Factors Affecting Surfactant Responsiveness

Influence of mode of administration and ventilation, disease stage and type of surfactant

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FAKTOREN DIE DE WERKING VAN SURFACTANT BEINVLOEDEN

Invloed van de manier van toediening en beademing, ziekte toestand en soort surfactant

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Introduction

Historically, Kurt von Neergaard [1] was the first to suggest that surface tension plays an important role in lung elasticity. He showed, in 1929, that the pressure necessary for filling the lung with liquid was less than half the pressure necessary for filling the lung with air, and concluded that two-thirds to three-fourths of the elasticity of the lung was derived from interfacial forces [1]. The problem with his discovery was that this paper was published in German and that, for 25 years, no scientists in the evolving field really took note of this publication. In 1954, Macklin [2] described the presence of a thin aqueous mucoid microfilm, formed from secretion of the granular pneumocytes, on the pulmonary alveolar walls and which is in constant slow movement toward the phagocytic pneumocytes and bronchioles. One year later, Pattle [3] noticed the remarkable stability of foam and bubbles from lung edema and healthy lung cut. He assumed that the walls of these bubbles consists of surface-active material which must lower the surface tension to nearly zero. In 1957, Clements [4] was the first to prove the direct evidence of surface active material in the lungs. He measured surface tension of a surface film derived from the lung by using a Wilhelmy balance and demonstrated that the surface tension was not a constant value; when the surface was stretched the tension was relatively high (40 dynes/cm), but when the surface area was decreased the tension fell to 10 dynes/cm. He pointed out that such a reduction in surface tension during deflation in the lung would tend to stabilize the air spaces by permitting them to remain open at low lung volumes. Two years later, Avery and Mead [5] demonstrated that lung extracts of very small premature infants and infants dying with hyaline membrane disease had much higher surface tension than normal lung extracts, due to a deficiency in surface active material. This was the first step towards extensive research on the surfactant system, and Fujiwara and colleagues, in 1980 [6], were the first to treat premature babies suffering from respiratory insufficiency with exogenous surfactant.

Pulmonary surfactant is a complex of phospholipids (80-90%), neutral lipids (5-10%) and at least four specific surfactant-proteins (5-10%) (SP-A, SP-B, SP-C and SP-D), lying as a monolayer at the air-liquid interface in the lung [7,8]. Surfactant is synthesized by the alveolar type II cells and secreted into alveolar spaces [7]. The surfactant lipids are lying in a thin aqueous film which coats the pulmonary alveolar walls and small airways. At the surface of this aqueous film, the phospholipid-molecules are lying as a monolayer and lower its surface tension; this reduce the

muscular effort necessary to breathe and prevents collapse of the alveoli at the end of expiration [9].

Since 1980, more than 100,000 premature infants suffering from respiratory distress syndrome (RDS) due to a surfactant deficiency are successfully treated with exogenous surfactant almost without any side-effects [10,11]. Biochemical and biophysical abnormalities of the pulmonary surfactant system is also seen in other diseases such as the adult respiratory distress syndrome (ARDS) [9,12,13], infectious lung disease [14] and after cardiopulmonary bypass surgery [15]. Furthermore, it could be demonstrated that non-optimal ventilation may lead to disturbance of alveolar surfactant [16].

However, only a few case reports and results of limited clinical pilot studies are available, in which patients other than neonates with RDS are treated with exogenous surfactant [17]. In this chapter, we describe the rationale for exogenous surfactant therapy in the different lung diseases by reviewing experimental and clinical findings.

Function of the pulmonary surfactant
The normal physiological functions of the pulmonary surfactant system include:
1) Mechanical stabilization of lung alveoli.

The force required to open alveoli is determined by surface tension at the air-liquid interface and by the radius of the terminal units of the lung in accordance with the LaPlace law ($P = 2\gamma/r$; P = pressure in the bubble, γ = surface tension, r = radius of the bubble). Pulmonary surfactant decreases the surface tension of the interface and thereby allows normal breathing with the least possible effort.

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 [18].
2) Stabilization of small airways.

Pulmonary surfactant also ensures stabilization of the peripheral airways and thus its lack might cause airway obstruction or collapse of the small bronchioli with air trapping [19]. Besides its role in mechanical stabilization, bronchial surfactant also has a transport function for mucus and inhaled particles [20]. Furthermore, bronchial surfactant acts as an antiglue factor, preventing the development of large adhesive

forces between mucus and the bronchial wall [21].

3) Protection against lung edema.

Another function of the pulmonary surfactant system is stabilization of the fluid balance in the lung and protection against lung edema [22]. In general, the forces that influence the circulation of liquid at the alveolar-capillary level in the lungs include: plasma colloid osmotic pressure on one side and capillary hydrostatic pressure, interstitial colloid osmotic pressure and alveolar surface tension on the other side (Figure 1). This means that a surfactant deficiency will increase the surface tension at the air-liquid interface and thereby the suction forces will increase, resulting in lung edema (Figure 1).

4) Surfactant and local defense mechanism.

It has also been demonstrated that surfactant plays a role in the lung's defense against infection [23]. Surfactant, and in particular SP-A, enhances the antibacterial and antiviral defense of alveolar macrophages [23].

We have demonstrated that the pulmonary 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 [24,25].

SCHEMATIC DIAGRAM OF WATER BALANCE IN THE LUNG

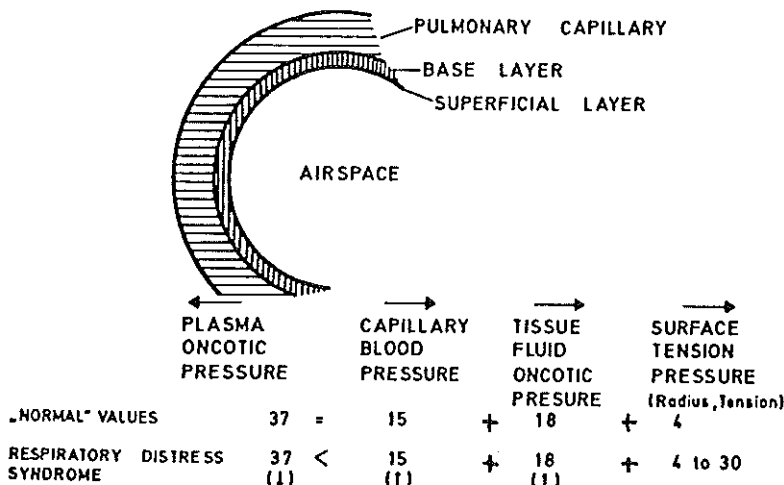|	PLASMA ONCOTIC PRESSURE		CAPILLARY BLOOD PRESSURE	TISSUE FLUID ONCOTIC PRESURE		SURFACE TENSION PRESSURE (Radius, Tension)
"NORMAL" VALUES	37	=	15	+ 18	+	4
RESPIRATORY DISTRESS SYNDROME	37 (↓)	<	15 (↑)	+ 18 (↑)	+	4 to 30

Figure 1. Simplified schematic diagram representing the factors influencing fluid balance in the lung (from reference [28]).

