rev Respir Dis.* 132: 880-884.
10. Dreyfuss D., P. Soler, G. Basset, and G. Saumon. 1988. High inflation pressure pulmonary edema: respective effects of high airway pressure, high tidal volume, and positive end-expiratory pressure. *Am Rev Respir Dis.* 137: 1159-1164.
11. Verbrugge S., G. Vazquez de Anda, D. Gommers, S. Neggers, V. Sorm, S. Böhm, and B. Lachmann. 1998. Exogenous surfactant preserves lung function and reduces alveolar Evans blue dye influx in a rat model of ventilation-induced lung injury. *Anesthesiology.* 89: 467-474.

12. Verbrugge S., B. Lachmann. 1999. Mechanisms of ventilation-induced lung injury: physiological rationale to prevent it. *Monaldi Arch Chest Dis.* 54: 22-37.
13. Taylor F., Abrams M., 1966. Effects of surface active lipoprotein on clotting and fibrinolysis and of fibrinogen on surface tension of surface active lipoprotein. *Am J Med.* 40: 346-352.
14. Holm B., Enhorning G., Notter R. 1988. A biophysical mechanism by which plasma proteins inhibit surfactant activity. *Chem Phys Lipids.* 49: 40-55.
15. Seeger W., Stohr G., Wolf H., Neuhof H. 1985. Alteration of surfactant function due to protein leakage: special interaction with fibrin monomer. *J Appl Physiol.* 58: 326-338.
16. Gommers D., C. Vilstrup, J. Bos, A. Larsson, O. Werner, E. Hannappel, and B. Lachmann. 1993. Exogenous surfactant therapy increases static lung compliance, and cannot be assessed by measurements of dynamic compliance alone. *Crit Care Med.* 21: 567-574.
17. Lachmann B., B. Robertson, and J. Vogel. 1980. In vivo lung lavage as an experimental model of the respiratory distress syndrome. *Acta Anaesthesiol Scand.* 24: 231-236.
18. Gruenwald P. 1963. A numerical index stability of lung expansion. *J Appl Physiol.* 88: 359-367.
19. van Daal G., J. Bos, E. Eijking, D. Gommers, E. Hannappel, and B. Lachmann. 1992. Surfactant replacement therapy improves pulmonary mechanics in end-stage influenza A pneumonia in mice. *Am Rev Respir Dis.* 145: 859-863.
20. Veldhuizen R., K. Inchley, S. Hearn, J. Lewis, and F. Possmayer. 1993. Degradation of surfactant associated protein B (SP-B) during in vitro conversion of large into small surfactant aggregates. *Biochem J.* 295: 141-147.
21. Bradford M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Ann Biochem.* 72:248-254.
22. Eijking E., D. Gommers, K. So, M. de Maat, J. Mouton, and B. Lachmann. 1993. Prevention of respiratory failure after hydrochloric acid aspiration by intratracheal surfactant instillation in rats. *Anesth Analg.* 76: 472-477.
23. Dreyfuss D., and G. Saumon. 1998. Ventilator induced lung injury. Lessons from experimental studies. *Am J Respir Crit Care Med.* 157: 294-323.
24. Faridy E. 1975. Effect of ventilation on movement of surfactant in airways. *Resp Physiol* 38:461-466.
25. Ito Y., R. Veldhuizen, L. Yau, L. McCaig, A. Bartlett, and J. Lewis. 1997. Ventilation strategies affect surfactant aggregate conversion in acute lung injury. *Am J Respir Crit Care Med* 155: 493-499.
26. Ito Y., S. Manwell, C. Kerr, R. Veldhuizen, L. Yao, D. Bjarneson, L. McCaig, A. Bartlett, and J. Lewis. 1998. Effects of ventilation strategies on the efficacy of exogenous surfactant therapy in a rabbit model of acute lung injury. *Am J Respir Crit Care Med.* 157: 149-155.
27. Lachmann B. 1987. The role of pulmonary surfactant in the pathogenesis and therapy of ARDS. In J.L. Vincent (ed.). Update in Intensive Care and Emergency Medicine, Springer-Verlag. pp 123-134.
28. Guyton A., D. Moffatt, T. Adair. 1984. Role of alveolar surface tension in transepithelial movement of fluid. In: Robertson B, van Golde LMG, Batenburg JJ (ed). Pulmonary surfactant. Elsevier, Amsterdam. pp. 171-185.
29. Walters D. 1992. The role of pulmonary surfactant in transepithelial movement of fluid. In: Robertson B, van Golde LMG, Batenburg JJ (ed). Pulmonary surfactant: from molecular biology to clinical practice. Elsevier, Amsterdam. pp. 193-213.

Chapter 6

Partial liquid ventilation improves lung function in ventilation-induced lung injury

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Summary

Background: Disturbances in lung function and lung mechanics are present after ventilation with high peak inspiratory pressures and low levels of PEEP. The combination of perfluorocarbon (PFC) with gas ventilation, better known as partial liquid ventilation (PLV), might be useful for treatment of ventilation-induced lung injury (VILI). Therefore, we investigated whether PLV can re-establish lung function after VILI has been induced.

Methods: Adult rats were exposed to high peak inspiratory pressures without PEEP for 20 min. Thereafter, the animals were randomly divided into five groups. The first group was killed immediately after randomisation and used as a non-ventilated control. The second group received only mechanical ventilation, and three groups received PFC (10 mL/kg, 20 mL/kg, and 20 mL/kg plus 5 mL/kg after one hour to compensate loss of PFC due to evaporation). Then the four groups were mechanically ventilated for two hours. Blood gases, lung mechanics, total protein concentration, minimal surface tension, and small surfactant aggregates/large surfactant aggregates ratio were determined.

Results: PLV improved gas exchange, dose and time dependent, and total lung compliance, but did not decrease the protein concentration or the small aggregates/large aggregates ratio in bronchoalveolar fluid after 2 hours mechanical ventilation.

Conclusion: PLV improves gas exchange and pulmonary compliance in VILI when evaporated PFC is replaced, but does not reduce the level of intra-alveolar protein concentration.

Introduction

It is known that modes of mechanical ventilation which allow end-expiratory alveolar collapse and/or end-inspiratory alveolar overstretching result in a decrease of lung compliance and gas exchange [1], and lead to atelectasis, pulmonary edema, pneumonitis and fibrosis [2,3]. Development of intra-alveolar protein-rich edema in healthy rats subjected to intermittent positive pressure ventilation at high inflation pressures, without positive end-expiratory pressure (PEEP), was first demonstrated by Webb and Tierney [4] and was later confirmed by Dreyfuss and colleagues who suggested that high inspiratory lung volumes induce endothelial and epithelial overstretching leading to microvascular injury [5,6]. Additionally, it is known that large changes in both volume and surface area result in surfactant depletion from the alveoli into the airways as well as to transformation from surface active large aggregates to inactive small aggregates [7-11]. Thus, loss of surfactant function will increase the surface tension at the air-liquid interface of the alveolar walls resulting in alveolar collapse and an increased suction force on the pulmonary interstitium which causes more alveolar edema. The epithelial/endothelial damage results mainly from the shear forces which appear in a non-homogeneous ventilated lung [6,10]. It is known that perfluorocarbons (PFCs) have a surfactant-like activity due to their low surface tension (18 mN/m) which in a surfactant-deficient lung decreases the high surface tension at the air liquid interface [12-15]. Based on this low surface tension, the resulting peak inspiratory pressures during volume controlled ventilation are reduced [12-15]. Another property of PFCs is their high density which, mainly in the dependent part of the lung, recruit collapsed alveolar units [12-16]. The combination of PFCs with gas ventilation, better known as partial liquid ventilation (PLV), finally also improves gas exchange in surfactant-deficient lungs [12,13]. Additionally, because PFCs might not be affected by the presence of plasma proteins in the alveolus, PFC might prove useful as treatment for VILI [17,18]. Therefore, the aim of this study was to establish whether PLV can re-establish lung function in ventilation-induced lung injury.

Material and methods

Animal Preparation

This study was approved by the local Animal Committee at the Erasmus University Rotterdam. The study was performed in 30 adult male Sprague-Dawley rats (body weight 280-

350 g). After induction of anesthesia with 2% enflurane and 65% nitrous oxide in oxygen, a polyethylene catheter was inserted into a carotid artery for drawing arterial blood samples and continuous monitoring of arterial blood pressure. Before tracheostomy, the animals received 30 ml/kg pentobarbital sodium, intraperitoneal (i.p.) (Nembutal®, Algin BV, Maassluis, the Netherlands). After tracheostomy, muscle relaxation was induced by pancuronium bromide 0.6 ml/kg, intramuscular (i.m.) (Pavulon®, Organon Teknika, Boxtel, the Netherlands) immediately followed by connection to a ventilator and a pressure transducer for continuous monitoring of arterial blood pressure. 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, postive end-expiratory pressure (PEEP) of 2 cm H_2O, and inspiratory/expiratory (I/E) ratio of 1:2. Anesthesia was maintained with pentobarbital sodium 30 mL/kg/h, i.p.; muscle relaxation was maintained with pancuronium bromide 0.6 mL/kg/h i.m. Body temperature was kept within normal range by means of a heating pad.

Experimental Design

In order to produce VILI, PIP was increased to 45 cm H_2O and PEEP was decreased to zero for 20 min, whereas the other ventilator settings were not changed. Thereafter, PIP was decreased to 26 cm H_2O and PEEP was increased to 6 cm H_2O for 5-min. Then, the animals were disconnected from the ventilator to ambient pressure to allow some edema fluid (1-2 ml) to flow from the lungs; after this procedure the animals were randomized.

Experimental Groups

The animals were randomised to one of five groups (n=6 per group). In the first group (Non-Ventilated) the animals were killed after the 5-minute ventilation period of 26/6 (PIP/PEEP) with an overdose of pentobarbital and were used as a non-treated, non-ventilated control group. The second group (Ventilated) received a sham bolus of air 28 mL/kg intra-tracheally and was mechanically ventilated at a PIP of 30 cm H_2O, PEEP of 10 cm H_2O, I/E ratio of 1:2, FiO_2 1.0, and respiratory rate of 40 bpm for two hours. These ventilator settings were chosen based on results of a preliminary study which showed that applied ventilation pressures of 26/6 cm H_2O (PIP, PEEP, respectively) and 28/8 cm H_2O were too low to keep animals alive for an observation period of 2 hours. Three groups received PFC at a dose of : 10 mL/kg (PFC_{10}), 20 mL/kg (PFC_{20}), or 20 mL/kg plus an extra dose of 5 mL/kg (PFC_{20+R}).

Treatment with PFC

The PFC used in this study (Liquivent®, Alliance Pharmaceutical, San Diego, CA, USA) is insoluble in water, has a specific gravity of 1.918 g/cm at 25°C, a surface tension of 18.1 dynes/cm, vapor pressure of 3.6 kPa at 20°C and 10.5 kPa at 37°C, an oxygen solubility of 53 mL/100 mL and CO_2 solubility of 210 mL/100 mL at 37°C, at 1 atmosphere pressure [12, 13]. The groups PFC_{10} and PFC_{20} received a single dose of PFC intratracheally. The PFC_{20+R} group received an initial dose of 20 mL/kg of PFC and, after 60 minutes, an extra dose of 5 mL/kg of PFC was instilled intratracheally to compensate loss of PFC due to evaporation. At instillation animals were disconnected from the ventilator and PFC was administered directly into the endotracheal tube over 3 to 5 sec; the animals were then immediately reconnected to the ventilator.

Gas Exchange and Hemodynamics

Arterial blood gas samples were taken in all groups before, after VILI, and at 5 min after the 26/6 period, and in the four ventilated groups at 5 min after the 30/10 period, and every 30 min for 2 h. The samples were analyzed for arterial oxygen tension (PaO_2) and arterial carbon dioxide tension ($PaCO_2$) by conventional methods (ABL 505, Radiometer, Copenhagen, Denmark). At the same time points, arterial pressure was recorded. Hemodynamic support was provided by infusion of 1 ml of saline (to a maximum of 2 ml per hour) when mean arterial pressure (MAP) decreased below 60 mmHg.

Pressure-Volume (P-V) Curves

At 120 min after administration of PFC all animals were killed with an overdose of pentobarbital sodium injected through the penile vene. Then static P-V curves were recorded. After the thorax and diaphragm were opened, the tracheostomy catheter was connected to a pressure transducer (Validyne model DP 45-32, Validyne Engineering Co., Northridge, CA, USA) with a syringe attached to it, and pressures were recorded on a polygraph (Grass model 7B, Grass Instrument Co., Quincy, MA., USA). Using a syringe filled with nitrogen (N_2) the lungs were first inflated (within 10 sec) to an airway pressure of 35 cm H_2O, which was maintained for 5 sec, followed by deflation to an airway pressure of 0 cm H_2O. Then the lungs were re-inflated in steps of 0.5 ml until an airway pressure of 35 cm H_2O was reached. Each inflation step took 1-2 sec followed by a 5-sec pause to allow pressure equilibration. After this, in the same way, the lungs

were then deflated until an airway pressure of 0 cm H_2O was reached. The volume of N_2 left in the syringe was recorded. The lower inflection point (LIP) was determined from the intersection of the lines representing the minimum slope of the compliance curve and the maximum slope of the compliance curve. Maximal compliance (C_{max}) was calculated from the steepest part of the deflation limb [19]. Total lung capacity (TLC_{35}) was defined as lung volume at inflation with a distending pressure of 35 cm H_2O [20].

Gruenwald Index

The Gruenwald index which characterises the surfactant system *in situ* [21], was calculated from the P-V curve, defined as $(2V_5+V_{10})/2V_{max}$, where V_5, V_{10} and V_{max} are the lung volumes at transpulmonary pressures of 5, 10 and 35 cm H_2O from the deflation limb, respectively.

Bronchoalveolar lavage (BAL)

After the P-V curve recordings a BAL (30 mL/kg) was performed five times with saline-$CaCl_2$ 1.5 mmol/litre (crude lavage). Thereafter, cell debris were removed from BAL by centrifugation at 400 g for 10 min. The active surfactant component in the BAL fluid was separated from the non-active surfactant component by differential centrifugation, followed by phosphorus analysis, and the ratio of non-active to active (small to large aggregate) surfactant was calculated [22]. Finally, the protein concentration of the BAL fluid was determined using the Bradford method (Bio-Rad protein-assay, Munich, Germany) [23].

Minimal Surface Tension

Minimal surface tension of the crude lavage was determined by means of a modified Wilhelmy balance (E. Biegler GmbH, Mauerbach, Austria). In this method, a tight-fitting teflon barrier reduces the surface area of a teflon trough from 100-20% at a cycle speed of 0.33/min. Saline is used as subphase and is kept at 37°C. The force on a platinum slide (1x1 cm) is measured by a force transducer and expressed as surface tension. Further, maximal surface tension is measured at 100% surface area and minimal surface tension at 80% surface compression and expressed as milli Newton/meter (mN/m). Surface tension characteristics of a BAL sample are measured after application on the surface of the saline-filled trough. In this study 300 µl of BAL fluid was applied to the surface of the trough; surface tension was measured after 3 cycles [24].

Statistical analysis

Statistical analysis was performed using the Instat 2.0 biostatistics package (GraphPad

software, San Diego, CA, USA). Intragroup comparisons were analysed with repeated measures ANOVA; intergroup comparisons were analysed with ANOVA. If a difference was found, a post-hoc test was performed (Tukey-Kramer). Statistical significance was accepted at p-values <0.05. All data are expressed as mean ± standard deviation.

Results

Figure 1 shows the PaO_2 levels during the whole study period. After VILI and after the ventilator settings were set at 26/6 cm H_2O for 5 minutes the PaO_2 decreased below 13.3 kPa in all groups. After PFC instillation and after increasing the pressures to 30/10 cm H_2O the PFC_{20} and PFC_{20+R} groups showed a significant increase in PaO_2 values to pre-VILI levels ($p < 0.001$), but only the PFC_{20+R} group maintained oxygen tension levels above 60 kPa during the 2-h study period. In both groups with a single dose of PFC (PFC_{10} and PFC_{20}) PaO_2 values decreased over time. There were significant differences between the values in the Ventilated and PFC_{10} groups compared with the values of the PFC_{20+R} throughout the study period ($p<0.001$).

Table 1 shows that the $PaCO_2$ values and MAP levels were comparable in all groups during the whole study period.

Table 2 shows data from BAL fluid and lung mechanics. Protein concentration was significantly higher in the PFC_{10} group compared with the Non-Ventilated group and the PFC_{20+R} group. The Gruenwald Index and the minimal surface tension of the crude lavage fluid, from all ventilated groups were not significantly different from the Non-Ventilated control group. For data on TLC_{35}, C_{max}, and LIP, see Table 2 and Fig. 2a/b. The total phosphorous concentration in the BAL fluid was not different between groups.

The ratio of small to large aggregates in BAL fluid was significantly higher in the four ventilated groups compared with the Non-Ventilated control group, and there was no significant difference between the ventilated control group and all the PFC-treated groups.

Figure 2a shows the inflation limbs from the P-V curves. Both PFC treated groups with 20 mL/kg have a significantly lower opening pressure (LIP) than both Non-Ventilated and the Ventilated control groups. Figure 2b shows the deflation limbs from the P-V curves. The three PFC treated groups had a significantly higher TLC_{35} and C_{max} than both the Non-Ventilated group and the Ventilated control groups (Table 2).

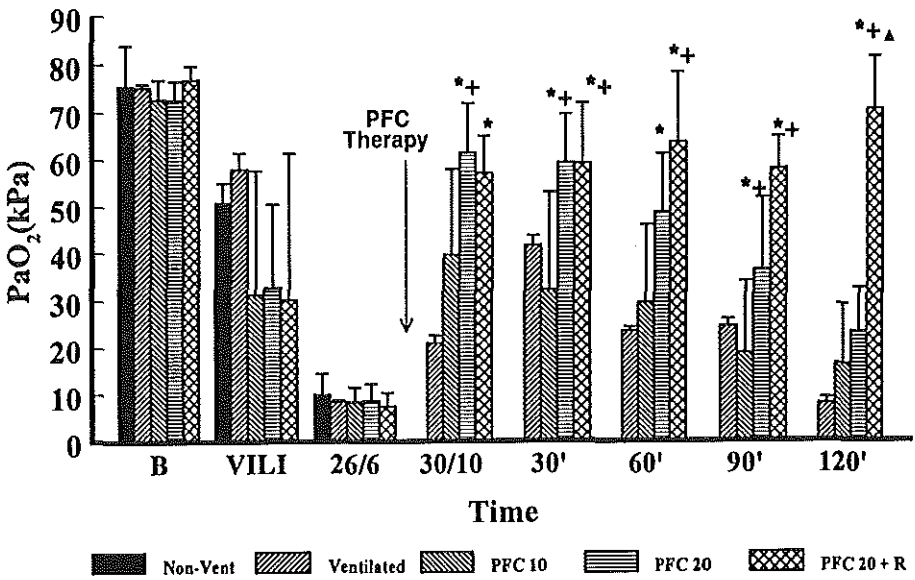

Figure 1. Arterial oxygen tension (mean ± standard deviation) during the whole study period. B = baseline, VILI = ventilation with Peak inspiratory pressure (PIP) of 45 cm H_2O without PEEP after 20 min, 26/6 = after 5 min at PIP 26 cm H_2O, 6 cm H_2O PEEP. * indicates significant difference with Ventilated control group, + indicates significant difference with PFC_{10}, ^ indicates significant difference with PFC_{20}.