Functional changes due to a 'disturbed' surfactant system

Disturbance of the surfactant system can result from different factors. Damage to the alveolo-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(ogen), albumin, globulin and transferrin, hemoglobin and cell membrane lipids [26]. These components are known to inhibit pulmonary surfactant function in a dose-dependent way [26]. Furthermore, the pulmonary surfactant may also be disturbed by the following mechanisms: breakdown of surfactant by lipases and proteases; phospholipid peroxidation by free radicals; loss of surfactant from the airways due to mechanical ventilation with large tidal volumes; disturbed synthesis, storage, or release of surfactant secondary to direct injury to type II cells [16,26,27].

Diminished pulmonary surfactant has far-reaching consequences for lung function. Independent of the cause, decreased surfactant function will directly or indirectly lead to [28]:

1. Decreased pulmonary compliance;
2. Decreased functional residual capacity (FRC);
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; and
6. Pulmonary edema with further inactivation of surfactant by plasma constituents.

Surfactant and 'adult' respiratory distress syndrome (ARDS)

In 1967 Ashbaugh and co-workers [29] described 12 adult patients with acute respiratory failure which did not respond to usual therapy. The clinical and pathological features were very similar to those seen in neonates with RDS, so the name Adult Respiratory Distress Syndrome (ARDS) was introduced. ARDS has become a well-recognized condition that can result from a number of different causes, e.g. sepsis, polytrauma, aspiration, multiple organ failure, burns, pneumonia, near-drowning, acute pancreatitis and many others [30]. Despite diverse etiologies in ARDS, the common pathological characteristic is increased alveolo-capillary permeability associated with damage to the alveolar epithelium. The mechanisms responsible for the injury to the alveolo-capillary membrane are complex and are still under discussion [31]. Active roles have been attributed to neutrophils, basophils, macrophages, platelets, arachidonic

acid metabolites, oxygen-derived free radicals, complement, proteases, interleukins, serotonin, platelet activating factor (PAF), tumor necrosis factor (TNF), surfactant inhibiting plasma-proteins, drugs, and many other substances [32]. But all these individual factors which can lead to a pulmonary edema do not, however, necessarily lead to ARDS. Therefore, another system must be involved to explain the functional changes as seen in ARDS. It is established 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 small airways which is, however, compensated by a release of stored surfactant from type II cells [26]. Thus, the progress of the disease depends on the balance between new production and release of surfactant into the alveoli and its inactivation/loss from the alveoli and airways. If the synthesis is reduced e.g. by influenza virus, hypoxia or hyperoxia, etc., an imbalance between new synthesis and demand will result. This will finally lead to a total loss of functional active surfactant, resulting in failure of the lung as a gas exchange organ [28,33]. Thus in ARDS the surfactant deficiency is a complication of lung injury rather than, as in neonatal RDS, a primary etiological factor. In spite increased sophistication in methods of respiratory support, mortality associated with ARDS currently remains between 48 and 75%, depending on the etiology [34,35]. Nowadays, it is more appropriate to speak about the Acute, rather than Adult, Respiratory Distress Syndrome (ARDS), since ARDS is not limited to adults [36].

Analyses of lung surfactant recovered in BAL from patients with ARDS, or from animal models of acute respiratory failure, demonstrate disturbances of the lung surfactant system [37]. Reduction of surfactant activity is associated with increased minimal surface tension of lung extracts or lung homogenates, and compositional changes of surfactant and/or decreased surfactant content of the lungs [7,17,33]. Ashbaugh and colleagues [29] were the first to demonstrate decreased lung compliance and increased minimal surface tension in lung extracts from two ARDS patients. Since then, several studies have demonstrated qualitative and quantitative changes of surfactant in BAL fluid from ARDS patients [38-41]. Recently, Gregory and colleagues [41] demonstrated that several of these alterations already occur in patients at risk of developing ARDS, suggesting that these abnormalities of surfactant occur early in the disease process.

The central role of surfactant deficiency can further be illustrated by recent studies in animal models of ARDS which demonstrated that exogenous surfactant

instillation dramatically improved blood gases and lung mechanics [9,42]. The models of surfactant deficiency in which these improvements could be demonstrated include acute respiratory failure due to in-vivo whole-lung lavage [43,44], neurogenic ARDS [45], respiratory failure as a result of oxygen toxicity [46,47] or oxidant-producing enzymes [48], acute respiratory failure after instillation of hydrochloric acid [49-51] or plasma instillation [52], and respiratory failure after intoxication with N-nitroso-N-methylurethane (NNNMU) [53] or paraquat [54].

Evidently, it is rational to administer exogenous surfactant in ARDS patients, but the question then arises why is this not yet a reality. Surfactant has been commercially available for neonates since 1987 [55]. Surfactant therapy in patients other than neonates with RDS is almost impossible due to the fact that there is not enough surfactant available and that current prices are too high (1 g of surfactant costs about US$ 3,000-5,000) [55]. Therefore, only a few case-reports and pilot studies have been performed up to now.

Lachmann *et al.* [56] treated a four-month-old boy with exogenous surfactant who had aspirated baby-oil and developed acute respiratory failure. In this case, the question was raised whether the detergent effect of the baby-oil could be overcome by administering exogenous surfactant. Using a Wilhelmy balance, it was first demonstrated that surfactant decreased surface tension despite the presence of baby-oil (Figure 2). Therefore, a natural surfactant (250 mg/kg; 40 mg/ml) was instilled intratracheally in this patient and arterial oxygenation was measured with an intra-arterial PO_2 catheter. Figure 3 shows that arterial oxygenation improved from 150 to 300 mmHg within two hours, despite a decrease of the peak airway pressure. Richman *et al.* [57] studied three adult patients with ARDS of different etiologies. These patients were treated with 4 g of a natural surfactant (60 mg/kg), delivered bronchoscopically in divided doses to each lobe. After surfactant instillation, arterial oxygenation improved immediately in two patients, but sustained for only one hour. In the third patient, arterial PO_2 improved over time and persisted throughout the observation period (72 hours). No significant changes were seen in compliance or functional residual capacity (FRC) in any of these patients. Joka and Obertacke [58] treated an 18-year-old victim of a motorcycle accident with a post-traumatic ARDS. Directly after exogenous surfactant instillation, PaO_2 improved, whereas mean pulmonary arterial pressure, FiO_2 and alveolar protein leakage in BAL were decreased. The surfactant was given in two doses (on days 9 and 13 after the accident), with a total concentration of
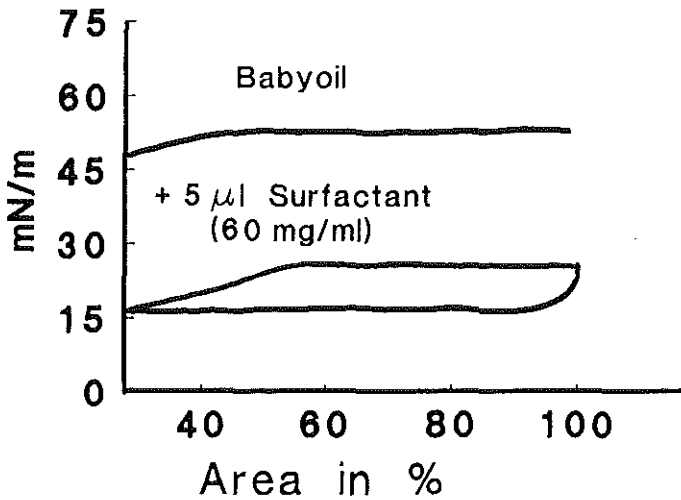
Figure 2. Surface pressure (mN/m) vs. area for baby-oil and baby-oil with surfactant, measured with a Wilhelmy balance (from reference [56]).
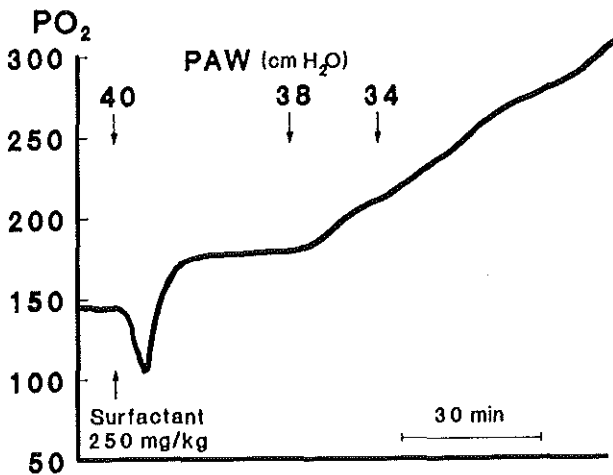
Figure 3. Effect of surfactant on arterial oxygenation (PO_2 in mmHg) in a child who had aspirated baby-oil and developed acute respiratory failure. PO_2 was measured continuously with an intra-arterial PO_2 catheter. The boy was mechanical ventilated with pressure controlled ventilation, 100% oxygen ($FiO_2 = 1.0$), 70% inspiration time, frequency 45/min, peak airway pressure (PAW) of 40 cm H_2O and a PEEP of 5 cm H_2O. After surfactant instillation, peak airway pressure (PAW) could first be reduced to 38 cm H_2O and 30 min later to 34 cm H_2O (from reference [56]).

about 50 mg/kg body weight. Nosoka and co-workers [59] demonstrated improvement in PaO_2 and chest X-ray after multiple instillations of surfactant in two adult patients with ARDS. The first patient received surfactant 20 times (240 mg each) during 38 days, whereas the second patient received three doses of surfactant (also 240 mg each) on three consecutive days (antibodies to the natural surfactant were not detected in either patient). Stubbig *et al.* [60] reported a case of surfactant therapy in a 21-year-old man who developed ARDS after severe lung contusion due to a car accident. No improvement occurred during conventional ventilatory treatment, including inverse-ratio ventilation and high-frequency ventilation. Immediately after instillation of a natural surfactant (on day 15 after the accident; 38 mg/kg), they observed the following changes: deterioration of the pulmonary function probably due to crusts in the lung; after aspiration of the crusts at bronchoscopy, there was a progressive improvement in respiratory parameters. The PaO_2 and chest X-ray improved, whereas FiO_2, inspiration time and PEEP level could be reduced. Marraro *et al.* [61] treated two adolescents who developed ARDS which appeared during leukemia treatment with surfactant (patient one, 60 mg/kg and patient two, 40 mg/kg). Arterial oxygenation improved within three hours (patient one: 60 to 350 mmHg; patient two: 160 to 300 mmHg) during mechanical ventilation with 100% oxygen. Haslem and colleagues [62] treated four adult patients with late stage of ARDS with a single bolus of synthetic surfactant (75 mg/kg) and found no sustained clinical improvement. In contrast to the results of Haslem and co-workers [62], Heikinheimo *et al.* [63] reported successful treatment of a 50-year-old patient suffering from ARDS with two doses of synthetic surfactant (total amount 104 mg/kg). McBrien *et al.* [64] treated a nearly drowned 9-year-old boy with synthetic surfactant. PaO_2/FiO_2 was increased from 57 to 293 mmHg while PIP was reduced from 40 to 25 cm H_2O and the patient was discharged successfully from the hospital two days later. Suzuki *et al.* [65] confirmed the rapid and dramatic effect of surfactant therapy on lung compliance, oxygenation and ventilation in a 3-year-old boy with refractory respiratory failure due to near-drowning. Knoch *et al.* [66] reported a case of surfactant therapy in a 48-year-old patient who developed respiratory insufficiency nine days after a bicycle accident. The left lung could not be ventilated even after separate artificial ventilation of each lung. After administration of a bolus of synthetic surfactant (50 mg/kg) and continued separate artificial ventilation on each side, there was a complete re-expansion of the left lung with an increase of arterial PO_2 values from 65 to 416 mmHg within a few hours ($FiO_2 = 1.0$). The results of first

clinical studies of surfactant therapy in ARDS patients are described in the next paragraph.

Surfactant and infectious lung diseases
Pneumonia is an important cause of respiratory failure and is associated with increased alveolar permeability leading to pulmonary edema, hemorrhage and atelectasis [67,68]. The pathophysiological changes in pneumonia include hypoxemia, decreased functional residual capacity (FRC), decreased total lung capacity (TLC), decreased lung compliance, and a diminished surfactant system [14,68-70].

As far back as 1964, Sutnick and Soloff [67] demonstrated that the surface tension of BAL fluid from lung tissue with pneumonia was increased; they suggested that the pulmonary surfactant became inactivated and was responsible for atelectasis. It has since been demonstrated that the surfactant system is also impaired in bacterial [14,70] and viral pneumonia [27], as well as in *Pneumocystis carinii* pneumonia [71,72]. In bacterial pneumonia surface tension of BAL fluid is increased, whereas SP-A content and total surfactant lipids are all significantly decreased [14]. In viral pneumonia, Stinson *et al.* [27] demonstrated that pulmonary surfactant activity is decreased; these workers suggested that injury and destruction of type II pneumocytes by the virus was the cause of reduced surfactant activity. Recently, two studies have demonstrated surfactant abnormalities in HIV positive patients with *Pneumocystis carinii* pneumonia [71,72]. In these patients qualitative and quantitative changes were seen in the surfactant composition, as well as increased phospholipase A_2 activity [72].

Bacteria, bacterial toxins, viruses, phospholipases, and proteinases released from inflammatory cells interact either directly with the surfactant film (Figure 4), or damage the endothelial and epithelial cells leading to high permeability edema [14]. It is well established that plasma proteins of the edema fluid inactivate the surfactant [26]. Due to the decreased surfactant activity, surface tension at the alveolar walls increases, leading to increased suction forces across the alveolo-capillary membrane [22]. This finally results in a vicious circle [13,73].