Table 1. Data on arterial carbon dioxide tension ($PaCO_2$) and mean arterial pressure (MAP) over time in the groups treated, Non-Ventilated = Non-ventilated control. Ventilated = Ventilated control. PFC_{10} = partial liquid ventilation (PLV) with perfluorocarbons (PFC) at dose 10 mg/kg, PFC_{20} = PLV with PFC at dose 20 mg/kg; PFC_{20+R}= PLV with PFC at dose 20 mg/kg plus 5 mg/kg after 60 minutes of ventilation. VILI = ventilation-induced lung injury, Baseline = measurement before VILI. Values are mean ± standard deviation.

	Time	Non-Ventilated	Ventilated	PFC_{10}	PFC_{20}	PFC_{20+R}
$PaCO_2$ (kPa)	**Baseline**	4.7±0.7	5.3±1.6	4.8±0.97	4.9±0.8	5.6±1.7
	VILI	2.5±0.3	2.4±0.6	2.5±0.35	2.7±0.4	2.9±0.5
	5' 26/6	4.7±0.7	5.3±1.0	5.0±2.1	5.2±0.89	6.7±1.7
	5' 30/10		5.3±1.3	4.3±0.4	4.8±0.7	5.7±0.8
	30'		5.6±1.3	3.9±0.4	4.4±0.9	5.4±0.9
	60'		6.3±1.6	4.4±1.3	4.4±0.7	4.7±1
	90'		6.5±1.3	4.8±1.2	4.7±0.5	5.6±0.8
	120'		6.9±1.8	4.8±2.1	4.9±0.3	4.4±0.9
MAP (mmHg)	**Baseline**	135±16	134±15	144±6	140±24	136±17
	VILI	75±25	84±32	72±25	85±22	71±21
	5' 26/6	74±30	63±42	47±24	67±36	89±32
	5' 30/10		111±12	88±24	106±25	97±14
	30'		91±24	80±20	89±21	90±9
	60'		89±19	78±17	95±15	95±37
	90'		86±29	76±17	97±15	97±12
	120'		89±27	85±9	73±24	80±13

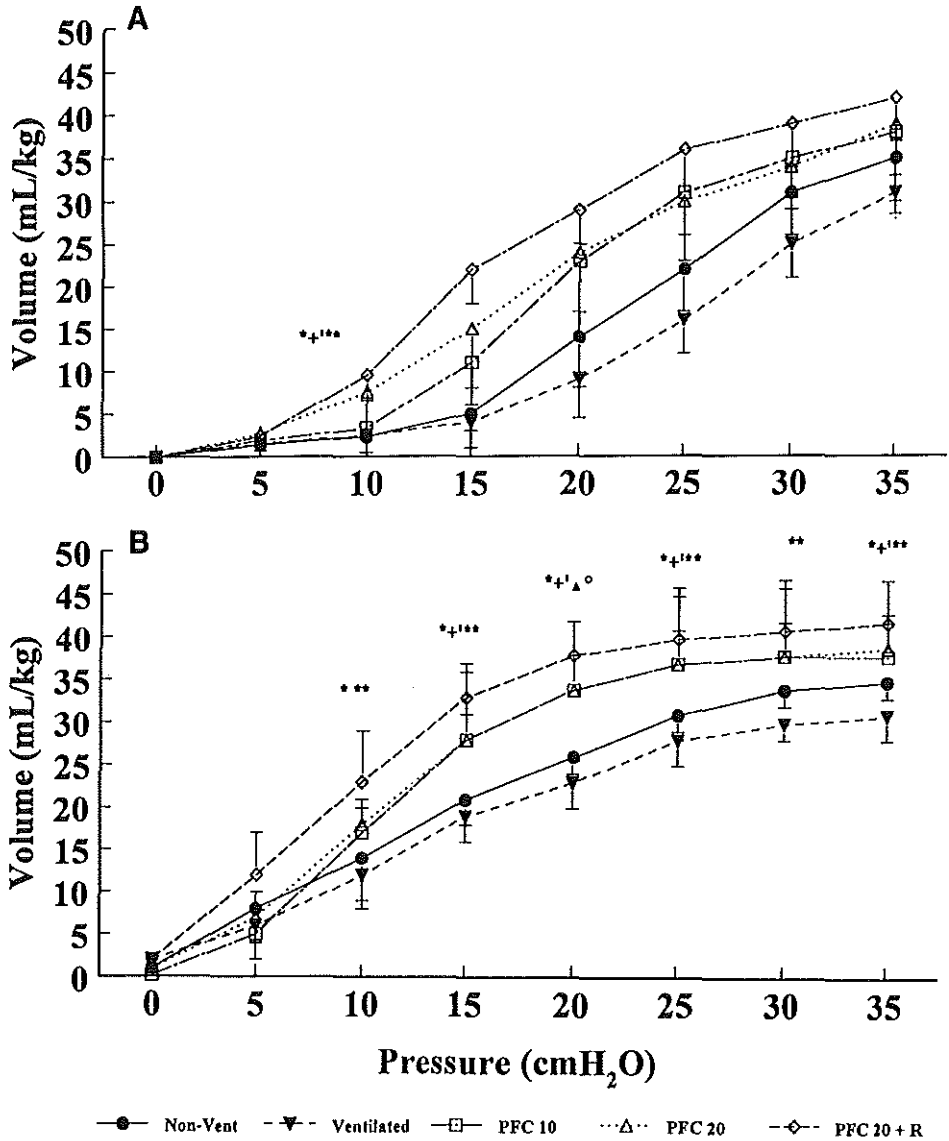

Figure 2. 2.a) Inflation limbs from the pressure-volume curves, (mean ± standard deviation). Non-Vent = Non-ventilated group. Lower inflection point. 2.b) Deflation limbs from the pressure-volume curves, (mean ± standard deviation). * indicates significant differences PFC_{20+R} vs Non-Ventilated, ** indicates significant differences between PFC_{20+R} vs Ventilated, + indicates significant differences between PFC_{20} vs Ventilated, ' PFC_{10} vs Non-Ventilated, ° indicates significant difference between PFC_{10} vs Ventilated, ▲ indicates significant difference between PFC_{20} vs Non-Ventilated.

Table 2. Amount of recovered broncho-alveolar lavage (BAL) fluid, protein concentration, lung volume above FRC at pressure 35 cm H_2O (TLC_{35}), maximum compliance (C_{max}), Gruenwald Index, lower inflection point of the pressure-volume curve (LIP), minimal surface tension (min surf) of crude BAL fluid, total phosphorus concentration, and small aggregates (SA)/large aggregates (LA) ratio. Non-Vent = Non-Ventilated Control group. Ventilated = Ventilated control group. PFC_{10} = PFC 10 mg/kg. PFC_{20} = PFC 20 mg/kg. PFC_{20+R} = PFC 20 mg/kg + 5 mg/kg at 60 min of study period to replace PFC loss due to evaporation. Values are mean ± standard deviation.

	Non-Vent	Ventilated	PFC_{10}	PFC_{20}	PFC_{20+R}
Recovery BAL fluid (%)	90±1	90±1	90±1	90±1	90±1
Prot. Conc. BAL (mg/ml)	1.3±0.3*	1.4±0.4	1.9±0.2	1.8±0.3	1.4±0.1*
TLC_{35}	35±2**	31±3^{*+**}	38±4	39±4	42±5
C_{max} (mL/kg)	1.5 ±0.3^{*+**}	1.4±0.2^{*+**}	2.4±0.2	2.6±0.2	2.8±0.6
Gruenwald Index	0.30±0.09	0.40±0.17	0.40±0.07	0.4±0.07	0.5±0.2
LIP (cm H_2O)	15.3±1.4^{*+**}	18.2±2^{*+**}	10.8±1.2**	10.6±3.3**	6.7±1.9*
Min surf (mN/m)	33±3.1	31±1.6	35±2.1	35±0.5	32±3.9
Total phosphorus (mmol)	2.0±0.6	1.4±0.5	1.7±0.4	1.5±0.3	1.5±0.2
SA/LA ratio	1.7±1.1	4.6±2.7	4.6±2.3	5.6±3.5	4.2±2.7

* vs PFC_{10} $p<0.05$
$^{+}$ vs PFC_{20} $p<0.05$
** vs PFC_{20+R} $p<0.05$

Discussion

This study shows that partial liquid ventilation improves PaO_2 and lung mechanics in ventilation-induced lung injury, despite the presence of a high intra-alveolar protein concentration.

In the present study, 20 min of ventilation with high peak-inspiratory pressures without PEEP resulted in pulmonary edema and hypoxemia, and in impairment of the pulmonary surfactant system. The latter is characterised by a decrease in lung mechanics and Gruenwald Index, and a high minimal surface tension in BAL fluid compared with healthy rat lungs [10]. The exact

mechanism by which the lung damage is produced by artificial ventilation is not yet entirely clear, but the role of surfactant changes is becoming increasingly realised [10,11,17]. Recently, our group showed that modes of mechanical ventilation with large tidal volume without PEEP disturb the surfactant system in the used animal model of VILI [10]. It has been demonstrated that loss of surface active molecules due to mechanical ventilation with high inspiratory lung volumes without PEEP is produced by displacement of surfactant from the alveolar air-liquid interface into the small airways [4,7]. Moreover, the surface area changes produced by the high inspiratory lung volumes lead to an increased rate of conversion of active into non-active surfactant subfractions [8-11]. These together will lead to alveolar collapse and protein infiltration [4-6,10,11] in which the latter leads to further inactivation of surfactant [25,26]. In the current study we used partial liquid ventilation to correct the lung function affected by VILI. The results shown that after VILI partial liquid ventilation produced an immediate improvement in PaO_2, dose and time dependent. In the group treated with 10 ml/kg of perfluorocarbon the pre-VILI values of PaO_2 were never reached, while in both groups treated with 20 mL/kg PFC within 5 min there was a significant increase in PaO_2 values compared with values after VILI, and these improved values were comparable with baseline values. However, PaO_2 decreased over time in both groups in which perfluorocarbon was not replaced. It has been shown that in surfactant-deficient animal lungs partial liquid ventilation provides adequate gas exchange as long as a sufficient amount of PFC is present in the lungs [12-15]. Our group has demonstrated that higher doses of PFC lead to higher levels of oxygenation in animals suffering from acute respiratory failure as a result of dose-dependent recruitment of collapsed atelectatic alveoli by PFC [12,13]. It is also known that oxygenation deteriorates over time if no additional doses of PFC are instilled; this is attributed to evaporation of PFC which will cause affected alveoli to collapse [12,13].

In the present study, the inflation limbs of the P-V curves showed on the one hand a significantly lower opening pressure in the three PFC-treated groups, and on the other a significantly higher total lung capacity and maximal compliance compared with both control groups. The reason for this is that in surfactant-deficient lungs, the decrease of surface tension at the air-liquid interface by PFC improves the mechanical properties of the lung [12,13,27-30]. Dreyfuss et al. advocated that an important benefit of PFC on lung mechanics was the reduction of the mechanical nonuniformity of flooded lungs and probably opposition to overinflation of the

more compliant, aereated zones [27]. But the results from the present study and other studies [29,30] would not support the findings of Dreyfuss and colleagues because our data, which characterise (indirectly) overinflation of alveoli i.e. a high SA/LA ratio [8,9] and high alveolar protein influx [5], just indicate overinflation.

A side effect of PFC may be the constant surface tension which does not change with the changes in surface area (which is a property of natural surfactant) so that the end-expiratory stability in the PFC-treated animals (characterised by the Gruenwald Index) was the same as in the Ventilated control animals [15].

As mentioned above, in the current study the protein level from the crude BAL fluid in all ventilated groups was as high as in the Non-Ventilated control group. Moreover, in both PFC treated groups without replacement, an increase in the total protein concentration was observed. Dreyfuss et al. showed in rats that PFC partially reversed the effects of alveolar flooding, but did not reduce the permeability changes on the alveolo-capillary membrane measured by ^{125}I-labeled serum albumin [27]. This is partially supported by our data showing that with a larger amount of PFC in the lung, the total amount of protein in the BAL fluid is less. How PFC prevents protein infiltration and alveolar flooding is not entirely clear. It has been suggested that as a result of the PFC-filled alveoli the suction forces on the interstitium more or less disappear thus preventing protein influx into the PFC-filled alveoli. However, alveoli which have only a PFC film at the air-liquid interface and which may collapse during expiration will promote alveolar flooding due to their high surface tension at end-expiration: this explains why the amount of proteins in the PFC group receiving only 10 mL/kg PFC is significantly higher than in the group treated with 20 mL/kg of PFC.

In conclusion, our results in this animal study show that, in VILI, partial liquid ventilation improves gas exchange and pulmonary function, despite the presence of a high intra-alveolar protein concentration. However, the loss of perfluorocarbon over time due to evaporation has to be replaced.

References

1. Verbrugge SJ, Lachmann B. Mechanisms of ventilation-induced lung injury and its prevention: role of surfactant. *Appl Cardiopulm Pathophysiol* 1998; **7**: 173-98.
2. Lachmann B, Danzmann E, Haendly B, Johnson B. Ventilator settings and gas exchange in respiratory distress syndrome: Applied Physiology in Clinical Respiratory Care. In Prakash O (ed.) Nijhoff, The Hague, 1982; pp 141-76.
3. Sykes MK. Does mechanical ventilation damage the lung? *Acta Anaesthesiol Scand* 1991; **35** [suppl]: 126-30.
4. Webb H, Tierney D. Experimental pulmonary edema due to high intermittent positive pressure ventilation with high inflation pressures. Protection by positive end-expiratory pressure. *Am Rev Respir Dis* 1974; **110**: 556-65.
5. Dreyfuss D, Basset G, Soler P, Saumon G. Intermittent positive pressure hyperventilation with high inflation pressures produces pulmonary microvascular injury in rats. *Am Rev Respir Dis* 1985; **132**: 880-4.
6. Dreyfuss D, Soler P, Basset G, Saumon G. High inflation pressure pulmonary edema: respective effects of high airway pressure, high tidal volume, and positive end-expiratory pressure. *Am Rev Respir Dis* 1988; **137**: 1159-64.
7. Faridy E. Effect of ventilation on movement of surfactant in airways. *Respir Physiol* 1975; **38**: 461-6.
8. Ito Y, Veldhuizen R, Yau L, et al. Ventilation strategies affect surfactant aggregate conversion in acute lung injury. *Am J Respir Crit Care Med* 1997; **155**: 493-9.
9. Ito Y, Manwell S, Kerr C, et al. Effects of ventilation on the efficacy of exogenous surfactant therapy in a rabbit model of acute lung injury. *Am J Respir Crit Care Med* 1998; **157**: 149-55
10. Verbrugge SJ, Böhm SH, Gommers D, Zimmerman L, Lachmann B. Surfactant impairment after mechanical ventilation with large alveolar surface area changes and effects of positive end-expiratory pressure. *Br J Anaesth* 1998; **80**: 360-4.
11. Verbrugge SJ, Vazquez de Anda G, Gommers D, et al. Exogenous surfactant preserves lung function and reduces alveolar Evans blue dye influx in a rat model of ventilation-induced lung injury. *Anesthesiology* 1998; **89**: 467-74.
12. Tütüncü AS, Akpir K, Mulder P, et al. Intratracheal perfluorocarbon administration as an aid in the ventilatory management of respiratory distress syndrome. *Anesthesiology* 1993; **79**: 1083-93.
13. Tütüncü AS, Faithfull NS, Lachmann B. Intratracheal perfluorocarbon administration combined with artificial ventilation in experimental respiratory distress syndrome: dose dependent improvement of gas exchange. *Crit Care Med* 1993; **21**: 962-9.
14. Hirschl RB, Tooley R, Parent A, et al. Evaluation of gas exchange, pulmonary compliance, and lung injury during total and partial liquid ventilation in the acute respiratory distress syndrome. *Crit Care Med* 1996; **24**:1001-8.
15. Verbrugge S, Lachmann B. Partial liquid ventilation. *Eur Respir* J 1997; **10**: 1937-9
16. Quintel M, Hirschl R, Roth H, et al. Computer tomographic assessment of perfluorocarbon and gas distribution during partial liquid ventilation for acute respiratory failure. *Am J Respir Crit Care Med* 1998; **158**: 249-55.
17. Dreyfuss D, Saumon G. Ventilator induced lung injury. Lessons from experimental studies. *Am J Respir Crit Care Med* 1998; **157**: 294-323.
18. Gauger PG, Overbeck MC, Chambers SD, Cailipan CI. Partial liquid ventilation improves gas exchange and increases EELV in acute lung injury. *J Appl Physiol* 1998; **84**: 1566-72.
19. Gommers D, Vilstrup C, Bos J, et al. Exogenous surfactant therapy increases static lung compliance, and cannot be assessed by measurements of dynamic compliance alone. *Crit Care Med* 1993; **21**: 567-74.
20. Lachmann B, Robertson B, Vogel J. In vivo lung lavage as an experimental model of the respiratory distress syndrome. *Acta Anaesthesiol Scand* 1980; **24**: 231-36.
21. Gruenwald P. A numerical index stability of lung expansion. *J Appl Physiol* 1963; **88**: 359-67.
22. Veldhuizen RAW, Inchley K, Hearn SA, et al. Degradation of surfactant associated protein B (SP-B) during in vitro conversion of large into small surfactant aggregates. *Biochem J* 1993; **295**: 141-7.
23. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Ann Biochem* 1976; **72**:248-54.
24. Eijking E, Gommers D, So K, et al. Prevention of respiratory failure after hydrochloric acid aspiration by intratracheal surfactant instillation in rats. *Anesth Analg.* 1993; **76**: 472-7.
25. Holm B, Enhorning G, Notter R. A biophysical mechanism by which plasma proteins inhibit surfactant activity. *Chem Phys Lipids* 1988; **49**: 40-55.