Evidence thus exists of a deficiency of active pulmonary surfactant in patients with pneumonia and this would be the rationale for exogenous surfactant therapy. We recently demonstrated the effectiveness of surfactant therapy in different animal models suffering from viral pneumonia or *Pneumocystis carinii* pneumonia [74-76]. In viral pneumonia, tracheal administration of exogenous surfactant led to improved lung

compliance and improved functional residual capacity (FRC) [74], as well as to restoration of gas exchange [75]. Similarly in rats with *Pneumocystis carinii* pneumonia, surfactant instillation led to an improvement in blood gases [76]. The therapeutic dosage in all three experimental studies was 200 mg surfactant/kg. Song *et al.* [77] recently confirmed the rapid effect of surfactant therapy (160 mg/kg) on arterial oxygenation in rats with *E.coli* pneumonia. These results show that there was a shortage of functional alveolar surfactant in these animals with infected lungs.
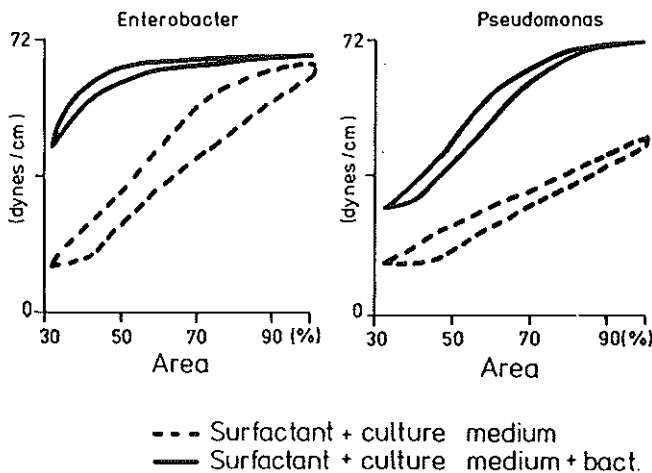
Figure 4. Surface tension (dynes/cm) vs. area for surfactant mixed with medium compared with surfactant mixed with medium and bacteria after incubation of 24 h. Surface tension is increased when surfactant is mixed with a bacterium. The surface tension is measured with a Wilhelmy balance.

A few preliminary reports indicate that instillation of exogenous surfactant might be efficacious in patients with infectious lung diseases. Lachmann [28] has treated a four-year-old patient with a bacterial pneumonia and acute respiratory failure. Surfactant was instilled three times in succession (150; 100; 50 mg/kg) and, after the last dose of surfactant, gas exchange improved dramatically. Chest X-ray taken four hours after surfactant instillation showed nearly 'normal' lungs. Buheitel *et al.* [78] reported exogenous surfactant therapy in two patients with acute respiratory failure due to viral pneumonia. In the first patient, 5.5 months old, gas exchange improved immediately after tracheal instillation of a natural surfactant (300 mg/kg) but was

sustained only for three hours. A second dose was given (215 mg/kg) 12 hours after the first one, arterial oxygenation improved slowly over time and after five weeks the boy could be extubated and discharged in good health. The second patient was almost four years old and was pressure-controlled ventilated as follows: peak airway pressure of 41 cm H_2O, PEEP of 12 cm H_2O, I/E ratio of 2:1 and FiO_2 of 0.95. Blood pressure decreased several times before surfactant instillation, probably as a result of the high ventilator pressures. After a natural surfactant (50 mg/kg) was instilled, arterial oxygenation improved immediately and peak pressure could be reduced from 41 to 30 cm H_2O. However, six hours after surfactant instillation the patient died, probably as a result of cardiovascular failure. Putz *et al.* [79] confirmed the successful treatment of ARDS caused by viral pneumonia in a 3-year-old boy with a bolus of a natural surfactant (200 mg/kg). Slater *et al.* [80] reported an infant with *Pneumocystis carinii* pneumonia associated ARDS who failed to respond to standard therapy, including corticosteroids, but improved dramatically with artificial surfactant (40 mg/kg).

Mikawa *et al.* [81] showed an improvement of oxygenation after *selective* instillation of exogenous surfactant in a 71-year-old man who developed lobar bacterial pneumonia and unsatisfactory oxygenation following abdominal surgery. On post-operative day five, surfactant was instilled via a bronchofiberscope which enabled deposition of a small amount of surfactant in the infected lobe only. This method of instillation was probably chosen due to the prohibitive price of surfactant and the non-availability of sufficiently large amounts of surfactant for use in adults. Immediately after surfactant application, oxygenation increased; this improvement was not dramatic but this may be attributed to the low dose of surfactant (240 mg) given. One may speculate that if surfactant had been administered to the whole right lung, the increase in oxygenation would be more striking.

The reported experimental and clinical findings support the role of exogenous surfactant therapy in bacterial, viral and *Pneumocytis carinii* pneumonia. Pneumonia and ARDS are closely associated. Not only is ARDS often complicated by nosocomial infections, but infection can also lead to ARDS [82].

Although these case-reports of surfactant therapy in ARDS and infectious lung diseases showed that some patients had only a transient improvement after a single dose of surfactant, better results are seen with higher or multiple surfactant doses. This was recently confirmed by two pilot studies [83,84]. Gregory *et al.* [83] studied four different dosing strategies in 48 adults with ARDS and the results showed that

maximum improvement in oxygenation, minimum ventilatory requirements, and the lowest mortality rate were obtained by using four doses of 100 mg/kg of a natural surfactant (total amount of 400 mg/kg). Walmrath and colleagues [84] reported an impressive acute improvement of arterial oxygenation in response to bronchoscopic application of a large quantity of natural surfactant (300 mg/kg) in 10 adult patients with severe ARDS and sepsis. In half of their patients, a second dose (200 mg/kg) was required within 24 h to achieve a prolonged effect on gas exchange. In contrast to these results, Anzueto *et al.* [85] demonstrated that administration of aerosolized artificial surfactant had no effect on mortality and lung function in a multicenter, randomized placebo-controlled trial in 725 patients with sepsis-induced ARDS. The authors speculated that one of the reasons for the lack of response could be that less than 25 mg surfactant per kg body weight was actually delivered into the lungs due to the method of administration, which is only one-sixteenth of the dosage used by Gregory and colleagues [83].

Thus, the reason for lack of response or only transient improvement after exogenous surfactant application in patients with ARDS has been attributed to the inhibition of the instilled surfactant by plasma components filling the alveolar space [26]. Therefore, the therapeutic goal must be to overcome the inhibitor capacity by large amounts of exogenous surfactant. This implies that if after surfactant instillation there is no, or only transient, improvement of blood gases in these patients (fibrotic lungs excluded), this does not mean that surfactant treatment does not work but only that the concentration of the exogenous surfactant used is too low in relation to the amount of surfactant inhibitors in the lung. We [86] have demonstrated in rats that approximately 1 mg surfactant phospholipids is required to overcome the inhibitory effect of 1 mg plasma proteins (see *Chapter 5*).

Surfactant and patients following cardiopulmonary by-pass

It has been seen that cardiopulmonary by-pass (CPBP) causes atelectasis, low pulmonary compliance, decreased diffusing capacity, and pulmonary hemorrhage [87]. Various explanations have been proposed including a loss or inhibition of surfactant. do Campo and colleagues [15] measured the total phospholipid concentration of BAL fluid of patients immediately after cardiac surgery with CPBP and found a significantly lower concentration of total phospolipids than in normal patients. Furthermore, radiological pictures taken immediately post-operatively appeared similar to ARDS

[88].

In this respect, the same investigators treated these patients with nebulised exogenous surfactant and found improved arterial oxygenation [89]. The magnitude of improvement was higher in the group treated with 30 mg/kg than in the group receiving 10 mg/kg. Strüber *et al.* [90] reported good result of surfactant therapy in a 38-year-old patient with extensive coronary disease in whom reperfusion injury of the lung developed after CPBP. Treatment was first started with inhaled nitric oxide at a concentration of 30 ppm and arterial PO_2 increased from 50 to 160 mmHg with 100% oxygen. However, lung compliance continued to drop and two days later, gas exchange deteriorated in spite of nitric oxide inhalation. Then, a bolus of exogenous synthetic surfactant was applied, resulting in an increase of compliance from 18 to 35 ml/mmHg and gas exchange improved from 70 to 220 mmHg. Because lung function had improved but heart failure remained, the patient was registered for heart transplantation. Finally, the patient was discharged from the hospital with excellent cardiopulmonary function.