26. Seeger W, Stohr G, Wolf H, Neuhof H. Alteration of surfactant function due to protein leakage: special interaction with fibrin monomer. *J Appl Physiol* 1985; **58**: 326-38.
27. Dreyfuss D, Martin-Lefèvre L, Saumon G. Hyperinflation-induced lung injury during alveolar flooding in rats. Effect of perfluorocarbon instillation. *Am J Respir Crit Care Med 1999*; **159**:1752-7.
28. Kaisers U, Kuhlen R, Keske U, et al.. Superimposing positive end-expiratory pressure during partial liquid ventilation in experimental lung injury. *Eur Respir J* 1988; **11**: 1035-42.
29. Hartog A, Kaisers U, Gommers D, Lachmann B. Comparing the effects of four different perfluorocarbons on gas exchange and lung mechanics in an animal model of acute lung injury. *Appl Cardiopulm Pathophysiol* (in press)
30. Hartog A, Vazquez de Anda G, Gommers D, et al. Comparison of exogenous surfactant therapy, mechanical ventilation with high end-expiratory pressure and partial liquid ventilation in a model of acute lung injury. *Br J Anaesth* 1999; **82**: 81-6.

Chapter 7

Protecting the lung during mechanical ventilation with The Open Lung Concept

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Introduction

Mechanical ventilation has been used during the last 30 years as the most important treatment of the acute respiratory failure (ARF) and neonatal respiratory distress syndrome (IRDS). The classical paper on ARF by Ashbaugh and colleagues, describes the consequences of closed lung units: Hypoxemia; intrapulmonary shunt (under the assumption that the hypoxic pulmonary vasoconstriction is inhibited) and atelectasis. Mechanical ventilation was used in all 12 patients to re-expand collapsed lung units, and to prevent re-collapse of those alveoli, positive end-expiratory pressure (PEEP) was initially applied (in only five patients) at levels of 5-7 cmH_2O. The authors conclude that "PEEP is most helpful in combating atelectasis and hypoxemia" [1].

The same group used positive pressure ventilation with PEEP at levels of 7-10 cmH_2O, in 21 patients with ARF. They found that once alveoli are collapsed, high pressure amplitudes are required for mechanical ventilation, in which the supplied gas is mainly distributed to healthy alveoli. Higher peak inspiratory pressures (PIP) between 60-70 cm H_2O were required to achieve tidal volumes of 400-500 ml. The combination of PIP and PEEP could improve oxygenation; when mechanical ventilation was applied without PEEP, alveolar collapse occurred during the expiratory phase [2].

Kirby et al. [3], used levels of PEEP $\geq$ 25 cm H_2O in 28 patients with ARF. They showed that very high inspiratory pressures (up to 160 cmH_2O) were initially required to deliver a tidal volume of 1000 ml. PEEP produced better values of oxygenation without hemodynamic compromise. A low rate of barotrauma was observed, and survival was reported to be 61%.

It is known that high pressure amplitudes, high tidal volumes, and low levels of PEEP can damage the lung. Many ventilator designs and ventilation strategies have been developed to improve oxygenation, which at the same time avoid ventilation-induced lung injury (VILI) [4]. Nowadays, to obtain reasonable gas exchange a recruitment manoeuvre to open most lung units, has been advocated. Thereafter, a mode of ventilaton with lower pressure amplitude, with low inspiratory pressures and sufficient levels of PEEP has been recommended [5]. It is believed that this ventilatory strategy may play an important role in modifying the disease process.

Physiologic background

The relation between airway pressures and lung volumes has been the focus of basic lung

physiology. This relation is determined by the interaction of millions of individual alveoli. To understand the behaviour of the entire lung it is, therefore, helpful to look first at a single alveolus.

The membrane of each alveolus is composed of different layers, starting with the capillary endothelium, the basement membranes, the connective tissue, the epithelial layer and finally the intraalveolar surfactant film. The tissue contains elastic and nonelastic fibers that limit the expansion of an alveolus beyond its elastic properties. The surface tension at the air-liquid interface adds to the retractive forces of the alveolar wall [6].

In 1929, von Neergard first called attention to the contribution of the alveolar surface tension to the retractive forces of the lungs [7]. He considered the formation of a bubble on the end of a capillary tube as an analogue for the surface geometry of an alveolus. For this model the law of LaPlace provides a mathematical explanation: $P = 2\gamma / r$; where P is the pressure inside the bubble, γ the surface tension of the liquid and r the radius of the bubble [6]. The physiological behaviour of the alveolus can be described by the following model (Figure 1). Before any pressure is applied, the fluid covers the orifice of the capillary tube as a flat perpendicular film. Increasing the pressure in the capillary will start the formation of a small bubble. The pressure within the system rises until the bubble's shape approaches that of a hemisphere. The bubble now has the same radius as the tube. Once the pressure within the bubble exceeds a critical pressure, the bubble overcomes the hemispheric state; it opens. Now the bubble can be kept open with a much lower pressure than the critical opening pressure. In an open bubble the pressure changes required to induce certain changes in volume are now significantly lower compared to the closed state [6].

Applying these concepts to the inflation of a surfactant-deficient collapsed alveolus, it becomes apparent that surface forces, as stated in the law of LaPlace, act predominantly at a low alveolar radius; they hinder alveolar opening. Once, the alveolus is opened, however, and while maintaining identical opening pressures the volume increases rapidly to about two thirds of the maximal volume up to the point where the tissue forces begin to oppose further expansion. The pressure within this newly expanded alveolus can now be decreased until the bubble reaches its unstable state, and collapses. In a healthy alveoli with a normal surfactant system this collapse pressure is reduced to 3-5 cm H_2O. In other words, due to the fact that at end-expiration surface tension decreases almost to zero, the required pressure to stabilise healthy alveoli is only 3-5 cm

H_2O which is equal to the applied transpulmonary pressure. This, in general, prevents a healthy lung from collapse. However, should the alveolus collapse once again, an active re-expansion is required to open it [8]. Thereafter, the pressures are reduced and kept at a value slightly above the previously determined collapsing pressure. This pressure level depends mainly on the function of the surfactant system [9]. In summary, the behaviour of alveoli is quantal: they are either open or closed. No stable state in between these endpoints exists. However, while maintaining identical opening pressures the volume increases rapidly to about two thirds of the maximal volume up to the point where the tissue forces begin to oppose further expansion. The pressure within this newly expanded alveolus can now be decreased until the bubble reaches its unstable state, and collapses. In a healthy alveoli with a normal surfactant system this collapse pressure is reduced to 3-5 cm H_2O. In other words, due to the fact that at end-expiration surface tension decreases almost to zero, the required pressure to stabilise healthy alveoli is only 3-5 cm H_2O which is equal to the applied transpulmonary pressure. This, in general, prevents a healthy lung from collapse. However, should the alveolus collapse once again, an active re-expansion is required to open it [8]. Thereafter, the pressures are reduced and kept at a value slightly above the previously determined collapsing pressure. This pressure level depends mainly on the function of the surfactant system [9]. In summary, the behaviour of alveoli is quantal: they are either open or closed. No stable state in between these endpoints exists. This quantal alveolar physiology was demonstrated by Mead [6]and Staub et al. [10] and was confirmed in computer tomography studies by Wegenius et al. [10-11].

Ventilatory strategies to avoid ventilation-induced lung injury

ARF and IRDS are characterised by atelectasis. Positive pressure ventilation has been used to re-expand alveoli and to minimise atelectasis. However, the application of high inspiratory pressures and volumes with the associated overdistention of open alveoli, combined with a low expiratory volume, increases the risk of barotrauma and volutrauma [4]. On the other hand, low levels of PEEP may also contribute to ventilation-induced lung injury by allowing alveoli to collapse and re-open during each respiratory cycle [4]. Shear forces are the result and may be responsible for a massive release of mediators into the alveoli [12] and into the pulmonary circulation [13]. In addition, an alteration of the surfactant function, and bacterial translocation at

low PEEP have been demonstrated [14].

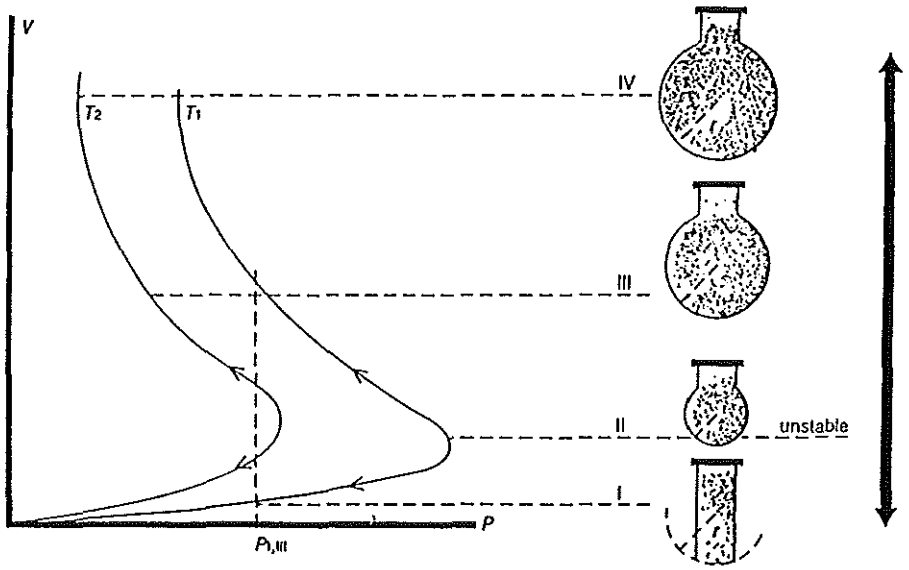

Figure 1. Physiological behaviour of the alveolus. The pressure (P)-volume(V) relation is displayed in X-Y axes. On the right side, the status of the broncho-alveolar unit. Its radius (r) reflects the pressure-volume relation (I-IV). Surface tension in pathological (T1) and normal conditions (T2). The arrows show the direction from closed (bottom) to open (top) states and vice versa (modified from Mead, 1961). For more detail see text.

To protect the lung against VILI, Lachmann et al. proposed that a protective ventilatory strategy based on the law of LaPlace should be used [5,8]. They showed that; raising airway pressures higher than 40 cm H_2O resulted in a recruitment of most functional alveolar units. Once opened these units should be kept open by the minimal PEEP level, and gas exchange can be kept in the normal range even at low pressure amplitude between PIP and PEEP. These low pressure amplitudes produce less shear forces, and thus protect against VILI. However, only a few clinical

studies have been performed using this ventilatory strategy [5,15]. This strategy produces a ventilatory condition which saves the lung from further damage, allows a reduction of FiO_2, promotes the resorption of interstitial and intrapulmonary edema, and finally reduces the pulmonary artery pressures by overcoming the hypoxic pulmonary vasoconstriction [5].

A similar protective ventilatory strategy can be applied using high frequency oscillatory ventilation (HFOV) at high levels of mean airways pressure, which results in low oscillation pressure amplitude, low tidal volumes and normal values of carbon dioxide ($PaCO_2$) [16]. Froese's group showed that HFOV is useful to protect the lung, but only after a re-expansion manoeuvre. The oscillation pressure amplitude itself is adjusted according to $PaCO_2$ values [17]. The ease of this intervention, makes this strategy a standard of ventilation in some neonatal intensive care units. However, its usefulness has been questioned by multicenter studies, in which no initial re-expansion manoeuvre was performed, showing no significant differences between HFOV and conventional mechanical ventilation [18].

With the intention to protect the lung against VILI, an international consensus conference compiled the following recommendations: The plateau pressure should be limited to 35 cm H_2O, the tidal volume should be as low as 5 ml/kg, permissive hypercapnia was allowed if normocapnia is not achievable at a limited plateau pressure, and the FiO_2 should be minimised. In addition, a re-expansion manoeuvre should be performed [19]. In relation to all these recommendations, some clinical studies have been performed. The effects of limited tidal volumes (Vt) and PIP were studied by two different groups; Brochard et al. [20], in a multicentric study, used a protective ventilatory strategy. It consisted of limiting the plateau pressure to 25 cm H_2O, and was compared with standard ventilation, Vt ≥ 10 ml/kg and PIP below 60 cm H_2O. It was shown that limiting PIP to 25 cm H_2O does not produce a protective effect, and no differences in mortality or multi-organ system failure were found between the groups. However, PIP was not reported in the standard ventilation group.

Stewart et al. [21] prospectively studied patients at high risk for ARDS or with ARDS. The authors compared a protective strategy of ventilation, with tidal volumes of 7 ml/kg, against a control group with tidal volume between 10-15 ml/kg. No differences in mortality were found between groups. This absence of differences could be attributable to the fact that in both groups plateau pressures were below 30 cm H_2O. In both studies, neither in the protective strategy nor

in the control group was a recruitment procedure done, and PEEP levels were not tritrated to prevent alveolar collapse. Thus, in these trials the lungs were allowed to "close". This ventilation at low lung volumes, in contrast to the protective low PIP, may have contributed to VILI.

Amato et al. [22] compared another ventilatory strategy against conventional ventilation in 53 patients with ARDS. Their ventilatory strategy aimed at lung protection. The PEEP level was set above the lower inflection point (P_{flex}) on the static pressure-volume curve. At the same time, tidal volume was limited to 6 ml/kg or PIP to 40 cm H_2O independent of the tidal volume. Permissive hypercapnia was allowed. Their study shows the importance of ventilating at low pressure amplitude between PIP and PEEP. A higher survival rate at 28 days in the protective ventilatory strategy, a lower incidence of barotrauma and a higher rate of weaning were observed. However, the open lung procedure with a PIP of 30-35 cm H_2O, could not effectively open all alveolar units and, therefore, these low tidal volumes produced hypoventilation and hypercarpnia.

Fort et al. [23], studied the effect of HFOV in adults with ARF in which conventional mechanical ventilation had failed to improve oxygenation. They showed, in this preliminary report, that applying a recruitment procedure by raising the Mawp in steps of 2 cm up to 45 cm H_2O (which was not applied during conventional mechanical ventilation), an oxygen saturation $\geq$ 90% at an $FiO_2 \leq 0.6$ could be reached. $PaCO_2$ could be kept within normal values by adjusting the differential pressure. This study showed that HFOV, applying a protective strategy, can be successfully used in adults with ARF. Higher levels of oxygenation and normal values of $PaCO_2$ without hemodynamic compromise could be obtained.

Using the same theoretical concept, conventional mechanical ventilation is very similar to HFOV only if "The Open Lung Concept" is applied. However, HFOV is noisier than conventional mechanical ventilation, in addition more clinical care, humidity of the circuit, and a huge amount of gases are required. Table 1 shows the main characteristics of the different procedures to open the lung.

Table 1. Different ventilatory strategies to open the lungs and to avoid ventilation-induced lung injury.

Strategy	*Mode of Ventilation*	*Criteria of Open Lung*	*Open Lung Procedure*	*Keep Open*	*CO_2 Removal*	*Clinical Application*
Open Lung Concept Lachmann et al. [5]	PCV PC-IRV	PaO_2/FiO_2 ≥ 450 mmHg or maximum	RR >20, tPEEP 15-20 cm H_2O, ↑ PIP to 50-60 cm H_2O for 15-20 s	tPEEP above closing pressure	↑↓ Δ P carefully controlled by RR	For all mechanically ventilated patients
High Lung Volume Strategy Froese et al. [16]	HFOV	PaO_2/FiO_2 ≥ 350 mmHg or SAT ≥ 90% at FiO_2 < 0.6	SI for 15 s at Mawp of 30-40 cmH_2O	Mawp above closing pressure	↑↓ Δ P of oscillation	IRDS ARDS
Open Lung Approach Amato et al. [15]	PSV PC-IRV VAPSV	PEEP above P_{flex} of the P-V curve	CPAP at 30-35 cmH_2O for 10-20 s	PEEP above P_{flex}, or PEEP at 16 cmH_2O and Vt < 6 ml/kg at PIP max of 40 cm H_2O	Vt < 6 ml/kg Permissive Hypercapnia	ARDS

PCV= pressure controlled ventilation. PC-IRV= pressure controlled inverse ratio ventilation. PaO_2/FiO_2 = arterial oxygen tension/inspired oxygen fraction. RR= respiratory rate. PIP= peak inspiratory pressure. Positive end expiratory pressure= PEEP. tPEEP= total positive end expiratory pressure (external PEEP+intrinsic PEEP).↑ = increasing, ↓= decreasing. Δ P= pressure amplitude (tPEEP-PIP). ARDS= acute respiratory distress syndrome. IRDS= neonatal respiratory distress syndrome. CO_2= arterial carbon dioxide. HFOV= high frequency oscillatory ventilation. SAT= arterial saturation of oxygen. SI = sustained inflation. Mawp = mean airway pressure. P-V= pressure-volume. P_{flex} = lower inflection point on P-V curve. PSV= pressure support ventilation. VAPSV= volume assured pressure support ventilation. CPAP = continuous positive airway pressure. Vt = tidal volume.

Nowadays, after reviewing the evidence of lung protection and VILI, "The Open Lung Concept" developed by Lachmann and colleagues some 20 years ago (Figure 2), still remains an effective lung protective strategy, which defines global treatment goals for optimal ventilator settings [5,8,24].

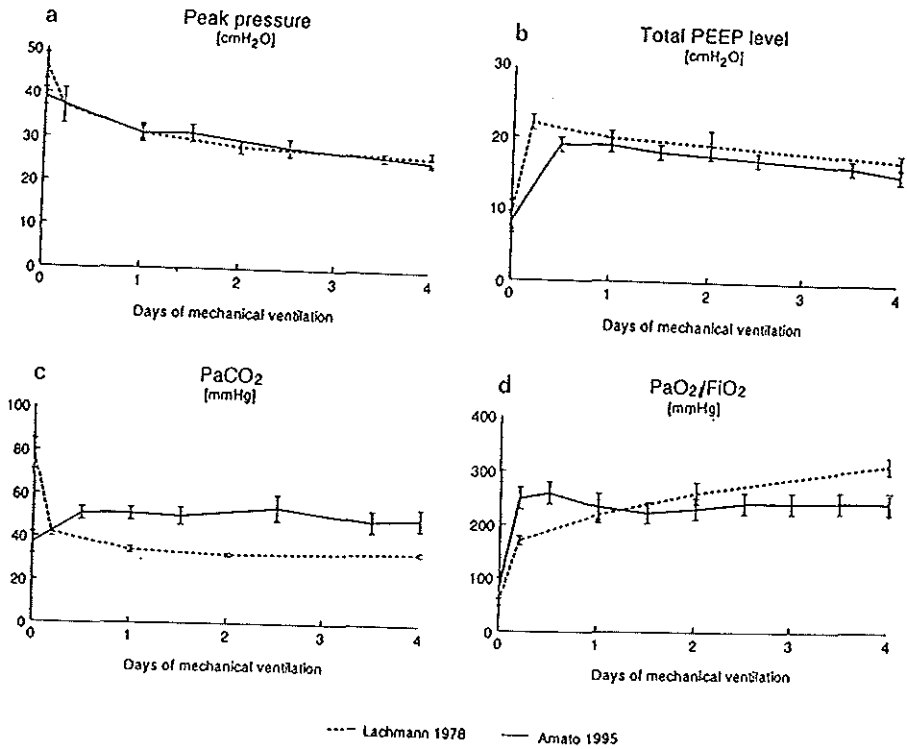

Figure 2. Example of two studies of mechanical ventilation based on lung protection strategies. "The Open Lung Concept" applied by Lachmann et al. [5] (dashed line) in 1978 in six patients with acute respiratory failure, and the "Open Lung Approach" applied by Amato et al. [15] in 1995 in 28 patients with acute respiratory distress syndrome (solid line). Airway pressures (a,b) and gas exchange (b,c) are displayed in function of days of mechanical ventilation. Note! Despite a smaller pressure amplitude in the Lachmann group, the $PaCO_2$ was lower in comparison to the Amato group. The reason for this is the use of more autoPEEP in the Lachmann group, which generally allows to better control CO_2 removal.

Practical considerations

The open lung state is characterised by an optimal gas exchange. The intrapulmonary shunt is ideally less than 10%, which corresponds to a PaO_2 of more than 450 mmHg on pure oxygen. At the same time, airway pressures are at the minimum that ensure the required gas exchange. Hemodynamic side-effects are minimised [5].

The three following statements by Lachmann and colleagues describe the treatment concept:

1) One must overcome a critical opening pressure during inspiration

2) This opening pressure must be maintained for a sufficiently long period of time

3) During expiration, no critical time that would allow closure of lung units should pass.

"The Open Lung Concept" is safe only when used with a pressure-control mode of ventilation; its application with a volume-control mode may even be considered a professional error. During the process of opening the lungs the PaO_2 helps to guide this effort, because it is the only parameter that reliably correlates with the amount of lung tissue that participates in gas exchange (Figure 3).

The objective of an initial intervention is to open up the lung. Therefore, it is important to manage: the level of both set PEEP and autoPEEP (tPEEP), respiratory rate (RR), I/E ratio, and PIP. PEEP should be at least 15-20 cm H_2O, increase the RR by 10-20 to 25-30 breath/min to get a certain autoPEEP (expiratory time too short to allow emptying the lungs from expiratory collapse), it will keep open those alveoli which are to be recruited by the peak inspiratory pressures. Thereafter, at an I/E ratio which guarantees an end-inspiratory flow of zero, peak pressures are incremented until 15-20 cm H_2O for 20 seconds, until the point where further increases in peak pressure do not lead to any further increases in PaO_2. If the lung disease is inhomogeneous, which is almost always the case, there may be a large difference in the pressures needed to open collapsed alveoli; some have always been open while others need further increments in pressure to overcome their closed state. The absolute level of PaO_2 at this point reflects the number of functional alveolar units. The lung is called "open"; a set of airway pressures is then recorded as "opening pressure".

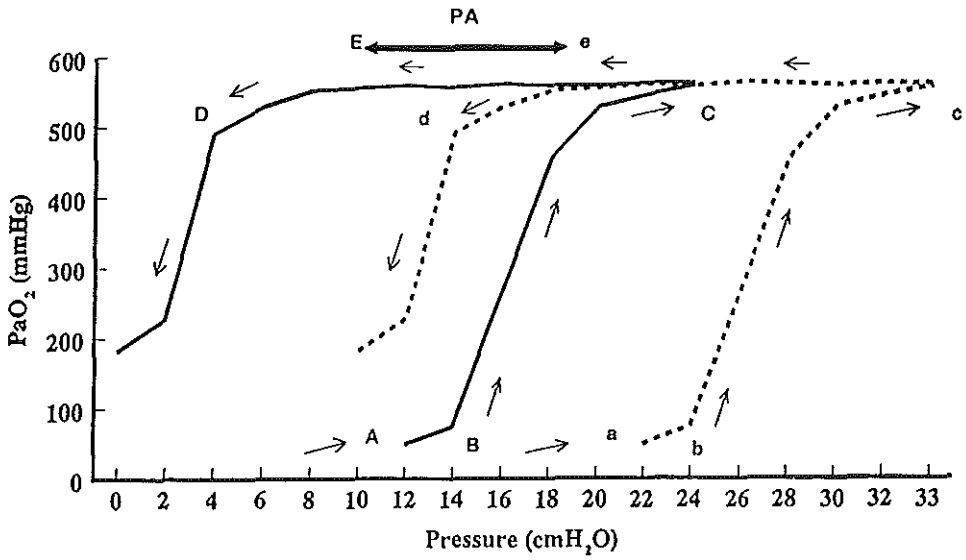

Figure 3. Results of a PaO_2 airway pressure curve from a rabbit suffering from acute respiratory failure. Arterial oxygen tensions are displayed as a function of PEEP (solid line) and peak pressure (dashed line). With a combination of PEEP (A)/PIP(a): at 12/22 cmH_2O, PaO_2 remains below 100 mmHg. The lung is closed. Raising PEEP (B)/ PIP(b) the lung starts to open at 14/24 cmH_2O. The lung is completely open at 23 (C)/33 (c) cmH_2O and PaO_2 is higher than 500 mmHg. Beyond 20/30 cmH_2O a few changes in PaO_2 occur. In the newly opened lung, pressures are decreased without changes in PaO_2 until the level of 6 (D)/16 (d) cmH_2O, the lung collapses and the PaO_2 drops. To re-open the lung, PEEP/PIP should be raised until 22/33 cmH_2O; to keep the lung open, PEEP should be kept ≥ 8 cmH_2O (E). PIP should be decreased to keep a pressure amplitude between PEEP and PIP (AP) normally between 8-12 cmH_2O (e).

The second intervention is to find the closing pressure. After achieving an "open lung", alveolar instability will occur only at low airway pressures. As a consequence, PIP and PEEP can be carefully reduced to a level which is safe, usually 2 cm H_2O above the closing pressure. During this phase the PaO_2 should, however, remain high despite the reduction in airway pressure, until the critical level of pressure is reached at which the least compliant parts of the lung start to collapse (closing pressure). Should this occur, then inspiratory pressure is immediately set back to the previously determined opening pressure and is kept there for a short period of 15-20 seconds (reopening). The lung tissue is yet again fully recruited, and the peak inspiratory pressures should then be reduced to 2-3 cm H_2O above the closing pressure.

After opening the lung and finding the closing pressures, the resulting pressure amplitude is minimised, normally between 8-12 cm H_2O and at the same time pulmonary gas exchange is maximised (keep open). In all open lung procedures performed with "The Open Lung Concept", hypercapnia never occurred, so permissive hypercapnia never had to be accepted. In the further course of the disease the ventilator can be adjusted carefully to any changes in the patient's respiratory condition. A reduction of the total level of support is generally possible after a successful alveolar re-expansion, within a few hours, especially if the patient is not mechanically ventilated for more than 48 hours.

It is important to realise that the lung has to be kept open at all times. Unnecessary disconnection and intrapulmonary suction have to be avoided. The fall in PaO_2 indicates that a renewed re-expansion manoeuvre has to be performed in the same way as previously described. Also, later in the weaning phase, one has to guarantee a sufficient level of PEEP to keep the entire lung open. This can be combined with a pressure support mode of ventilation to ensure adequate CO_2 removal. Both levels of support should be reduced according to the improvement of the patient's condition.

Alveolar re-expansion should almost always be possible during the first 48 hours on mechanical ventilation. Even if not all of the lung tissue may be fully re-expanded for gas exchange, as in consolidating pneumonia, this ventilatory strategy will prevent further damage to the re-aerated part of the lung.

Conclusion

The basic treatment principles are:

1. Open up the lung with high inspiratory pressures
2. Keep the lung open with tPEEP levels above the closing pressures
3. Maintain optimal gas exchange at the smallest possible and at the lower pressure amplitude to guarantee lung protection against VILI without circulatory compromise.

With the strict application of these principles, prophylactic treatment is aimed at preventing ventilator-induced lung injury and pulmonary complications. Ongoing and future clinical trials will have to provide further evidence for this treatment concept.

References

1. Ashbaugh DG, Bigelow DB, Petty TL, Levine BE. Acute respiratory distress in adults. Lancet 1967; 2: 319-323.
2. Ashbaugh DG, Petty TL, Bigelow DB, et al. Continuous positive pressure breathing (CPPB) in the adult respiratory distress syndrome. J Thorac Cardiovasc Surg 1969; 57:31-41.
3. Kirby R, Downs J, Civetta J, et al. High level positive end expiratory pressure (PEEP) in acute respiratory insufficiency. Chest 1975; 67:156-163.
4. Dreyfuss D, Saumon G. Ventilator-induced lung injury. Am J Respir Crit Care Med 1998; 157:294-323.
5. Lachmann B, Danzmann E, Haendly B, Jonson B. Ventilator settings and gas exchange in respiratory distress syndrome. In: Prakash O (ed). Applied physiology in clinical respiratory care. Nijhoff, 1982. The Hague pp 141-176.
6. Mead J, Takishima T, Leith D. Stress distribution in lungs: a model of pulmonary elasticity. J Appl Physiol 1970; 28: 596-608.
7. Von Neergaard K. Neue Auffassungen über einen Grundbegriff der Atemmechanik; Die Retraktionskraft der Lunge, abhängig von der Oberflächenspannung in den Alveolen. Z Ges Exp Med 1929; 66:373-394.
8. Lachmann B. Open up the lung and keep the lung open. Intensive Care Med 1992; 118:319-321.
9. Taskar V, John J, Evander E, et al. Surfactant dysfunction makes the lungs vulnerable to repetitive collapse and reexpansion. Am J Physiol 1997; 155:313-320.
10. Staub N, Nagano H, Pearce, Spring ML. Pulmonary edema in dogs, especially the sequence of fluid accumulation in lungs. J Appl Physiol 1967; 22:227-240.
11. Wegenius G. Wickerts CJ, Hedenstierna G. Radiological assessment of pulmonary edema. A new principle. Eur J Radiol 1994; 4:146-154.
12. Tremblay L, Valenya F, Ribeiro SP, Jingfang L, Slutsky AS. Injurious ventilatory strategies increase cytokines and c-fos m-RNA expression in an isolated rat lung model. J Clin Invest 1997; 99:944-952.
13. von Bethmann A, Brash F, Müler K Wndel A, Uhlig S. Prolonged hyperventilation is required for release of tumor necrosis factor-alpha but not IL6. Appl Cardiopulm Pathophysiol 1996; 6:171-177.
14. Verbrugge S, Sorm V, Van't Veen A, Mouton J, Gommers D, Lachmann B. Lung overinflation without positive end-expiratory pressure promotes bacteremia after experimental Klebsiella pneumoniae inoculation. Intensive Care Med 1998; 24:172-177.
15. Amato MBP, Barbas CSV, Medeiros DM, et al. Beneficial effects of the "Open Lung Approach" with low distending pressures in acute respiratory distress syndrome. Am J Respir Crit Care Med 1995; 152:1835-1846.
16. Froese A, Bryan Ch. High frequency ventilation. Am Rev Respir Dis 1987; 135:1363-1374.
17. Froese A. High-frequency oscillatory ventilation for adult respiratory distress syndrome: let's get it right this time. Crit Care Med 1997; 25:906-908.
18. HIFI study group. High-frequency oscillatory ventilation compared with conventional mechanical ventilation in the treatment of respiratory failure in preterm infants. N Engl J Med 1989; 320: 88-93.
19. Slutsky A. Consensus conference on mechanical ventilation January 28-30, 1993 at Northbrook, Illinois, USA. Part 1. Intensive Care Med 1994; 20:64-79, and part 2; 20: 150-162.
20. Brochard L, Roudot-Thoraval F, collaborative Group on VT reduction. Tidal volume (Vt) reduction in acute respiratory distress syndrome (ARDS); a multicenter randomized study. Am J Respir Crit Care Med 1997; 155 suppl: A505.
21. Stewart TE, Meade MO, Cook DJ, et al. Evaluation of a ventilation strategy to prevent barotrauma in patients at high risk for acute respiratory distress syndrome. N Engl J Med 1998; 338:355-361.
22. Amato MBP, Barbas CSV, Medeiros DM, et al. Effect of a protective ventilation strategy on mortality in the acute respiratory distress syndrome. N Engl J Med 1998; 338:347-54.
23. Fort P, Farmer C, Westerman J, et al. High-frequency oscillatory ventilation for adult respiratory distress syndrome- A pilot study. Crit Care Med 1997; 25:937-947.
24. Böhm SH, Vazquez de Anda GF, Lachmann B. "The Open Lung Concept". In: Vincent JL (ed). Yearbook of intensive care and emergency medicine. Springer-Verlag, 1998, pp 430-440.

Chapter 8

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 and mechanically ventilated with 100% oxygen, and randomly divided into two groups (n=6 each): The open lung group underwent 6 saline lavages at different ventilator settings that prevented alveolar collapse. The settings were (frequency/PIP/PEEP/I:E): 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 6 saline lavages with settings 30/26/6/1:2. After the lavages, PIP and PEEP were increased in this group by 2 cm H_2O each for the remaining study period. An additional group of 6 animals was sacrified 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 h study period, pressure-volume curves were constructed from which total lung capacity at a distending pressure of 35 cm H_2O (TLC_{35}) was determined. Subsequently, total lung volume at a distending pressure of 5 cm H_2O (V_5) was determined, followed by broncho-alveolar lavage (BAL).

Results: In the ventilated control group, Pao_2, V_5 and TLC_{35} were significantly decreased, and protein concentration of BAL was significantly increased compared to 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 BAL were comparable with the healthy controls.

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

Introduction

In the 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 in excess of 50% [2]. New strategies that are currently under clinical investigation include 'open lung' ventilation strategies, which aim to re-open collapsed but recruitable lung units and to keep them open by applying a sufficiently high positive end-expiratory pressure (PEEP) [3-6]. Recent studies show that such strategy improves oxygenation in ARDS patients, and provide the first results that indicate that the technique 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]. Since 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, 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 a ventilation strategy with a high PEEP and a high frequency was applied 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 (NIH publication No. 86-23, revised 1985) were followed.

The study was performed in eighteen adult male Sprague-Dawley rats (body weight 280-340 g). After induction of anesthesia 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) set at 12 cm H_2O, positive end-expiratory pressure (PEEP) set at 2 cm H_2O, and inspiratory/expiratory (I/E) ratio of 1:2. Anesthesia 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 anesthesia 6 animals were sacrificed and served as healthy, non-ventilated controls. The remaining animals subsequently underwent 6 whole lung lavages with warm saline (37°C, LAV 1 to 6), according to Lachmann et al. [14]. During lavage, different ventilator settings were used in both groups, that are shown in table 1. After lavage, PIP and PEEP 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 h (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.

Table 1. Ventilator settings in the two ventilated groups.

	Ventilated control group				**Open lung group**			
	LAV 1	LAV 2-3	LAV 4-6	> LAV 6	LAV 1	LAV 2-3	LAV 4-6	> LAV 6
PIP (cmH_2O)	26	26	26	27	26	28	33	33
PEEP (cmH_2O)	6	6	6	8	6	10	15	15
Frequency	30	30	30	30	100	100	100	100
I/E	1/2	1/2	1/2	1/2	½	1/1	1/1	1/1

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

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 a blood gas analyser (ABL 505, Radiometer, Copenhagen, Denmark).

After the animals were sacrificed by administering an overdose of pentobarbital, static pressure-volume curves (P/V curves) were recorded 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 (Validyne model DP 45-32, Validyne Engineering Co., Northridge, CA, USA), and pressures were recorded on a polygraph (Grass model 7B, Grass Instrument co., Quincy, Mass., USA). The lungs were first quickly inflated with 100% nitrogen (N_2) from the syringe to an airway pressure of 35 cm H_2O, which was maintained for 5 seconds, followed by deflation to an airway pressure of 0 cm H_2O. Then, the lungs were re-inflated with N_2 from the syringe in steps of 0.5 ml until an airway pressure of 35 cm H_2O was reached. For each step, the bolus of N_2 was administered quickly, and was followed by a 5 seconds pause to allow pressure equilibration. After this, the lungs were deflated likewise, until an airway pressure of 0 cm H_2O was reached. The volume of N_2 left in the syringe was recorded. From the P/V curves total lung capacity (TLC_{35}) was determined, which was defined as lung volume above collapsed volume at a distending pressure of 35 cm H_2O, and maximal compliance (C_{max}) which was calculated from the steepest part of the deflation limb.

After construction of the P/V curves, the lungs were removed *en bloc* and weighted, and lung volume at an airway pressure of 5 cm H_2O (V_5) was determined by fluid displacement. A positive pressure of 5 cm H_2O was choosen 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 functional residual capacity (FRC).

After assessment of lung mechanics, the lungs were lavaged with saline-$CaCl_2$ 1.5 mmol/liter. 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 [17]. The protein concentration of the BAL fluid was determined using the Bradford method (Bio-Rad protein-assay, Munich, Germany) [18].

Statistical analysis was performed using the Instat statistical package. For bloodgases 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

In the lavage fluid from the 6 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 vs 5.28 ± 1.38 μmol), respectively.

Blood gas values before lavage were comparable for both ventilated groups (Table 2). After 6 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 to 45 torr [4.7 to 6 kPa]) in the open lung group (Table 2). During the 4-hour 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 to 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 to 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 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). There was no difference between both ventilated groups in total lung volume at a distending pressure of 5 cm H_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).