Surfactant and asthmatic attack
In an asthmatic attack, increased mucus secretion, transudation of proteinaceous fluid and mucociliary disturbance causes mucus plug formation [91]. Even 23 years ago, Macklem and co-workers [19] concluded that the existence of bronchial surfactant is a prerequisite for normal lung function and that disturbance of the bronchial surfactant leads to airway obstruction and impaired bronchial clearance. It is tempting, therefore, to speculate on the possible role of surfactant in reversing airway obstruction in asthma attack [92]. Furthermore, it has been demonstrated that beta-adrenergic agents and glucocorticoids, which are two of the most widely used medications for the treatment of asthma, stimulate the release of surfactant and/or the production of surfactant [93,94].

Liu *et al.* [95] showed that surfactant dysfunction developed in sensitized guinea-pigs challenged with aerolized ovalbumin. Surface activity of BAL from immunized, challenged animals was significantly reduced, but there was no change in the concentration nor in the composition of the surfactant phospholipids. However, the BAL fluid showed a substantial increase in the concentration of proteins, and that was the likely reason for the increased surface tension. In addition, Liu *et al.* [96] recently reported that repeated challenges of immunized guinea-pigs resulted in decreased

synthesis and storage of pulmonary surfactant in type II cells. Further, the possibility that surfactant might play an important role in the pathogenesis of asthma was substantiated first by Lachmann *et al.* [97] and later confirmed by Liu *et al.* [98] who showed that the increase in airway resistance, following the challenge of immunized guinea-pigs, was less prominent when surfactant had been instilled into the airway prior to the challenge.

At this moment, human data are rare. Kurishima and co-workers [99] showed in a pilot study of 11 patients that surfactant inhalation had a therapeutic effect in asthmatic attack. Adult patients were assigned to either placebo (=saline) or surfactant inhalation (1 ml; 10 mg/ml). After surfactant inhalation (by a hand jet nebulizer), respiratory functions markedly improved; FVC, FEV_1 and PaO_2 increased with $11.7 \pm 1.3\%$, $27.3 \pm 4.4\%$ and $13.4 \pm 0.8\%$, respectively. However, Bambang Oetomo *et al.* [100] found that inhalation of 100 mg nebulized natural surfactant did not alter airflow obstruction and bronchial responsiveness to histamine in 12 asthmatic children with severe airflow obstruction. In that study [100] exogenous surfactant was applied during a stable phase of the disease process and not during an asthma attack. Lemarchand and colleagues [101] found that the bronchial clearance of DTPA is only increased in asthmatic patients during attacks but not increased in asthmatic patients with chronic airflow limitation (i.e. stable phase), or asthmatics without airflow limitation but with bronchial hyperresponsiveness to metacholine. Further, it was shown that the bronchial clearance of DTPA decreased toward normal levels after recovery from the acute attack. Kurashima *et al.* [102] recently reported that in the acute phase of an asthma attack minimal surface tension and total protein of airway fluid increased significantly in these patients, and decreased again in the recovery phase of the attack. Further, these authors demonstrated no difference in surface activity of sputum between patients with stable asthma and healthy controls. In addition, it was shown that total phospholipids of airway fluid increased significantly in the recovery phase of an attack, indicating an increased secretion of surfactant from type II cells. In conclusion, these results suggest that the many different facets of surfactant function in the airways need to be considered in the interpretation of the pathogenesis of bronchial asthma.

Surfactant and artificial ventilation

Studies have shown that ventilator modes with large tidal volumes and high peak inspiratory pressures during artificial ventilation affect the pulmonary surfactant system

[16,103]. The exact mechanism by which the surfactant system is affected by artificial ventilation is not yet entirely clear. One factor is that the surfactant in the alveolar lining is actively removed from the alveolus towards the larger airways; this can lead to a shortage of surfactant at alveolar level causing the changes in surface tension characteristics in the lung seen during or after prolonged periods of mechanical ventilation [104].

During end-expiration the surfactant molecules covering the alveolar epithelium are compressed on the small alveolar area (leading to low surface tension or a high surface pressure) thus preventing the alveoli from collapse [16]. If the surface of the alveolus is smaller than the surface occupied by the surfactant molecules, the molecules are squeezed out of the surface of the alveolus and forced towards the airways. These surfactant molecules are then 'lost' for the alveoli and are eventually cleared from these alveoli. During the following inflation of the alveoli, the surface is replenished with surfactant molecules coming from the underlying hypophase where surfactant molecules in micelles are 'stored' for later use. During the next expiration, the mechanism repeats itself and again surfactant molecules are forced out of the alveolus and subsequently replenished from the hypophase; this is a continuing cycle (Figure 5) [16].

The amount of surfactant that must be produced and subsequently secreted by the alveolar type II cells is proportional to the loss of surface active molecules during the breathing cycle. When production and secretion of new surfactant molecules keep pace with consumption, no surfactant deficiency can occur, as in a normal healthy lung.

Thus, artificial ventilation should take place at a lung volume equal or higher as the functional residual capacity level with the smallest possible volume and pressure changes. Another factor that might be of some importance is that mechanical ventilation especially in non-homogeneous lungs, creates severe shear forces between open and closed airways and possible overstretch of the epithelium during the breathing cycle, resulting in necrosis and desquamation of bronchiolar and alveolar epithelium [105]. The overstretch of the intercellular junctions of the epithelium leads to an increased permeability with the result of surfactant inhibition (as described earlier).

Insp.   Exp.

A

5 cm H₂O

5 cm H₂O

Inspiration   End-inspiration

B

30 cm H₂O

5 cm H₂O

C   Exp.   Insp.

5 cm H₂O

30 cm H₂O
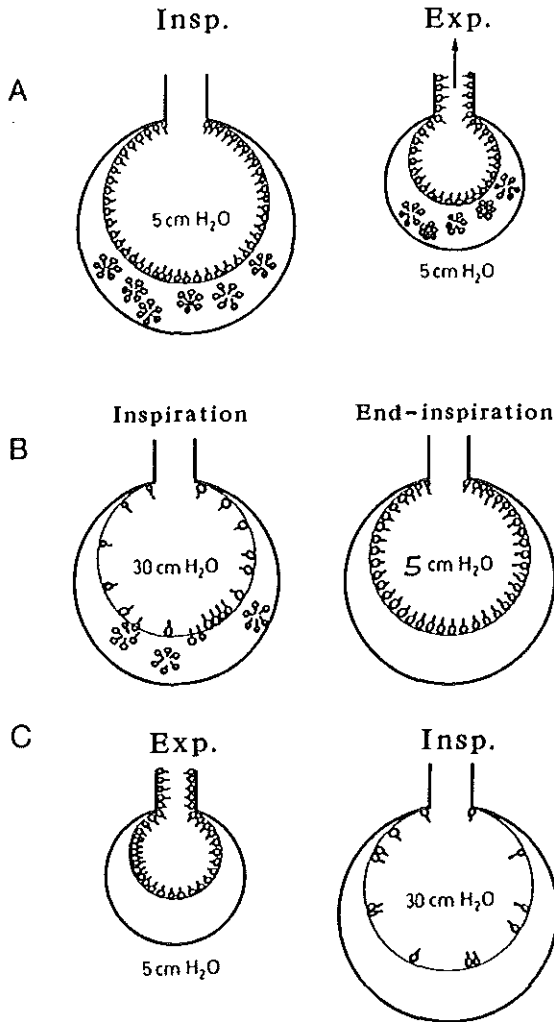
Figure 5. (A) Balance between synthesis, release and consumption of surfactant in the healthy lung. The pressure values given represent the intrapulmonary pressure needed to open up this alveolus. At the surface and in the hypophase (micelles), there are sufficient molecules of surfactant. These micelles deliver the surfactant necessary to replace the molecules squeezed out during expiration (Exp.). (B) Imbalance between synthesis, release and consumption of surfactant due to artificial ventilation. At the beginning of inspiration, there exists an apparent deficiency of surfactant molecules but there is a respreading of molecules stored in the hypophase to the surfactant layer. At the end of inspiration there is, in principle, enough surfactant on the surface. (C) With the next expiration (Exp.), 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. Insp. = inspiration (from reference [16]).

Delivery techniques, timing, type of surfactant, and ventilatory support

The optimal method to deliver exogenous surfactant is not yet known. Possibilities include aerosol delivery, continuous infusion, lung lavage or bolus administration. The latter method has been used in most animal studies, clinical case reports, as well as in neonates with RDS [9,11]. The advantage of this method of instillation is that it is rapid and capable of delivering large quantities of surfactant that are necessary, especially in ARDS, to overcome the inhibitory effects of the serum proteins present in the alveoli. Van der Bleek *et al.* [106] also demonstrated that the distribution of endotracheal instilled surfactant is more homogeneous after a large bolus than after a smaller one. This could be confirmed by Segerer and colleagues [107] who demonstrated a homogeneous pulmonary surfactant distribution after bolus instillation, whereas distribution after slow tracheal infusion of exogenous surfactant was extremely uneven. In this study, it was shown that the distribution of surfactant was closely related to its effect on pulmonary gas exchange [107]. Results from studies in premature animals showed that administration of surfactant directly after birth gives a more homogeneous distribution than in animals ventilated before treatment [108]. Instillation of surfactant into lungs which are filled with intrapulmonary fluid could be compared with instillation of a very large bolus of surfactant in sick lungs. Therefore, one may speculate that one has to fill up at least the total dead space of the lungs with surfactant suspension for a more even distribution. However, the disadvantage of bolus instillation technique is the relatively large amount of fluid which has to be instilled. However, Gilliard *et al.* [109] demonstrated that the volume of fluid in which surfactant is administered is rapidly absorbed; 30 minutes after surfactant instillation, there was no significant difference between the lung weights of animals with lung injury receiving 5 ml and those of animals receiving 50 ml of surfactant suspension. Thus, studies in which exogenous bolus instillation show heterogeneous distribution may be explained by too small an amount of fluid of each single bolus [109-111].

In addition, exogenous surfactant delivered as an aerosol has been investigated [110,111]. The rationale was that by this method of instillation less volume of liquid will be instilled into the lungs at one time and that the distribution will be more homogeneous. In two different animal models, Lewis *et al.* [110,111] could demonstrate that the distribution pattern was more homogeneous after aerosolized surfactant administration. However, they found that tracheally instilled surfactant was superior to aerosolized surfactant in improving blood gases, whereas there was no

difference concerning improvement of lung mechanics. They suggested that 'the low quantities of aerosolized surfactant deposited in the lungs limited the physiological responses'. In this study, only $6.1 \pm 2.2\%$ of the total aerosolized surfactant was recovered in the peripheral lung tissue whereas after bolus instillation $51 \pm 2\%$ of the instilled surfactant was recovered [111]. They concluded that: 'a disadvantage of aerosolized surfactant administration is the relatively long time period required to administer significant quantities of exogenous surfactant due to the inefficiencies of aerosol deposition'. Also, aerosolized surfactant could not be considered cost-effective because large quantities of surfactant are required. Recently, the same group of investigators also showed that it was impossible to improve gas exchange after aerosolized surfactant in a non-uniform pattern of lung injury [112]. In this study the less injured areas of the lung received relatively more surfactant than the severely injured areas. They conclude that 'one should be cautious in administering aerosolized surfactant to patients with ARDS who have non-uniform infiltrates on chest radiograph' but this a contradiction because ARDS lungs are always injured in a non-uniform way [113].

An alternative approach to administer surfactant is by lung lavage. We [51] have shown that lavaging the lung with a diluted surfactant suspension (3.3 mg/ml, 30 ml/kg) was as effective as high-bolus administration (200 mg/kg) to improve gas exchange in a model of acid aspiration. In lung lavaged rabbits, Balaraman *et al.* [114] demonstrated that the effectiveness of a synthetic surfactant, which has not been shown to be highly effective in various animal models [115,116], was enhanced by administering the surfactant by means of a lavage procedure compared to normal bolus administration. Further, Enhorning [117] has suggested that saline lavage can also be used to reduce the protein content intra-alveolar, which would be beneficial in the treatment of ARDS. This idea has been investigated by Kobayashi *et al.* [49] and these workers demonstrated that a relative low dose of exogenous surfactant (75 mg/kg) could only improve gas exchange after intra-alveolar edema was removed by lung lavage with saline in rabbits with severe respiratory failure due to acid aspiration. However, lung lavage with saline will also remove the endogenous surfactant, leading to further deterioration of the pulmonary function [118]. Therefore, we [119] performed lavage with a diluted surfactant suspension prior to surfactant therapy and showed that this combination was the optimal treatment regime compared to the alternatives in a model of severe respiratory failure (see *Chapter 6*).

Another important aspect of surfactant response is the time at which surfactant is given. In a model of acid aspiration, we [50] showed that respiratory failure can be prevented when exogenous surfactant was given before deterioration of lung function (i.e. within 10 min after acid aspiration), whereas after development of respiratory failure exogenous surfactant served only to prevent further deterioration of lung function but did not restore gas exchange (see *Chapter 4*). In the model of repeated saline lavage, Ito *et al.* [120], and later confirmed by our group [119], demonstrated that exogenous surfactant at an early stage of lung injury resulted in a sustained improvement of lung function whereas lung function deteriorated when surfactant was given at a relative late time point in lung injury, due to increased amount of proteins (see also *Chapter 6*). Therefore, it is expected that early treatment of patients with ARDS may require smaller amounts of exogenous surfactant and the outcome results will probably be better.

Various surfactant preparations are already available on the market and have been used successfully in worldwide clinical trials in neonates with RDS [9]. In lung lavaged rats, we [121] compared eight clinically used surfactants under standardized conditions and confirmed previous results of several animal studies [115,116] and studies in neonates with RDS [122,123] that the natural surfactant preparations, which contain the hydrophobic peptides SP-B and SP-C, are more effective in improving lung function immediately after instillation than the artificial surfactant preparations without surfactant proteins. In the same study [121], we showed that the effect of surfactants on oxygenation was, in general, dose-dependent and we found even marked differences in response pattern between the natural surfactants, especially when PEEP was reduced at the end of the study protocol (see *Chapter 3*).