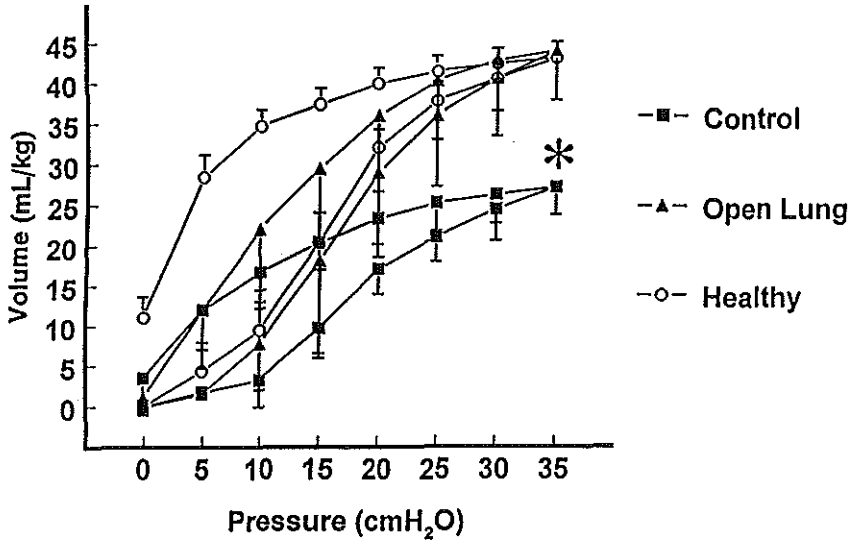

Figure 1. Pressure-volume curves, mean ± SD. Volume (mL/kg) is lung volume above functional residual capacity. *$p<0.01$ vs. healthy control group. C_{max} was decreased in the ventilated control group compared to the open lung group (5.4 ± 1.0 versus 8.6 ± 2.8 mL/cmH_2O/kg, $p<0.05$), but in both ventilated groups C_{max} was significantly decreased compared to the healthy control group (13.4 ± 1.1 mL/cmH_2O/kg, $p<0.001$ vs ventilated control, $p<0.05$ vs open lung group). TLC_{35} = total lung capacity at a distending pressure of 35 cmH_2O. C_{max} = maximal compliance.

Table 2. Pao_2 and $Paco_2$ in the two ventilated groups (mean ± SD, torr)

	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 h	109.4 ± 120.5§¶	599.3 ± 36.7	62.7 ± 19.2§¶	40.0 ± 8.9
2 h	104.8 ± 125.5§¶	602.9 ± 30.7	65.5 ± 20.7§¶	43.9 ± 11.1
3 h	100.5 ± 112.0§¶	598.2 ± 37.3	66.9 ± 22.0§¶	44.1 ± 11.6
4 h	100.8 ± 114.7§¶	600.5 ± 41.6	68.8 ± 24.6§¶	44.0 ± 9.8

To convert torr to kPa, multiply the value by 0.1333
h, hour. § $p<0.05$ vs. open lung group; ¶ $p<0.05$ vs. before lavage

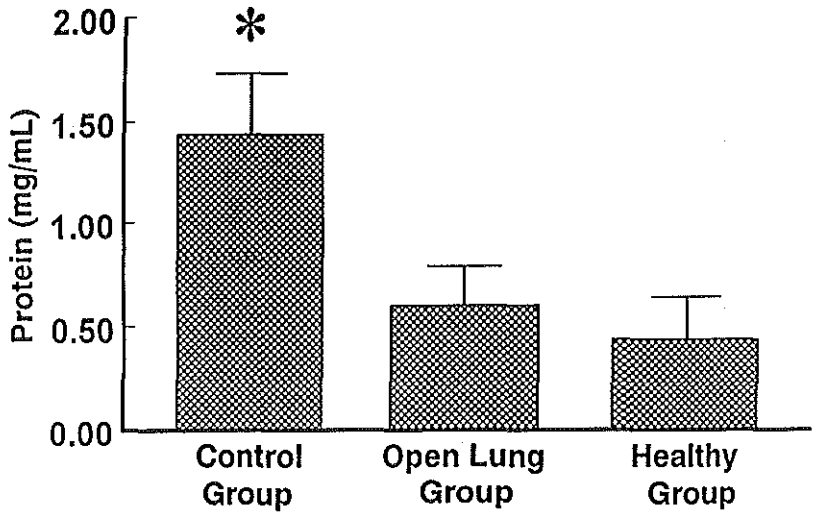

Figure 2. Mean protein concentration ± SD (mg/mL) of the BAL fluid of the three study groups. * $p<0.05$ vs. healthy control group.

The concentration of protein in the BAL 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 BAL fluid, which was 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 non-active and active surfactant components (SA/LA ratio) was significantly increased in both ventilated groups compared to the healthy control group ($p<0.001$) (Table 4).

Table 3. V_5 (mean ± SD) and lung weight (mean ± SD, g).

	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*

* $p<0.05$ vs. healthy control group. V_5= total lung volume at a distending pressure of 5 cmH_2O.

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

	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 *

*$p<0.05$ vs. Healthy control group

Discussion

In this study, the lungs were defined to be open when Pao_2/Fio_2 was above 500 torr [67 kPa], indicating that there is no intra-pulmonary shunting. We have previously shown that surfactant-deficient lungs can be opened and kept open when the open lung concept that applies a high PEEP and high frequency is used [19,5,6]. These same settings were therefore used 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 to the healthy control group. In the ventilated control group, however, all these parameters deteriorated. Compared to healthy controls, lung volume at a distending pressure of 5 cm H_2O (V_5), which was taken to be FRC, was significantly decreased only in the ventilated control group, although no significant differences in V_5 were found between the open lung and ventilated control groups.

An important determinant of protein transport across the alveolar-capillary membrane is integrity of the alveolar epithelium. Repeated alveolar collapse has been shown to compromise the integrity of the alveolar epithelium due to the occurrence of shear forces [20,21]. Application of "the open lung concept" in an animal model after lung lavage was previously demonstrated to decrease protein leakage [19], [for review see (21)]. In addition, application of PEEP 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 PEEP of 15 cm H_2O.

No differences in surfactant quantity and quality (SA/LA ratio) were found between the open lung group and the ventilated control group (Table 4). It has been demonstrated that the alveolar area cycling, which is dependent on the pressure difference between inspiration and expiration, is responsible for conversion of active LA into non-active 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, significant differences between both ventilated groups were found in lung mechanics (TLC_{35} and maximal compliance). We attribute this discrepacy between lung mechanics and surfactant parameters to the difference in protein leakage, since it has been established that plasma proteins inhibit surfactant function in a dose-dependent way [23]. We therefore 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 application of a high inspiratory airway pressure for a brief period. 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; r=alveolar radius) it follows that the pressure necessary to keep alveoli open, and the pressure difference to induce volume changes in the alveoli, is 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 PEEP and smaller pressure difference, and hence a lower PIP, than prior to alveolar recruitment. If PEEP is kept above the critical closing pressure, alveolar collapse will not occur and reperated application of higher PIP 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 PEEP in high-risk patients had no effect on the incidence of ARDS. However, in that study a PEEP of 8 cmH_2O was used, and PEEP was removed for eight minutes 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. Also in this study, which

reported no reduction in mortality and possibly an increase in morbidity, an average PEEP of <10 cm H_2O was used. We hypothesize that the PEEP levels used in these later studies were not high enough to prevent alveolar collapse, which may have resulted in an increase in protein leakage. This hypothesis is supported by results from Gattinoni et al., who, in a computed tomography study of ventilated ARDS patients, showed that re-inflated lung tissue could only be kept open at end-expiration at PEEP levels of 15 cmH_2O and higher [26]. This might explain the results by Kirby et al. [27] and Douglas et al. [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, since 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.

References

1. Lewis JF, Jobe AH: Surfactant and the Adult Respiratory Distress Syndrome. *Am Rev Resp Dis* 1993; 147: 218-233
2. Artigas A, Carlet J, Le Gall JR, et al: Clinical presentation, prognostic factors and outcome of ARDS in the European Collaborative Study (1985-1987): a preliminary report. *In:* Adult respiratory distress syndrome. Zapol WM, Lemaire F. (Eds). New York, Marcel Dekker, 1991, pp 37-64
3. Lachmann B: Open up the lung and keep the lung open. *Intensive Care Med* 1992; 18: 319-321
4. Jonson B. Positive airway pressure: Some physical and biological effects. In: Applied Physiology in Clinical Respiratory Care, Martinus Nijhoff Publishers, 1982
5. Lachmann B, Danzmann E, Haendly B, et al: Ventilator settings and gas exchange in respiratory distress syndrome. *In:* Applied Physiology in Clinical Respiratory Care. Prakash O, (Ed). The Hague, Nijhoff, 1982, pp 141-176
6. Lachmann B, Jonson B, Lindroth M, Robertson B. Modes of artificial ventilation in severe respiratory distress syndrome: Lung function and morphology in rabbits after wash-out of alveolar surfactant. *Crit Care Medicine* 1982; 10: 724-732.
7. Kesecioglu J, Tiboel D, Lachmann B: Advantages and rationale for pressure control ventilation. *In:* Yearbook of Intensive Care and Emergency Medicine. Vincent JL (Ed). Berlin-Heidelberg-New York, Springer-Verlag, 1994, pp 524-533
8. Amato MBP, Barbas CSV, Medeiros DM, et al: Beneficial effects of the "open lung approach" with low distending pressures in acute respiratory distress syndrome *Am J Resp Crit Care Medicine* 1995; 152: 1835-

1846
9. Amato MBP, Barbas CSV, Medeiros DM, et al: Effect of a protective-ventilation strategy on mortality in the acute respiratory distress syndrome. *N Eng J Med* 1998; 338: 347-354
10. Muscedere G, Mullen JBM, Gan K, et al: Tidal ventilation at low airway pressures can augment lung injury. *Am J Resp Crit Care Medicine* 1994; 149: 1327-1334
11. Dreyfuss D, Soler P, Basset G, et al: High inflation pressure pulmonary edema: respective effects of high airway pressure, high tidal volume, and positive end-expiratory pressure. *Am Rev Resp Dis* 1988; 137: 1159-1164.
12. Verbrugge SJC, Böhm SH, Gommers D, et al: Surfactant impairment after mechanical ventilation with large alveolar surface area changes and the effects of positive end-expiratory pressure. *Br J Anaesth* 1998; 80: 360-364
13. Verbrugge SJC, Vazquez de Anda GF, Gommers D, et al: Exogenous surfactant preserves lung function and reduces alveolar Evans blue dye influx in a rat model of ventilation induced lung injury. *Anesthesiology* 1998; 89: 467-474
14. Gregory TJ, Longmore WJ, Moxley MA, et al: Surfactant chemical composition and biophysical activity in acute respiratory distress syndrome. *J Clin Invest* 1991; 88: 1976-1981
15. Lachmann B, Robertson B, Vogel J: In vivo lung lavage as an experimental model of the respiratory distress syndrome. *Acta Anaesthesiol Scand* 1980; 24: 231-236
16. van Daal GJ, Bos JAH, Eijking EP, et al: Surfactant replacement therapy improves pulmonary mechanics in end-stage influenza A pneumonia in mice. *Am Rev Resp Dis* 1993; 145: 859-863
17. Veldhuizen RAW, Inchley K, Hearn SA, et al: Degradation of surfactant associated protein B (SP-B) during in vitro conversion of large into small surfactant aggregates. *Biochem J* 1993; 295: 141-147
18. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Ann Biochem* 1976; 72: 248-254.
19. Hartog A, Vazquez de Anda GF, Gommers D, et al: Comparison of exogenous surfactant therapy, mechanical ventilation with high end-expiratory pressure and partial liquid ventilation in a model of acute lung injury. *Br J Anaesth* 1999; 82: 81-86
20. Mead J, Takishima T, Leith D: Stress distribution in lungs: a model of pulmonary elasticity. *J Appl Physiol* 1970; 28: 596-608
21. Dreyfuss D, Saumon G: Ventilator-induced lung injury: Lessons from experimental studies. *Am J Resp Crit Care Med* 1998; 157: 294-323
22. Permutt S: Mechanical influences on water accumulation in the lungs. *In:* Pulmonary edema. Clinical Physiology series. Fishman AP, Renkin EM, (Eds). Bethesda, American Physiology Society, 1979, pp 175-193
23. Lachmann B, Eijking EP, So KL, et al: In vivo evaluation of the inhibitory capacity of human plasma on exogenous surfactant function. *Intensive Care Med* 1994; 20: 6-11
24. Pepe PE, Hudson LD, Carrico CJ: Early application of positive end-expiratory pressure in patients at risk for the adult respiratory distress syndrome. *N Eng J Med* 1984; 311: 281-286
25. Steward TE, Meade MO, Cook DJ, et al: Evaluation of a ventilation strategy to prevent barotrauma in patients at high risk for acute respiratory distress syndrome. *N Eng J Med* 1998; 338: 355-361
26. Gattinoni L, Pelosi P, Crotti S, et al: Effects of positive end-expiratory pressure on regional distribution of tidal volume and recruitment in adult respiratory distress syndrome. *Am J Resp Crit Care Med* 1995; 151: 1807-14
27. Kirby RR, Downs JB, Civetta JM, et al: High Level positive end-expiratory pressure (PEEP) in acute respiratory insufficiency. *Chest* 1975; 67: 156-163
28. Douglas ME, Downs JB. Pulmonary function following severe acute respiratory failure and high levels of positive end-expiratory pressure. *Chest* 1977; 71: 18-23

Chapter 9

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

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Summary

Background: Changes in pulmonary edema infiltration and surfactant after intermittent positive pressure ventilation with high peak inspiratory lung volumes have been well described. To further elucidate the role of surfactant changes, the authors tested the effect of different doses of exogenous surfactant preceding high peak inspiratory lung volumes on lung function and lung permeability.

Methods: Five groups of Sprague-Dawley rats (n = 6 per group) were subjected to 20 min of high peak inspiratory lung volumes. Before high peak inspiratory lung volumes, four of these groups received intratracheal administration of saline or 50, 100, or 200 mg/kg body weight surfactant; one group received no intratracheal administration. Gas exchange was measured during mechanical ventilation. A sixth group served as nontreated, nonventilated controls. After death, all lungs were excised, and static pressure-volume curves and total lung volume at a transpulmonary pressure of 5 cm H_2O were recorded. The Gruenwald index and the steepest part of the compliance curve (C_{max}) were calculated. A bronchoalveolar lavage was performed; surfactant small and large aggregate total phosphorus and minimal surface tension were measured. In a second experiment in five groups of rats (n = 6 per group), lung permeability for Evans blue dye was measured. Before 20 min of high peak inspiratory lung volumes, three groups received intratracheal administration of 100, 200, or 400 mg/kg body weight surfactant; one group received no intratracheal administration. A fith group served as nontreated, non-ventilated controls.

Results: Exogenous surfactant at a dose of 200 mg/kg preserved total lung volume at a pressure of 5 cm H_2O, maximum compliance, the Gruenwald Index, and oxygenation after 20 min of mechanical ventilation. The most active surfactant was recovered in the group that received 200 mg/kg surfactant, and this dose reduced minimal surface tension of bronchoalveolar lavage to control values. Alveolar influx of Evans blue dye was reduced in the groups that received 200 and 400 mg/kg exogenous surfactant.

Conclusions: Exogenous surfactant preceding high peak inspiratory lung volumes prevents impairment of oxygenation, lung mechanics, and minimal surface tension of brochoalveolar lavage fluid and reduces alveolar influx of Evans blue dye. These data indicate that surfactant has a beneficial effect on ventilation-induced lung injury.

Introduction

The development of pulmonary edema and alveolar flooding in healthy rats after overinflation of the lungs with peak inspiratory pressures of 45 cm H_2O without positive end-expiratory pressure (PEEP) were first demonstrated by Webb and Tierney [1] and was later confirmed by Dreyfuss et al. [2]. The main determinant for development edema is the peak inspiratory lung volume [3]. Experiments with thoracic restriction in this rat model have clearly shown that high peak inspiratory pressures themselves when not accompanied by high peak inspiratory lung volumes (HIPPV), do not induce lung injury [3].

Peak inspiratory overstretching by overinflation of the lungs alone, however, can not explain ventilation-induced lung injury, because 10 cm H_2O of PEEP at the same degree of overdistension (e.g. the same peak inspiratory pressure) in this animal model has been shown to reduce permeability edema and to prevent lung parenchymal injury almost completely [2,3]. One study attributed this reduction in permeability edema by PEEP to a decrease in the pulmonary capillary hydrostatic pressure [4], which reduces formation of edema when the pressure balance between (1) plasma colloid oncotic pressure, (2) capillary hydrostatic pressure, (3) interstitial oncotic pressure and (4) alveolar surface tension at the alveolo-capillary barrier is shifted away from the alveolar direction [5]. A recent study by our group in the same rat model, however, showed a reduction in the amount of surface-tension reducing surfactant components after 20 min of overinflation of the lungs without PEEP. Impairment of the surfactant system could be prevented with 10 cm H_2O of PEEP [6] which prevented the conversion of surface active tubular myelin-like forms of surfactant (large aggregates) into nonactive components that represent small vesicular structures (small aggregates). Gross and Narine were the first to show that conversion of active into nonactive surfactant subfractions is dependent on cyclic changes in surface area *in vitro* [7]. Studies by Veldhuizen *et al. in vivo* have confirmed that conversion is dependent on the change in alveolar surface area associated with mechanical ventilation [8]. These studies suggest that the reduction in alveolar flooding by PEEP is partially caused by its preservation of the surfactant system suggesting that ventilation-induced surfactant changes play a role in the development of alveolar flooding.

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

Materials and methods

The study protocol was approved by the local animal committee, and the care and handling of the animals conformed with the principles approved by the Council of the American Physiologic Society. Sixty-six adult male Sprague Dawley rats (body weight 290-350 grams) were used.

Studies on the effects of exogenous surfactant

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

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

Thirty minutes after tracheotomy, the animals were reanesthetised whit gaseous anesthesia (see previous description) and a polyethylene catheter (0.8 mm OD) was inserted into a carotid artery. After this surgical procedure, gaseous anesthesia was discontinued and anesthesia was replaced with 60 mg/kg pentobarbital sodium given intraperitoneally (Nembutal®; Algin BV, Maassluis, the Netherlands) during the remainder of the experiment. Muscle relaxation was attained with 2 mg/kg pancuronium bromide given intramuscularly (Pavulon®; Organon Technika, Boxtel, the Netherlands). After muscle relaxation, the animals were connected for 20 min to a ventilator (Servo Ventilator 300, Siemens-Elema, Solna, Sweden) set in a pressure-controlled mode at a peak inspiratory pressure of 45 cm H_2O without PEEP, a frequency of 25 breaths/min, an I/E ratio of 1:2; and a fractional inspired oxygen tension of 1.0.

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

After 20 min of mechanical ventilation, all animals were killed with an overdose of pentobarbital sodium through the penile vein. A sixth group of animals was killed immediately after tracheotomy in an identical way. Static pressure-volume diagrams were then recorded using conventional techniques [10]. For these measurements the thorax and diaphragm were opened. The animals were placed into a volume-and temperature-constant body box, and the lungs were reexpanded with pure nitrogen up to a pressure of 35 cm H_2O and subsequently deflated again. This procedure was performed to reopen lung areas that became atelectatic after this surgical procedure. The lungs were then reinflated immediately, starting from a pressure of 0 cm H_2O and proceeding in steps of 1 cm H_2O up to an intra-alveolar pressure of 35 cm H_2O and subsequently deflated in steps of 1 cm H_2O. This was done by changing the PEEP level on the ventilator while in continuous positive airway pressure mode (Servo Ventilator 300). Pressure changes in the body box were recorded (Validyne model DP 45-32, Validyne Engineering Co., Northridge, CA, USA) at a sampling rate of 10 Hz using a (12-bit) analog-to-digital converter (DAS 1800, Keithley MetraByte, Taunton, MA) and stored in a computer. With the rat still in the body box, the pressure signals from the bodybox were calibrated for known volume changes immediately after pressure- volume recordings, by injection of known volumes of air into the body box, using a precise syringe. The maximal compliance (C_{max}) was defined as the steepest part of the pressure-volume deflation curve, and was determined separately for each animal [9]. The Gruenwald index, defined as $(2 \cdot V_5 + V_{10}) / 2 \cdot V_{max}$, where V_5, V_{10} and V_{max} are the lung volumes higher than functional residual capacity at transpulmonary pressures of 5, 10 and 35 cm H_2O was calculated [11]. Functional residual capacity was estimated by measuring total lung volume at a transpulmonary pressure of 5 cm H_2O (V_5) as previously described [12]. For this measurement the lungs and the heart were removed from the thorax. After dissection from the heart, the lungs were reexpanded with nitrogen up to a pressure of 35 cm H_2O to reopen lung areas that became atelectatic during excision. The lungs were then left to deflate against a positive pressure of 5 cm H_2O, which was chosen to compensate for the loss of negative intra-thoracic pressure. The total weight of lungs (W) was registered and the lungs were then immersed in saline at a preset depth to measure the upward force (F). According to the principle of Archimedes, this force is caused by fluid displacement equal to the volume of the lungs.

V_5 was then calculated as 0.99•F -0.94•W [12].

Thereafter, the lungs were lavaged with saline/1.5 mM $CaCl_2$ (30 ml/kg) five times. The percentage recovered lung lavage fluid was calculated. The obtained lavage fluid was first centrifuged at 400 X *g* (Beckman GPR, Beckman Instruments Inc., Palo Alto, CA) for 10 min at 4°C to remove cells and cellular debris. The supernatant of this 400 X *g* fraction (crude lavage) was then centrifuged at 40,000 X *g* for 15 min at 4°C (Beckman L8-70M) to separate a surface-active surfactant pellet (large aggregates) from a non-surface-active supernatant fraction (small aggregates) [13]. The large aggregates were resuspended in 2 ml conversion buffer (0.15 M NaCl/10mM Tris/1 mM $CaCl_2$/0.1 mM EDTA, *p*H 7.4) [11]. Total phosphorus of the small and large aggregates was determined by extraction of phospholipid [14] followed by subsequent phosphorus analysis [15]. Twenty microliters of crude lavage and the resuspension of the active surfactant part were used for biophysical analysis of minimal surface tension after 50 cycles on a pulsating bubble surfactometer (PBS; Electronetics Corporation, Tonowanda, New York) as described by Enhorning [16]. This apparatus records pressure across the surface of a bubble, expanded in the sample fluid and communicating with ambient air. The bubble pulsated within a sample chamber at a frequency of 20 pulsations/min between defined radius limits. The sample temperature was set at 37 °C. From the known pressure gradient across the bubble surface and the minimal bubble radius, the minimal surface tension was calculated according to the law of LaPlace ($P = 2\gamma/r$).

Permeability studies

To further elucidate the exact mechanism of the effect of surfactant in HIPPV shown in the first part of the study, a second set of studies was performed. Thirty rats were randomly divided into five groups of six rats each and tracheotomized as described earlier. Identical to the way described earlier, three groups received exogenous surfactant at a dose of 100, 200, or 400 mg/kg body weight (groups S100, S200, and S400, respectively) and one group did not receive any intratracheal instillation. After recovery from anesthesia and spontaneous breathing, a carotid artery was cannulated, and the animals were connected to the ventilator to receive mechanical ventilation. A fifth group of animals served as non-treated, nonventilated healthy controls (control group).

Vascular permeability was quantified by the extravasation of Evans blue dye over 19 min (Sigma, Steinheim, Germany) which correlates well with the extravasation of radiolabeled albumin at high rates of plasma leakage [17]. The dye (30 mg/ml) was filtered with a 0.22 μm Millipore filter

(MILLEX-GV; Millipore Products Division, Bedford, MA) before use [18]. One minute after starting mechanical ventilation and after tracheotomy in the control group, Evans Blue dye (30 mg/kg) was injected through the penile vein. Nineteen minutes after injection of Evans Blue dye, the lungs were lavaged once with warm saline (30 ml/kg). The lavage was centrifuged at 400 X *g* to remove cells and cellular debris. The high amount of surfactant dissolved in the broncho-alveolar lavage (BAL) was shown to disturb photospectrometric measurements of concentration of Evans Blue dye. Pilot experiments (not reported) measuring the extinction of the chloroform layer at 620-nm at various concentrations of Evans Blue dye in saline after Bligh Dyer extraction, demonstrated that Evans Blue dye does not dissolve in chloroform but completely dissolves in a water-methanol phase. Therefore, 1 ml BAL was used for extraction of phospholipid according to Bligh and Dyer [14] to separate phospholipids in a chloroform layer from Evans Blue dye in the water-methanol phase.

After BAL, the tissue content of Evans Blue dye was determined by perfusing the lung circulation *via* the pulmonary artery with 20 ml warm saline (37 °C) to remove intravascular dye. For this purpose, the aorta was cut at the level of the diaphragm and the left auriculum was removed from the heart before lung vascular perfusion. Evans Blue dye was extracted from the lungs by incubation at room temperature for 3 days in 12 ml formamide (Sigma) in stoppered tubes [18].

The absorbance of water-methanol extracts of Evans Blue dye from BAL and of the formamide tissue extracts of Evans Blue dye were determined against a water-methanol and formamide blank at 620-nm wavelength and by interpolation from a standard curve of Evans Blue dye in the range 0.5-10 μg/ml in water-methanol and formamide, respectively [18]. It was demonstrated (data not reported) that after Bligh Dyer extraction there are no substances in the BAL of animals with lung edema not given Evans Blue dye that affect the absorbance for water-methanol at 620-nm. The total amount of Evans Blue dye milligrams recovered from the BAL and in the tissue was calculated.

Statistical analysis

Intergroup comparisons were analyzed with ordinary analysis of variance (ANOVA). Intragroup comparisons were analyzed with repeated measures ANOVA. If ANOVA resulted in a probability value <0.05, a Student-Newman-Keuls *post-hoc* test was performed. All data are

reported as mean ± SD.

Results

Table 1 gives data on arterial oxygen and carbon dioxide tensions over time in the five ventilated groups in the studies on lung function. After 20 min, oxygenation decreased in the two groups that did not receive exogenous surfactant. Oxygenation was preserved over time in the group that received 200 mg/kg body weight surfactant.

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

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

Table 1. Data on Blood Gas Tension (mmHg) of the Five Ventilated Groups during the Study Period in the Lung Function Experiments.

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

Values are mean ± SD. Intergroup and intragroup comparisons ANOVA with Student-Newman-Keuls post-hoc test if ANOVA $p < 0.05$.

* Statistical significance *versus* $t = 20$ min.

† Statistical significance *versus* group S200.

‡ Statistical significance *versus* group S100.

§ Statistical significance *versus* group 45/0.

¶ Statistical significance *versus* $t = 10$ min.

Table 2. Recovery of BAL Fluid and Postmortem Data for C_{max}, Gruenwald index, V_5, Total Lung Weight, Total Phosphorus of Small Aggregates (SA) and Large Aggregates (LA) and Minimal Surface Tension (min. surf.Tens.) of Crude Lavage and Large Aggregate Resuspension in the Lung Function Experiments.

Group	Control	45/0	saline	S50	S100	S200
Recovery BAL fluid (%)	74.6±4.7	76.6±4.2	73.3±2.9*	71.6±1.3*	75.6±4.7	81.3±6.6
C_{max} (ml/kg)	3.9±0.7	2.3±0.5	2.5± 0.7†	2.9±0.6*	3.1±0.6	4.2±0.9§
Gruenwald Index	0.47±0.13	0.25±0.08*†	0.28±0.10*†	0.23±0.09*†	0.37±0.07	0.52±0.21
V_5 (ml)	18.2±4.1	6.0±2.5†	4.1±2.4*†‡	7.4 ±3.2	9.2±2.9§	15.4±3.5§
SA (mmol)	0.8±0.4	1.3±0.3	1.2±0.2*‡	3.0±0.9*†‡	4.9±1.3†§	5.8±2.7†§
LA (mmol)	3.0 ±1.6	2.0±0.7*	2.6±1.0*	6.3±1.6§	7.7±2.5*	17.0±6.5†§
Min. surf. Tens. crude (mN/m)	28.5±6.5	40.0±1.5*†	46.1±0.7*†‡	39.5±6.8*†	37.7±7.7*†	29.7±2.6
Min. surf. Tens. LA (mN/m)	24.8±2.9	38.4±5.9†	45.0±3.2*†‡¶	14.8±10*†‡§	6.2±7.9†§	1.8±1.5†§

Values are mean ± SD. Inter-group comparisons ANOVA with Student-Newman-Keuls post-hoc test if ANOVA $p < 0.05$.

* Statistical significance *versus* group S200.

† Statistical significance *versus* group control.

‡ Statistical significance *versus* group S100.

§ Statistical significance *versus* 45/0.

¶ Statistical significance *versus* group S50.

Table 3. Data on blood Gas Tension (mmHg) and Permeability Indices in the Five Different Groups in the Permeability Studies.

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

Values are mean ±SD. Intergroup and intragroup comparisons ANOVA with Student-Newman-Keuls post hoc test if ANOVA $P < 0.05$

* Statistical significance *versus* $t = 1$ min.

† Statistical significance *versus* group control.

‡ Statistical significance *versus* group 45/0.

§ Statistical significance *versus* group S100

Discussion

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

Changes in permeability of the alveolocapillary barrier to protein have been attributed to epithelial stretching. Equivalent pore radii indicate that the epithelium, rather than the endothelium, is primarily responsible for restricting solute transport from the capillaries across the alveolocapillary membrane into the alveolus [19,20]. As the epithelium is progressively stretched there is an opening of water-filled channels between alveolar cells [21,22].

Important evidence regarding the role of capillary hydrostatic pressure in inducing edema in the HIPPV rat model comes from the effect of 10 cm H_2O PEEP, which was shown to reduce edema infiltration [1,3]. This effect was attributed to hemodynamic alterations resulting from PEEP, which reduce filtration pressure over the alveolocapillary membrane [4]. Infusion of dopamine to correct the decrease systemic arterial pressure that occurs with PEEP ventilation was shown by Dreyfuss and Saumon to abolish partially the reduction in pulmonary edema induced by PEEP [4]; however, this effect was only partial, and because pulmonary artery pressure was not recorded in their study it cannot be excluded that the transpulmonary filtration pressure after infusion of dopamine, was higher than in the animals ventilated without PEEP [4]. Therefore, the possibility that other factors contribute to the development of intraalveolar edema cannot be excluded.

Loss of surfactant function with an increase in surface tension at the air-liquid interface on the alveolar walls has been shown to direct the net driving force across the alveolocapillary membrane to the alveolar side, resulting in accumulation of intra-alveolar fluid and protein [5,23,24]. Based on such observations, a recent study by our group postulates a different mechanism for the effect of PEEP on the reduction of lung permeability edema in HIPPV [6]. It describes the mechanisms of surfactant impairment after HIPPV, which include surfactant displacement from the alveolar air-liquid interface into the small airways and increased conversion of active into non-active surfactant subfractions, and it shows that PEEP reduces such HIPPV-induced surfactant impairment [6]. Surfactant preservation by PEEP reduces the contribution of

surface tension to fluid and particle transport across the alveolocapillary barrier, which is a different explanation for the reduction in permeability edema induced by PEEP [6]. If this mechanism is valid, then exogenous surfactant preceding HIPPV should be able to reduce permeability edema after HIPPV. The current study shows that this is the case and that doses of 200 and 400 mg/kg body weight of exogenous surfactant are able to reduce intraalveolar influx of Evans blue dye. This is a substantial amount given the normal total surfactant phospholipid pool size of 10 mg/kg body weight in rats [25]. The current data demonstrate that, although peak inspiratory epithelial pore overstretching and capillary hydrostatic pressure are important determinants of permeability edema, surfactant actively stabilizes the fluid balance in the lung and protects the lung from permeability edema at the level of the BAL-accessible space. Such findings are consistent with recent findings in a model of mild surfactant pertubation by dioctyl sodium sulfosuccinate, which was shown to initiate protein infiltration [26], and previous findings on the rate-limiting effect of supraphysiological amounts of (exogenous) surfactant on solute permeability of normoventilated rabbits [27]. The contribution of surface tension to fluid and particle transport across the alveolocapillary barrier appears to be most prominent on transudation across the alveolo-capillary barrier, as demonstrated by the reduced Evans Blue dye in the BAL accessible space, and appears to be less prominent on exudation from the capillary, evidenced by the equal amount of Evans Blue dye recovered from the tissue.

Once protein infiltration has started, plasma-derived proteins dose-dependently inhibit surfactant [28,29], resulting in a vicious circle of more inlux of fluid and protein as a result of increased surface tension with further surfactant inactivation by plasma-derived proteins and more destabilisation of the small airways. In the current study, exogenous surfactant at a dose of 200 mg/kg preceding HIPPV prevented a decrease in arterial oxygenation after 20 min of HIPPV and preserved the Gruenwald index, C_{max} and V_5 at control values. These findings indicate that exogenous surfactant preceding HIPPV is able to preserve normal end-expiratory lung stability even after 20 min of HIPPV. This end-expiratory alveolar stabilization attributable to exogenous surfactant is likely caused by a more advantageous protein-phospholipid ratio, which is a critical factor for normal surfactant function [29]. There are two reasons for this more beneficial ratio. First, there was a higher amount of surfactant present in the BAL-accessible space, as evidenced by the higher amount of total phosphorus of surface-active large and nonsurface-active small aggregates in

the animals given exogenous surfactant (Table 2). Second, the reduction in surface tension over the alveolocapillary barrier towards normal levels by exogenous surfactant reduced influx of protein. The large aggregate resuspension of the group given 200 mg/kg exogenous surfactant showed more potential to reduce surface tension than in nonventilated controls (Table 2). When the influence of surfactant inhibiting proteins in the BAL-accessible space was included, however, the net surface tension-reducing potential was normalized to the level of controls, as evidenced by the normalization of the minimal surface tension of the crude lavage on the pulsating bubble surfactometer in group S200 (Table 2).

Such disturbance of surfactant function may be the reason for repeated collapse and reexpansion of the lung and, thus, for ventilation-induced lung parenchymal damage [26]. It may be suggested, therefore, that surfactant changes are (partially) responsible for the lung parenchymal damage previously demonstrated in this animal model [2]. Such a relationship has been shown previously by Nilsson *et al.* in prematurely delivered rabbits. It was shown that exogenous surfactant preceding mechanical ventilation with both constant tidal volumes (10 ml/kg) and constant peak inspiratory pressures increases lung-thorax compliance and reduces epithelial lesions [31]. Further studies need to be conducted to test such a hypothesis in this HIPPV-induced lung injury model.

The current data show that there is an important interaction between mechanical ventilation and surfactant changes in inducing lung injury. Such changes occur in a model of acute lung injury of prematurely delivered animals characterized by an immature surfactant system [30] and, as our data show, in a model of acute lung injury in adult animals, in which surfactant changes are induced by mechanical ventilation itself [6]. It has now been demonstrated that high amounts of exogenous surfactant have a beneficial effect on lung function and, possibly, survival in patients with acute respiratory distress syndrome [31]. Our data suggest that administration of high amounts of exogenous surfactant may beneficially influence further impairment of lung function attributable to mechanical ventilation in such patients by protecting the healthy lung areas not yet affected by the disease process.

Our data show that administration of exogenous surfactant preceding 20 min of overinflation of the lungs without PEEP reduces Evans blue dye in the BAL-accessible space and preserves end-expiratory lung stability. These data indicate that exogenous surfactant

changes have a beneficial effect on ventilation-induced lung injury.