Several experimental studies have shown that ventilator pattern strongly influences exogenous surfactant efficacy [124-129]. Recently, Froese *et al.* [126] have demonstrated that in lung lavaged rabbits the effect of exogenous surfactant on arterial oxygenation remained stable only in combination with high-frequency oscillatory ventilation (HFOV) at high-lung volume, whereas not with HFOV at low-lung volume or conventional mechanical ventilation (CMV) at high or low lung volume. High-lung volume means that lungs are actively opened (re-expanded) and then kept expanded by using relative high mean airway pressures [128]. These results are in contrast to our findings [129] in which surfactant therapy in combination with HFOV was not superior to CMV in increasing lung function and/or reducing lung injury in lung lavaged rabbits

(see *Chapter 7*). Further, it has been shown that HFOV has also a beneficial effect on exogenous surfactant composition by reducing the conversion of exogenously administered surfactant into small aggregates (non-active) forms [126]. This has been attributed to the small alveolar volume changes with HFOV. However, we have shown in another study [130] that this can also be obtained with CMV by using small pressure amplitudes and high end-expiratory pressures (see *Chapter 8*).

Contra-indications for exogenous surfactant therapy
From clinical experience, it has been shown that there are also contra-indications for exogenous surfactant treatment. For example; exogenous surfactant was instilled in a patient who got a virus pneumonia after near-drowning in sweet water and who was ventilated for more than two weeks. However, immediately after surfactant instillation, blood gases decreased and, after increasing mean airway pressure, blood gases deteriorated still further. Considering these observations, it was concluded that the lungs may be fibrotic and that the airways had been filled up with surfactant suspension. The fact that the increase in mean airway pressure led to a further deterioration may be explained by impairment of the capillary perfusion. After reducing the mean airway pressure to levels lower than the pre-treatment period, at least the same blood gas values were found. Unfortunately, a few hours later the patient died of multi-organ failure. The autopsy showed almost totally fibrotic lungs. Thus, the few parts of the lung in which some gas exchange took place were filled with exogenous surfactant. This could be the reason why blood gases deteriorated after surfactant therapy.

The rationale for giving surfactant is always to recruit collapsed alveoli and to stabilise them with the applied ventilator settings. Before exogenous surfactant therapy is applied, one therefore has to evaluate by lung function tests whether or not sufficient parts of recruitable lung areas are still available. Surfactant should thus not be given to patients with heavily consolidated and/or fibrotic lungs in which surfactant could not effectively improve lung function.

Conclusion
Results from these experimental and first clinical case reports show that the pulmonary surfactant system is at least involved in other diseases than the neonatal RDS, such as ARDS, pneumonia, etc. We conclude that after injury to the alveolo-capillary

membrane, followed by capillary leakage, the surfactant system will be responsible for further pathophysiological changes (Figure 6) [131]. These well-documented functional disturbances in the lung will finally result in the failure of the lung as a gas-exchange organ.
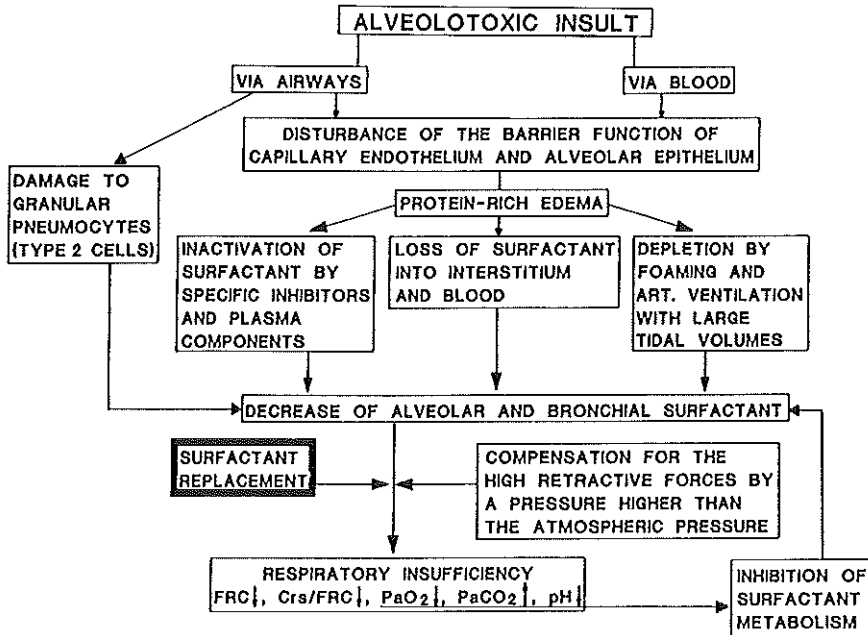
Figure 6. Pathogenesis of ARDS with special reference to the surfactant system, including suggestions to compensate for a damaged surfactant system. (from reference [131]).

Surfactant therapy seems a promising approach for the treatment of acute respiratory failure in ARDS and ARDS-like syndromes. However, many questions remain unanswered: for example, which patients should be treated (e.g. in extended pulmonary fibrosis no effect can be expected after surfactant administration), when should surfactant treatment start, which dosage and type of surfactant should be used, the method of administration, the type of ventilatory support, and many others. Some of these questions have been addressed in the following chapters.

Surfactant has been commercially available for infants for about six years; if one would apply only a dosage as used for neonates (100 mg surfactant/kg) in adult patients, the amount of surfactant needed would be at least 7-10 g. At current prices,

the costs of one treatment for one adult would then be US$ 30,000-50,000. So the price of exogenous surfactant preparations has first to be lowered before exogenous surfactant therapy in adults can become a reality.

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Chapter 2

Exogenous surfactant therapy increases static lung compliance, and cannot be assessed by measurements of dynamic compliance alone

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Summary

Objective: To study the immediate effects of exogenous surfactant therapy on blood gases, lung volumes and lung mechanics in adult rabbits with experimentally induced respiratory distress syndrome.

Design: Prospective randomised, controlled study.

Setting: Laboratory and animal facility of a large university.

Subjects: Twelve adult New Zealand white rabbits.

Interventions: Respiratory failure was induced by repeated bilateral whole-lung lavage with saline (30 ml/kg body weight). After the last lavage, the animals were randomly assigned to two groups. Group 1 received surfactant (120 mg/kg body weight) that was suspended in a 0.6% sodium chloride solution. Group 2 received comparable volumes of the same hypotonic solution and served as controls.

Measurements and main results: Before and after endotracheal surfactant instillation, blood gases and functional residual capacity were measured, and lung mechanics from tidal volumes and pressure-volume curves were calculated. Functional residual capacity was measured with a computerized, multiple-breath, washin-washout method using sulfur hexafluoride (SF_6) as tracer gas. The pressure-volume curves were obtained by an occlusion technique originally described for measuring static breath-by-breath compliance. The technique was modified for present use and fully computerized. Within 60 min after surfactant instillation, there was a marked improvement in PaO_2 (61 ± 7 mmHg [8.2 ± 0.9 kPa] to 470 ± 47 mmHg [62.6 ± 6.2 kPa]) and in functional residual capacity (7.6 ± 1.4 to 17.7 ± 1.6 ml/kg body weight) at unchanged ventilator settings. The pressure-volume curves became steeper over time and the pressure-volume curves for total lung volume were restored to an almost normal state. Maximum compliance calculated from the pressure-volume curves increased by 92% but there was no significant change in dynamic compliance. In the control group, no improvements in any measured or calculated lung parameters were seen.