References

1. Webb HH, Tierney DF: Experimental pulmonary edema due to high intermittent positive pressure ventilation with high inflation pressures: Protection by positive end-expiratory pressure. Am Rev Respir Dis 1974; 110: 556-65.
2. Dreyfuss D, Basset G, Soler P, Saumon G: Intermittent positive-pressure hyperventilation with high inflation pressures produces pulmonary microvascular injury in rats. Am Rev Respir Dis 1985; 132: 880-4.
3. Dreyfuss D, Soler P, Basset G, Saumon G: High inflation pressure pulmonary edema. Respective effects of high airway pressure, high tidal volume, and positive end-expiratory pressure. Am Rev Respir Dis 1988; 137: 1159-64.
4. Dreyfuss D, Saumon G: Role of tidal volume, FRC, and end-inspiratory volume in the development of pulmonary edema following mechanical ventilation. Am Rev Respir Dis 1993; 148: 1194-203.
5. Gommers D, Lachmann B: Surfactant therapy in the adult patient. Curr Opinion Crit Care 1995; 1: 57-61.
6. Verbrugge SJC, Böhm SH, Gommers D, Zimmerman LJI, Lachmann B: Surfactant impairment after mechanical ventilation with large alveolar surface area changes and the effects of positive end-expiratory pressure. Br J Anaesth 1998; 80: 360-64.
7. Gross NJ, Narine KR: Surfactant subtypes in mice: metabolic relationship and conversion in vitro. J Appl Physiol 1989; 66: 414-21.
8. Veldhuizen RAW, Marcou J, Yao LJ, McCraig L, Ito Y, Lewis JF: Alveolar surfactant aggregate conversion in ventilated normal and injured rabbits. Am J Physiol 1996; 270: L152-58.
9. Gommers D, Vilstrup C, Bos JAH, Larsson A, Werner O, Hannappel E, Lachmann B: Exogenous surfactant therapy increases static lung compliance and, cannot be assessed by measurements of dynamic compliance alone. Crit Care Med 1993; 21: 567-74.
10. Nilsson R, Grossmann G, Robertson B: Bronchiolar epithelial lesions induced in the premature rabbit neonate by short periods of artificial ventilation. Acta Path Microbiol Scand 1980; 88: 359-67.
11. Gruenwald P: A numerical index of the stability of lung expansion. J Appl Physiol 1963; 18: 665-7.
12. van Daal GJ, Bos JAH, Eijking EP, Gommers D, Hannapel E, Lachmann B: Surfactant replacement therapy improves pulmonary mechanics in end-stage influenza A Pneumonia in mice. Am Rev Respir Dis 1992; 145: 859-63.
13. Veldhuizen RAW, Inchley K, Hearn SA, Lewis JF Possmayer F: Degradation of surfactant associated protein B (SP-B) during in vitro conversion of large into small surfactant aggregates. Biochem J 1993; 295: 141-7.
14. Bligh EG, Dyer WJ: A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959; 37: 911-7.
15. Rouser G, Fleischer S, Yamamoto A: Two-dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. Lipids 1970; 5: 494-6.
16. Enhorning G. Pulsating bubble technique for evaluating pulmonary surfactant. J Appl Physiol 1977; 43: 198-203.
17. Rogers DF, Boschetto P, Barnes PJ: Plasma exudation: Correlation between Evans blue dye and radiolabeled albumin in guinea pig airways in vivo. J Pharmacol Methods 1989; 21: 309-15.
18. Kageyama N, Miura M, Ichinose M, Tomaki M, Ishikawa J, Ohuchi Y, Endoh N, Shirato K: Role of endogenous nitric oxide in airway microvasular leakage induced by inflammatory mediators. Eur Resp J 1997; 10: 13-9.
19. Gorin AB, Stewart PA: Differential permeability of endothelial and epithelial barriers to albumin flux. J Appl Physiol 1979; 47: 1315-24.
20. Effros RM, Mason GR, Silverman P, Reid E, Hukkanen J: Movements of ions and small solutes across endothelium and epithelium of perfused rabbit lungs. J Appl Physiol 1986; 60: 100-7.
21. Egan EA, Nelson RM, Olver RE: Lung overinflation and alveolar permeability to non-electrolytes in the

adult sheep in vivo. J Appl Physiol 1976; 260: 409-24.
22. Egan EA: Response of alveolar epithelial solute permeability to changes in lung inflation. J Appl Physiol 1980; 49: 1032-6.
23. Albert RK, Lakshminarayan S, Hildebrandt J, Kirk W, Butler J: Increased surface tension favors pulmonary edema formation in anesthetized dogs' lungs. J Clin Invest 1979; 63: 1015-8.
24. Bredenberg CE, Nieman GF, Paskanik AM, Hart AKE: Microvascular membrane permeability in high surface tension pulmonary edema. J Appl Physiol 1986; 60: 253-9.
25. Dethloff LA, Gladen BC, Gilmore LB, Hook GER: Kinetics of pulmonary surfactant phosphatidylcholine metabolism in the lungs of silica-treated rats. Toxicol. Appl Pharmacol 1989; 98:1-11.
26. Taskar V, John J, Evander E, Robertson B, Jonson B: Surfactant dysfunction makes lungs vulnerable to repetitive collapse and reexpansion. Am J Resp Crit Care Med 1997; 155: 313-20.
27. Bos JAH, Wollmer P, Bakker WH, Hannappel E, Lachmann B: Clearance of ^{99m}Tc-DTPA and experimentally increased alveolar surfactant content. J Appl Physiol 1992; 72: 1413-7.
28. Seeger W, Stöhr G, Wolf HRD, Neuhof H: Alternation of surfactant function due to protein leakage: specific interaction with the fibrin monomer. J Appl Physiol 1985; 58: 326-38.
29. Lachmann B, Eijking EP, So KL, Gommers D: In vivo evaluation of the inhibitory capacity of human plasma of exogenous surfactant function. Intensive Care Med 1994; 20: 6-11.
30. Nilsson R, Grossmann G, Robertson B: Pathogenesis of neonatal lung lesions induced by artificial ventilation: Evidence against the role of barotrauma. Respiration 1980; 40: 218-25.
31. Gregory TJ, Steinberg KP, Spragg R, Gadek JE, Hyers TM, Longmore WJ, Moxley MA, Cai GZ, Hite RD, Smith RM, Hudson LD, Crim C, Newton P, Mitchell BR, Gold AJ: Bovine surfactant therapy for patients with acute respiratory distress syndrome. Am J Resp Crit Care Med 1997; 155: 1309-15.

Summary and Conclusions

As outlined in *Chapter 1* the acute respiratory failure (ARF) is characterised by respiratory dysfunction including hypoxemia, low lung compliance, enlargement of functional right-to-left shunt, decreased functional residual capacity, atelectasis, and pulmonary edema. In ARF a shortage of surfactant at the alveolar level is observed. It has been suggested that the capillary leakage combined with damage to the alveolar epithelium leads to an immediate, or moderately slow, loss of active surfactant by inactivation or depletion from the alveoli and the small airways. Normally, the loss of active surfactant will be compensated by release of stored surfactant from type II cells. However, when the balance between production/release and loss/inactivation of surfactant favors the latter, the surface tension will rise at the air liquid interface leading to higher retracting forces of the alveoli. Therefore, an alveolus with surfactant impairment would be predisposed to end-expiratory alveolar collapse and prone to be affected by shear forces. It is shown that these "shear forces" contribute to a great extent to ventilation-induced lung injury. Therefore, the treatment of ARF should include prevention of end-expiratory alveolar collapse by a protective mode of mechanical ventilation, which is nowadays called The Open Lung Concept. Additionally, perfluorocarbons can be used to eliminate the air liquid interface by filling the lung with a fluid that is capable to maintain gas exchange at the alveolar capillary membrane, thereby providing fluid-filled alveoli which can not collapse ("fluid PEEP"). Finally, the most rational therapy would be to replenish the active surfactant at the alveolocapillary membrane with exogenous surfactant. The literature concerning these issues is briefly reviewed in *Chapter 1*. In the first part of this thesis (Chapters 2 to 6) we investigated some aspects of the treatment strategies in ARF, and in the second part (Chapters 7 to 9) we studied some preventive strategies for ARF.

Chapters 2 and 3 show the effect of The Open Lung Concept during pressure-controlled time-cycled mechanical ventilation, and high frequency oscillatory ventilation, on lung function. The aim of the study in *Chapter 2* was to demonstrate that under well-defined conditions, commercially available ventilators allow settings which are as effective as high frequency oscillatory ventilation with respect to improve lung function. It was shown that in lung-lavaged rats, pressure-controlled time-cycled ventilation in combination with a recruitment maneuver results in the same level of oxygenation, carbon dioxide elimination, protein infiltration and lung mechanics as high frequency oscillatory ventilation. In another study, pressure-controlled time-cycled ventilation was compared with high frequency oscillatory ventilation to observe their ability to preserve the function of exogenous surfactant in lung-lavaged rats (*Chapter 3*). The results show that pressure-controlled time-cycled ventilation with sufficient level of PEEP and small driving pressure amplitudes is as effective as high frequency oscillatory ventilation, when applied to fully aerated lungs, to prevent protein influx and conversion of active into nonactive surfactant aggregates. Both these studies indicate that achieving and maintaining alveolar expansion is more important than the type of mechanical ventilation

In *Chapter 4* exogenous surfactant therapy, mechanical ventilation with high PEEP, and partial liquid ventilation were compared for their effect on gas exchange, lung mechanics, lung injury, protein leakage into the alveoli and surfactant system, in a model of acute lung injury. The results show that the

three strategies opened up the lungs and kept them open, as indicated by the high levels of oxygenation, but the impact on protein infiltration, lung injury, and surfactant composition differed markedly. Only with exogenous surfactant therapy was there an improvement in all variables.

The studies in *Chapter 5* and *Chapter 6* used an established model of ventilation-induced lung injury (VILI) with high inspiratory lung volumes at peak inspiratory pressures of 45 cm H_2O without PEEP. In *Chapter 5* it was investigated whether exogenous surfactant might be used to re-establish the surfactant function after VILI, and in *Chapter 6* whether the combination of perfluorocarbon with gas ventilation (partial liquid ventilation; PLV) might also be used as an alternative therapy to treat VILI. The results show that both exogenous surfactant and PLV therapy re-established gas exchange and lung mechanics after VILI. However, with exogenous surfactant lung mechanics were improved to near healthy values.

Thus we have shown that established alveolar collapse can be treated by the above- mentioned therapies. In the subsequent studies we investigated preventive strategies for acute respiratory failure.

Chapter 7 briefly describes how to manage The Open Lung Concept. This strategy produces a ventilatory condition which saves the lung from further damage, allows a reduction of FiO_2, promotes the resorption of interstitial and intrapulmonary edema, and finally reduces the pulmonary artery pressures by overcoming the hypoxic pulmonary vasoconstriction.

To evaluate whether The Open Lung Concept can prevent acute respiratory failure, this mode was compared with conventional settings of ventilation in an animal model of surfactant deficiency (*Chapter 8*). The results show that keeping the lung open during surfactant depletion prevented a decrease in gas exchange, attenuated the deterioration in lung mechanics, and prevented 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 protein inhibition.

Chapter 9 presents a study on the effect of exogenous surfactant therapy before acute respiratory failure was induced by lung overinflation without PEEP. It was demonstrated that high doses of exogenous surfactant, preceding ARF, preserved lung mechanics and gas exchange, and reduced infiltration of Evans blue dye into the alveolar spaces. These data suggest that large amounts of exogenous surfactant may limit further impairment of lung function due to mechanical ventilation in patients with acute respiratory failure.

The work presented in this thesis contributes to the knowledge that treatment and prevention of end-expiratory alveolar collapse may beneficially influence functional impairment of the lung due to mechanical ventilation in patients suffering from acute respiratory failure.

Samenvatting en Conclusies

Zoals beschreven in *Hoofdstuk 1*, wordt Acuut Respiratoir Falen (ARF) gekenmerkt door respiratoire dysfunctie met als belangrijkste kenmerken hypoxie, verlaagde compliantie van de long, vergroting van de functionele rechts-links shunt, verlaagde functionele residuale capaciteit, atelectase en pulmonaal oedeem. Bij ARF is er sprake van een tekort aan surfactant op alveolair niveau. Tegenwoordig wordt verondersteld dat de capilaire permeabiliteit gecombineerd met de schade aan het alveolaire epitheel leidt tot een acuut danwel geleidelijk verlies van actief surfactant, door inactivatie en verlies van surfactant uit de alveoli naar de kleinere luchtwegen. In de normale situatie zal dit verlies worden gecompenseerd door afgifte van opgeslagen surfactant uit de type II cel. Echter wanneer het evenwicht tussen aanmaak/ afgifte enerzijds en verlies/ afbraak anderzijds doorslaat naar de laatstgenoemde zal dit een stijging van de oppervlakte spanning aan de lucht-vloeistof overgang veroorzaken. Deze stijging van de oppervlakte spanning veroorzaakt verhoogde retractieve krachten welke op de alveoluswand aangrijpen. Het bovengenoemde proces maakt meteen duidelijk waarom een alveolus met een gestoord surfactant systeem bloot staat aan eind- expiratoire collaps en de daaruit voortvloeiende "shear" krachten. Het is aangetoond dat "shear" krachten een van de belangrijkste veroorzakers zijn van beademings-geïnduceerde longschade. De therapie voor ARF zal daarom ook gericht zijn om deze eind-expiratoir collaps te voorkomen door een "beschermende" vorm van mechanische beademing toe te passen, zoals omschreven in "Het Open Long Concept". Daarnaast kan er gebruik gemaakt worden van perfluorcarbonen, die de lucht-vloeistof overgang elimineren en tegelijkertijd gasuitwisseling over de alveolair- capilair membraan mogelijk maken. Bij dit laatste proces worden de alveoli gevuld met vloeistof zodat ze niet kunnen samenvallen ("vloeibare PEEP"). Tenslotte is de meest voor de hand liggende therapie om alveolaire collaps te voorkomen, het aanvullen op alveolair niveau met exogeen surfactant. In *Hoofdstuk 1* van dit proefschrift wordt een kort literatuur overzicht gegeven over ARF en de behandelings mogelijkheden. In het eerste deel van dit proefschrift (hoofdstukken 2-6) wordt onderzoek beschreven over verschillende behandeling strategieën voor ARF. In het tweede deel (hoofdstukken 7-9) wordt een korte uiteenzetting gegeven van enkele onderzoeken die deze strategieën preventief toepassen.

Hoofdstuk 2 en 3 laten het effect zien van "Het Open Long Concept" toegepast, tijdens druk gestuurde tijd gereguleerde mechanische beademing en "high frequency oscillatory" beademing op de longfunctie. De doelstelling van de studie in *Hoofdstuk 2* was het aantonen dat de thans commercieel verkrijgbare ventilatoren net zo effectief zijn in het verbeteren van longfunctie als "high frequency oscillatory" ventilatoren. De resultaten lieten zien dat er geen verschil was tussen druk gestuurde tijd gereguleerde mechanische beademing gecombineerd met een openingsprocedure en "high frequency oscillatory" ventilatie met betrekking tot de oxygenatie, koolstofdioxide uitwas, eiwit-influx en longmechanica. In de volgende studie (*Hoofdstuk 3*) werd het effect op exogeen surfactant bestudeerd tussen beide beademingsvormen. De resultaten toonden aan dat, in volledig geopende longen, druk gestuurde tijd gereguleerde mechanische beademing met voldoende PEEP en kleine druk amplitudes net zo effectief is als "high frequency oscillatory" beademing, in het voorkomen van zowel eiwit-influx als van de omzetting van actieve in niet-actieve surfactant aggregaten. Beide studies lieten zien dat het openen en open houden van alveoli belangrijker is dan de beademingsvorm.

In *Hoofdstuk 4* werden 1) exogeen toegediend surfactant, 2) mechanische ventilatie met hoge PEEP drukken en 3) partiële vloeistof beademing met elkaar vergeleken in een model van acute longschade, waarbij vooral gelet werd op het effect op de gasuitwisseling, longmechanica, longschade, alveolaire eiwit-influx en surfactant inactivatie. Alle 3 de therapieën bleken instaat de long te openen en open te houden, wat te zien was aan de hoge arterieële zuurstofspannings waarden. Wel werd onderling een duidelijk verschil gezien tussen de eiwit-influx, de longschade en surfactant omzetting. Een verbetering van al de bestudeerde variabelen werd alleen bij surfactant therapie waargenomen.

Voor de studies beschreven in *Hoofdstukken 5 en 6* werd gebruik gemaakt van een bestaand experimenteel model van beademings geïnduceerde longschade door middel van hoge piek-inspiratoire longvolumes zonder PEEP. In *Hoofdstuk 5* werd onderzocht of exogeen surfactant gebruikt kan worden om de surfactant functie te herstellen in ventilatie geïnduceerde longschade. Vervolgens werd in *Hoofdstuk 6* gekeken of partiële vloeistof beademing een alternatief kan zijn voor de behandeling van ventilatie geïnduceerde longschade. Beide therapieën bleken de gasuitwisseling en de longmechanica te verbeteren, echter alleen bij exogeen surfactant verbeterde de longmechanica tot "gezonde" waarde.

De voorafgaande studies hebben laten zien dat bestaande alveolaire collaps behandeld kan worden door middel van de hierboven beschreven therapieën. In het volgende gedeelte zal preventieve toepassing van deze therapieën voor ARF bestudeerd worden

In *Hoofdstuk 7* wordt kort uiteengezet hoe "Het Open Long Concept" in de praktijk kan worden toegepast. Dit concept behelst een beademingsstrategie waarbij het voorkomen van additionele longschade voorop staat met daarbij een verlaging van de inspiratoire zuurstof toediening, resorptie van interstitieël en intrapulmonaal oedeem, met uiteindelijk een verlaging van de pulmonale arterieële druk door het overwinnen van de hypoxische pulmonale vasoconstrictie.

In *Hoofdstuk 8* werd onderzocht of acuut respiratoir falen voorkomen kan worden door het toepassen van "Het Open Long Concept" tijdens de surfactant depletie in ratten. Door het openhouden van de long gedurende surfactant depletie werd 1) een verlaging van de gasuitwisseling vooorkomen, 2) de achteruitgang in long mechanica beperkt en 3) geen toename van eiwit lekkage gezien, zoals gezien werd in de controle groep. Het voorkomen van de eiwit lekkage is vooral van belang in longen met een verminderde surfactant concentratie, omdat dan deze kleine hoeveelheden surfactant geïnactiveerd worden door de aanwezige plasma eiwiten.

Tenslotte werd in *Hoofdstuk 9* onderzocht of het toedienen van surfactant ARF kan voorkomen in het model van beademings geïnduceerde longschade. De resultaten toonden aan dat een hoge dosis exogeen surfactant, de long mechanica en gasuitwisseling instandhoudt en dat de instroom van Evans blue kleurstof naar de alveoli verminderde in vergelijking met een controle groep. Derhalve menen wij dat de toediening van een hoge dosis exogeen surfactant de verdere achteruitgang van de longfunctie die ten gevolge van kunstmatige beademing in ARF patiënten optreed gunstig kan beïnvloeden.

In conclusie, het werk dat gepresenteerd wordt in dit proefschrift draagt bij aan het inzicht dat behandeling en preventie van eind-expiratoire alveolaire collaps, een gunstig effect heeft op de longfunctie, die door mechanische beademing verminderd is in patiënten met acuut respiratoir falen.

Resumen y Conclusiones

En el *Capítulo 1* se incluye una descripción de la insuficiencia respiratoria aguda (IRA), la cual se caracteriza por la presencia de disnea, hipoxemia, baja distensibilidad pulmonar, incremento en los "cortocircuitos" intrapulmonares de derecha a izquierda, disminución en la capacidad funcional residual, atelectasias y edema pulmonar no cardiogénico. Se ha observado que durante la IRA existe un déficit de surfactante a nivel alveolar. Se ha propuesto que la fuga capilar en combinación con daño al epitelio alveolar conduce a una pérdida inmediata o progresiva de surfactante activo, ya sea por inactivación o déficit en el alvéolo y vias aéreas. En condiciones normales la pérdida de surfactante activo será compensada por la liberación de surfactante almacenado en las células alveolares tipo II. Sin embargo, cuando el balance entre producción/liberación y pérdida/inactivación de surfactante favorece a éste último, la tensión de superficie alveolar a nivel de la interfase aire-líquido se incrementa produciendo elevadas fuerzas de retracción alvéolar. Por lo tanto, un alvéolo con déficit de surfactante estará predispuesto a colapsarse al final de la espiración y propenso a ser afectado por fuerzas de tracción. Se ha demostrado que estas fuerzas de tracción contribuyen en gran medida al daño pulmonar inducido por la ventilación mecánica. Por lo tanto, el tratamiento de la IRA debe incluir prevención del colapso alveolar al final de la espiración mediante un modo de ventilación mecánica que proteja al pulmón contra las fuerzas de tracción. Dicho modo ventilatorio debe incluir una maniobra que permita reacrear (abrir) las unidades alveolares colapsadas, esto se logra mediante el incremento de las presiones en la via aérea hasta que sobrepase el punto de apertura alveolar. Una vez que todos los alvéolos se encuentran reacreados (abiertos), las presiones en la via aérea deberan reducirse al nivel mínimo necesario para contrarestar las fuerzas de retracción alveolar y mantener dichos alvéolos abiertos durante el período espiratorio. A éste modo ventilatorio se le ha denominado "Open Lung Concept". Aunado a este modo de ventilación, otra terapia que evita el colapso alveolar al final de la espiración es por la eliminanción de la interfase aire-líquido a nivel alveolar mediante la aplicación de perfluorocarbonos. El perfluorocarbono mantiene al alvéolo lleno de líquido el cual no podra colapsarse al final de la espiración ("PEEP-liquido") permitiendo el intercambio gaseoso a nivel de la membrana alvéolo-capilar. Finalmente, el tratamiento básico de la IRA consiste en restituir el surfactante activo a nivel de la membrana alvéolo capilar mediante surfactant exógeno. En el *Capítulo 1* se describen algunos de estos aspectos basados en una revisión de la literatura.

En la primera parte de esta tesis (*capítulos 2-6*), nosotros investigamos algunos aspectos sobre las medidas de tratamiento de la IRA, y en la segunda parte (*capítulos 7-9*) estudiamos algunas medidas para prevenir la IRA.

En los estudios incluidos en los *Capítulos 2* y *3* se muestra el efecto del "Open Lung Concept" en la función pulmonar durante la ventilación mecánica ciclada por tiempo y controlada por presión, y la ventilación oscilatoria de alta frequencia. El objetivo del estudio descrito en el *Capítulo 2* fue; demostrar que bajo condiciones bien definidas, ventiladores comerciales permiten ajustes en la técnica ventilatoria, los cuales son tan efectivos como la ventilación oscilatoria de alta frequencia con respecto a la mejoría de la función pulmonar en en un modelo de lesion pulmonar aguda. Se demostró que la ventilación ciclada por tiempo y controlada por presión, en combinación con una maniobra de reaereación alveolar resultó en el mismo nivel de oxigenación, eliminación de bióxido de carbono,

infiltración de proteinas al alvéolo, y mecánica pulmonar que la ventilación oscilatoria de alta frequencia. En otro estudio, la ventilación ciclada por tiempo y controlada por presión fue comparada con la ventilación oscilatoria de alta frequencia en la preservación del surfactante exógeno (*Capítulo 3*). Los resultados muestran que en ratas sometidas a lavado pulmonar, la ventilación ciclada por tiempo y controlada por presión con suficiente nivel de PEEP y corta amplitud entre las presiones inspiratorias y espiratorias, es tan efectiva como la ventilación oscilatoria de alta frequencia cuando se aplica en pulmones completamente aereados, previniendo la infiltración de proteinas al alvéolo y la transformación de surfactante de componentes activos en componentes no activos. Estos capítulos indican que obteniendo la reapertura alveolar y manteniendo los alveolos abiertos durante todo el ciclo ventilatorio es más importante que el tipo de ventilación mecánica.

En el estudio descrito en el *Capítulo 4* se utilizó un modelo de lesión pulmonar aguda para comparar; la terapia con surfactante exógeno, la ventilación mecánica con altos níveles de PEEP, y la ventilación parcial líquida, en su efecto sobre el intercambio gaseoso, la mecánica pulmonar, la lesión pulmonar, el infiltrado de proteinas al alvéolo, y la composición de surfactante. Los resultados mostratron que las tres estrategias lograron "abrir" los pulmones y lograron mantenerlos "abiertos", como lo indico el elevado nivel de oxigenación, sin embargo, el impacto sobre la infiltración de proteínas, la lesión pulmonar y la composición de surfactante difirio marcadamente. Solamente con la terapia a base de surfactante exógeno hubo mejoría en todas las variables.

En los estudios descritos en el *Capítulo 5* y *Capítulo 6* usamos un modelo establecido de lesión pulmonar inducida por la ventilación mecánica (LIVM) el cual consiste en ventilar con altos volumenes inspiratorios a altas presiones inspiratorias de 45 cm H_2O sin PEEP. En el estudio descrito en el *Capítulo 5* investigamos si la terapia con surfactante exógeno puede ser usada para reestablecer la función del surfactante pérdido o inactivado después de la LIVM, y en el estudio descrito en el *Capítulo 6* estudiamos si la combinación de perfluorocarbonos con ventilación mecánica (ventilación parcial líquida) puede ser utlizada para tratar la LIVM. Los resultados mostraron que tanto la terapia con surfactante exógeno como la ventilación parcial líquida reestablecierón el intercambio gaseoso y la mecánica pulmonar después de la LIVM. Aun más, la terapia con surfactante exógeno mejoró la mecánica pulmonar a valores cercanos a los registrados en animales sin daño pulmonar.

En los capítulos descritos hasta ahora hemos mostrado que el colapso alveolar puede ser tratado con las terapias mencionadas anteriormente. En los siguientes tres capítulos mostraremos estrategias para prevenir la IRA.

En el *Capítulo 7* se describe la técnica del "Open Lung Concept". Esta estrategia produce una condición ventilatoria que proteje al pulmón de un mayor daño, permite una reducción de la fracción inspirada de oxígeno, promueve la reabsorción de edema intersticial e intraalveolar, y finalmente, reduce las presiones de la arteria pulmonar debidas a vasoconstricción pulmonar por hipoxía.

Para evaluar si el "Open Lung Concept" puede prevenir IRA, se comparó ésta técnica con la ventilación convencional en un modelo animal de deficiencia de surfactante (*Capítulo 8*). Los resultados muestran que manteniendo el pulmón "abierto" durante la deficiencia de surfactante se previno la disminución en el intercambio gaseoso, se atenuó el deterioro en la mecánica pulmonar, y

se previno el infiltrado de proteínas al alvéolo. En pulmones deficientes de surfactante es muy importante prevenir la infiltración de proteinas debido a que la escasa cantidad de surfactante remanente en el pulmón esta propenso a ser inhibido por las proteínas.

Nosotros estudiamos en el *Capítulo 9* el efecto de la terapia con surfactante exógeno antes de que la IRA fuera inducida por sobredistensión pulmonar sin PEEP. En este estudio demostramos que altas dosis de surfactante exógeno administrado antes de la IRA, preservó la mecánica pulmonar y el intercambio gaseoso, además redujo el infiltrado de colorante azul de Evans dentro del espacio alveolar. Estos datos sugieren que grandes cantidades de surfactante exógeno podrían limitar el daño pulmonar debido a la ventilación mecánica en pacientes con IRA.

El trabajo presentado en esta tesis contribuye al conocimiento sobre el tratamiento y prevención del colapso alveolar al final de la espiración, el cual puede tener un efecto benéfico en el impedimento de la función pulmonar debido a la ventilación mecánica en pacientes que desarrollan insuficiencia respiratoria aguda.

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Diciembre de 1999.

Curriculum vitae

Gilberto Felipe Vazquez de Anda was born on 5th of February, 1963, in Monterrey, state of Nuevo León, México. After receiving the high school diploma in 1980 from the State of Mexico Autonomous University, that same year he began medical studies at the same University and graduated as a Medical Doctor on 10 April, 1989. From 1984 to 1989 he worked with an ambulance service of Toluca City, first as a paramedic and later as a physician. From February 1989 to February 1990 he worked as a general practitioner in Atlacomulco, México. In 1989 he passed the National Residency Examination, and in 1990 he started critical care training at the Mexican Institute of Social Security (*IMSS*), from 1990-1991 in the *Hospital General Regional No. 8* of Toluca City, and from 1991 to 1993 in the *Hospital de Especialidades Dr. Bernardo Sepulveda G.* of the National Medical Center *Siglo XXI* in Mexico City, under the supervision of Dr. Jorge Castañón González. On February 28 1993, he received the Critical Care Medicine diploma from the *IMSS*, and on March 13 the certification for Critical Care Medicine from the *Consejo Mexicano de Medicina Critica*. On May 1st that same year, he became a staff member at the Intensive Care Unit of the same hospital. In 1994, he got his Diploma in Critical Care Medicine from the National Autonomous University of Mexico. From 1994-1996 he studied clinical research in the medical faculty of the State of Mexico Autonomous University, under the supervision of Dr. Rogelio Miranda Ruiz and Dr. Jorge Castañón González. He got his Master's degree on 13th February, 1997. In July 1996 he received study grants from the *Consejo Mexicano De Ciencia y Tecnología*, from the *Dirección General de Relaciones Internacionales de la Secretaria de Educación Pública* and from *IMSS* to complete PhD studies at the Department of Anesthesiology, Erasmus University Rotterdam (the Netherlands), in which from March 1997 until now, he has been working under the supervision of Prof. Dr. B. Lachmann. During this time, he has had international collaboration with: Dept. of Pathology of the Ruhr-Universität Bochum, Germany; Dept. of Anesthesiology and Intensive Care Medicine, Virchow Clinics, Humboldt University, Berlin, Germany. In March 2000 he will continue his work in the *IMSS*, in the Intensive Care Unit of the *Hospital de Especialidades Dr. Bernardo Sepulveda G.* of the National Medical Center *Siglo XXI*, México.

Curriculum vitae

Gilberto Felipe Vazquez de Anda nació el 5 de Febrero de 1963, en Monterrey, NL, México. En 1980 terminó su bachillerato en Ciencias de la Salud en la Universidad Autonoma del Estado de México, e inició sus estudios en médicina en la misma universidad , el 10 de Abril de 1989 recibio el título de Médico Cirujano. De 1984 a 1989 trabajó como paramédico y después como médico general en una unidad de emergencia de la ciudad de Toluca. De 1989 a Febrero de 1990 trabajó como médico general en una clínica de medicina familiar en Atlacomulco, México. En 1989 aprobó el XIII Examen Nacional de Aspirantes a Residencias Medicas y en 1990 inició su especialidad en Médicina del Enfermo en Estado Crítico en el Instituto Mexicano del Seguro Social (IMSS), de 1990 a 1991 en el Hospital General Regional No. 8 de la ciudad de Toluca, y de 1991 a 1993 en el Hospital de Especialidades "Dr. Bernardo Sepulveda G." del Centro Médico Nacional siglo XXI (ciudad de México), bajo la supervisión del Dr. Jorge Castañón González. El 28 de Febrero de 1993 recibió el diploma de especialista y el 13 de Marzo obtuvo la certificación por el Consejo Mexicano de Medicina Crítica A.C. El primero de Mayo del mismo año fue nombrado médico adscrito en la unidad de cuidados intensivos del mismo hospital de especialidades. En 1994 recibió el diploma universitario (Universidad Nacional Autónoma de México) de especialista.. De 1994 a 1996 estudió la maestría en investigación clínica en la Facultad de Medicina de la Universidad Autónoma del Estado de México, bajo la supervisión del Dr. Rogelio Miranda Ruiz y del Dr. Jorge Castañón González. El 13 de Febrero de 1997 obtuvo el grado de Maestro en Investigación Clínica. En Julio de 1996 obtuvo becas del Consejo Nacional de Ciencia y Tecnología, de la Dirección General de Relaciones Internacionales (Secretaria de Educación Pública) y del Instituto Mexicano del Seguro Social, para realizar estudios de doctorado en el Departamento de Anestesiología de la Universidad Erasmus de Rotterdam (Holanda). De Marzo de 1997 a la fecha, ha estado bajo la supervisión del Dr. B. Lachmann. Durante este tiempo ha recibido colaboración internacional del Departamento de Patología de la *Ruhr-Universität Bochum,* Alemania, y del Departamento de Anestesiología y Cuidados Intensivos de *Virchow Clinics, Humboldt University, Berlin,* Alemania. El primero de Marzo del año 2000 continuará con su trabajo en el Instituto Mexicano del Seguro Social, en la Unidad de Cuidados Intensivos del Hospital de Especialidades "Dr. Bernardo Sepulveda G." del Centro Médico Nacional Siglo XXI, México.

PUBLICATION LIST

Articles

1. Soler-Morejón C, Colmenero-Zubiate, **Vazquez de Anda GF**, Castañon-González JA. Acute Respiratory Distress Syndrome in Adults. In: Cuidados Intensivos. Clinicas Médicas Mexicanas. González Antonio (ed), Interamericana-Mc Graw-Hill, México 1995; pp 301-307
2. Castañón-González JA, **Vazquez de Anda GF**, Gallegos H, Miranda RR, Hernández G, Eid Lidth G. Acute fatty liver of pregnancy complicated with pancreatitis. Gaceta Med Mex 1997: 133:253-258
3. Böhm S, **Vazquez de Anda GF**, Lachmann B. Iatrogenic Lung Damage by Artificial Ventilation: What is the Role of the Pulmonary Surfactant System? In A Gullo (ed) Anaesthesia, Pain, Intensive Care and Emergency Medicine-A.P.I.C.E., 1998, Springer-Verlag, pp 141-146
4. Böhm S, **Vazquez de Anda GF**, Lachmann B. The Open Lung Concept. In: Vincent JL (ed) Yearbook of Intensive Care and Emergency Medicine. 1998. Springer-Verlag, Heidelberg, pp 430-440
5. **Vazquez de Anda GF**, Lachmann B. Protecting the lung during mechanical ventilation with The Open Lung Concept. Acta Anaesthesiol Scand 1998; 112: S63-66
6. Verbrugge SJC, **Vazquez de Anda GF**, Gommers D, Neggers S, Vera Šorm, Böhm S, Lachmann B. Exogenous surfactant dose-dependently preserves lung function and reduces intra-alveolar Evans blue dye influx in a rat model of ventilation-induced lung injury. Anesthesiology 1998; 89: 467-474
7. **Vazquez de Anda GF**, Hartog A, Verbrugge SJC, Gommers D, Lachmann B. The Open Lung Concept: Conventional mechanical ventilation is as effective as high frequency oscillatory ventilation in improving gas exchange and lung mechanics in surfactant-deficient animals. Intensive Care Med 1999; 25: 990-996
8. Verbrugge SJC, de Jong JW, Keijzer E, Neggers S, **Vazquez de Anda GF**, Lachmann B. Purine in broncho-alveolar lavage fluid as marker of ventilation induced lung injury. Critical Care Med 1999; 27:779-783
9. Tusman G, Böhm SH, **Vazquez de Anda GF**, do Campo JL, Lachmann B. "Alveolar Recruitment Strategy" improves arterial oxygenation during general anaesthesia. Br J Anaesth 1999; 82: 8-13
10. Hartog A, **Vazquez de Anda GF**, Gommers D, Kaisers U, Verbrugge SJC, Schnabel R, Lachmann B. Comparison of exogenous surfactant therapy, mechanical ventilation with high end-expiratory pressure and partial liquid ventilation in a model of acute lung injury. Br J Anaesth 1999; 82: 81-86
11. Hartog A, **Vazquez de Anda GF**, Gommers D, Kaisers U, Lachmann B. Prophylactic use of "The Open Lung Concept" during surfactant depletion attenuates the deterioration of pulmonary function. Critical Care Med (in press)

12. Kunst PWK, Böhm SH, **Vazquez de Anda GF**, Lachmann B, Amato M, Postmus PE, de Vries PMJM. Monitoring of respiratory dynamics by electrical impedance tomography in a model of ARDS. Critical Care Med (in press)
13. **Vazquez de Anda GF**, Gommers D, Verbrugge SJC, De Jaegere A, Lachmann B. Conventional mechanical ventilation with high end-expiratory pressure and small pressure amplitude is as effective as high frequency oscillatory ventilation to preserve the function of exogenous surfactant in lung-lavaged rats. (Submitted for publication)
14. **Vazquez de Anda GF**, Lachmann R, Verbrugge S, Gommers D, Lachmann B. Treatment of ventilation-induced lung injury with exogenous surfactant. (Submitted for publication)
15. **Vazquez de Anda GF**, Lachmann R, Verbrugge S, Gommers D, Lachmann B. Partial liquid ventilation improves lung function in ventilation-induced lung injury (Submitted for publication)
16. Kunst PWA, **Vazquez de Anda GF**, Böhm SH, Faes TJC, Lachmann B, Postmus PE, de Vries PMJM. Monitoring of recruitment and derecruitment by electrical impedance tomography in a model of acute lung injury (Submitted for publication)

Abstracts

1. Böhm S, **Vazquez de Anda GF**, Rombouts C, Amato M, Barbas C, Hendrik E, Rajan G, Staas N, Kunst P, Lachmann B. Comparison between two experimental models of alveolar recruitment (Preliminary Report). Intensive Care Med 1997; 23 (Suppl): S1-S206 (Abstr)
2. **Vazquez de Anda GF**, Verbrugge S, Gommers D, De Jaegere A, Lachmann B. Pressure control ventilation (PCV) with small pressure amplitudes is as effective as high frequency oscillatory ventilation (HFOV) to preserve surfactant function. Diab Nutr Metab 1997; 10 (Suppl): S-30
3. **Vazquez de Anda GF**, Majoor S, Lachmann RA, Verbrugge S, Lachmann B. Treatment of ventilation-induced lung injury with exogenous surfactant. Br J Anaesth 1999; 82 (Suppl): S-182 (Abstr A 607)
4. **Vazquez de Anda GF**, Majoor S, Lachmann RA, Verbrugge S, Lachmann B. Treatment of ventilation-induced lung injury with partial liquid ventilation. Intensive Care Med 1999, 25 (Suppl): S-14 (Abstr 38)