Conclusions: The findings indicate that during mechanical ventilation, the effects of surfactant therapy on lung mechanics are best characterized by changes in functional residual capacity and maximum compliance obtained from static pressure-volume curves and not by dynamic compliance.

Introduction

Patients with respiratory distress syndrome have impaired surfactant function; the physiologic abnormalities associated with respiratory distress syndrome may be related to this diminished function [1]. Exogenous surfactant therapy should, therefore, result in improved lung function, which has already been demonstrated by the improvement of arterial oxygenation in clinical trials [2] with premature babies and a few studies [3,4] on patients with adult respiratory distress syndrome. It seems reasonable to assume that such improvement in gas exchange is caused by an immediate improvement in lung volumes and lung mechanics. However, studies [5-8] in which respiratory compliance was calculated from tidal volumes (dynamic compliance) during mechanical ventilation in surfactant-treated infants did not show any immediate changes. These seemingly paradoxical results may be explained by a surfactant-induced left-shift and increase of the pressure-volume curve.

Thus, after exogenous surfactant therapy with unchanged positive end-expiratory pressure levels and unchanged ventilatory settings, the lung would operate on the upper, more flattened part of the pressure-volume curve. We investigated this hypothesis by measuring blood gases and functional residual capacity, and by calculating lung mechanics from tidal volumes and pressure-volume curves both before and after endotracheal surfactant instillation in adult rabbits with acute respiratory distress syndrome.

Materials and methods

Preparation of animals. This study was approved by the local Animal Committee at the Erasmus University, Rotterdam, and the care and handling of the animals were in accord with the European Community guidelines (86/609/EEG). In 12 adult New Zealand white rabbits (CPB, Zeist, The Netherlands) (body weight 2.7 ± 0.3 kg), anesthesia was induced with sodium pentobarbital (50 mg/kg intravenously) and maintained by intermittent intraperitoneal injection of the same drug (5 mg/kg/h). For muscle relaxation, pancuronium bromide (0.3 mg/kg/h intramuscularly) was given. After tracheostomy was performed, the animals were pressure-control ventilated by a Servo Ventilator (900C, Siemens-Elema, Solna, Sweden) at the following settings: frequency of 20 cycles/min; inspiratory/expiratory ratio of 1:2; FiO_2 of 1.0; peak pressure of 9 to 12 cm H_2O and zero end-expiratory pressure. The left carotid artery was cannulated for continuous monitoring of arterial pressure and to allow for blood

sampling. Maintenance fluid consisted of 5 ml/kg/h of 2.5% glucose. Five millilitres of sodium bicarbonate (0.8%) was given when base excess was <-5 mmol/l. Body temperature was maintained within the normal range by means of a heating pad.

Lung lavage. Respiratory failure was induced by repeated lung lavage [9]. Each lavage was performed with saline (30 ml/kg body weight) heated to 37 °C. During lavage, tracheal pressure was measured and not allowed to increase to >40 cm H_2O. Lung lavage was repeated five to eight times at 5-min intervals until PaO_2 was <80 mmHg (10.6 kPa) at a peak pressure of 26 cm H_2O and a positive end-expiratory pressure (PEEP) of 6 cm H_2O with 100% oxygen; inspiratory-expiratory ratio and frequency were not changed. The animals were randomly assigned to two groups 10 min after the last lavage. Group 1 (n=6) received surfactant 120 mg/kg body weight, which was suspended in a 0.6% sodium chloride solution. Group 2 (n=6) received comparable volumes of the same hypotonic solution (0.6% sodium chloride) and served as controls. Immediately after disconnection from the ventilator, the animals received via a syringe either 3 ml surfactant and 10 ml air per kg body weight, or 3 ml hypotonic solution and 10 ml air per kg body weight. Peak pressure was varied to keep the $PaCO_2$ at 30 to 45 mmHg (4.0 to 6.0 kPa) but peak pressure was never increased to >35 cm H_2O. PEEP was increased when PaO_2 was <60 mmHg (<8.0 kPa).

Surfactant. Natural surfactant was isolated from the lungs of recently slaughtered cows. The lungs were minced and the tissue fragments were washed with normal saline. The mixture of saline and tissue fragments was filtered, and the filtrate was centrifuged to remove cell debris. The supernatant was then ultra-centrifuged to obtain pellets of 'crude' surfactant. This 'crude' surfactant was extracted by the method of Bligh-Dyer [10]. The chloroform phase was evaporated and suspended in acetone to remove the neutral lipids. After centrifugation, the pellet was suspended in water and finally freeze-dried.

The surfactant consists of approximately 90 to 95% phospholipids and 1% hydrophobic proteins (surfactant-protein B and C), with the remainder being other lipids such as cholesterol, glyceride and free fatty acids. There is no surfactant-protein A in this surfactant preparation. The freeze-dried preparation was resuspended in a 0.6% sodium chloride solution to a total concentration of 40 mg phospholipids/ml.

Measurements. Data were collected at the following times: before lavage at PEEP of 0, 4, 6 and 8 cm H_2O; 5 min after lavage; 5, 15 and 30 min after surfactant or hypotonic solution instillation; and every 30 min for 3 h. At each data collection

point, PaO_2, $PaCO_2$ base excess, pH, functional residual capacity and dynamic compliance measurements were obtained. Pressure-volume curves were recorded before and after lavage and at 15 min, 1, 2, and 3 h after surfactant or hypotonic solution instillation. PaO_2, $PaCO_2$, base excess and pH of the samples were measured by conventional methods (ABL 330, Radiometer, Copenhagen, Denmark).

Functional residual capacity was measured using a computerized, multiple-breath, washin-washout method, with sulfur hexafluoride (SF_6) as the tracer gas. The method has been described previously for studies of functional residual capacity and gas washout in adults, children and small neonates [11-13]. The components of the system (Figure 1) included a computer (PDP 11/23, Digital Equipment, Maynard, Marlboro, MA), a ventilator (Servo 900C, Siemens-Elema), electromagnetic inspiratory and expiratory auxiliary valve (360P0121, Neptune Research, Maplewood, NS), and inspiratory and expiratory heated Fleisch pneumotachograph no. "00" (Gould, Lausanne, Switzerland; linearity flow range of 0 to 100 ml/sec) with differential pressure transducers (MP 45-1-871, Validyne, Northridge, CA).

The tracer gas concentration was measured using an infrared analyser(Siemens) [14]. The transducer of the analyser was placed over a cuvette with windows near the T-piece in the expiratory part of the ventilatory tubings. Sulfur hexafluoride was washed in until the alveolar concentration was about 1.5%. Sulfur hexafluoride washout was continued until the concentration was <0.01%. Signals representing expired flow and sulfur hexafluoride concentration were fed into the computer, which calculated functional residual capacity as the value of sulfur hexafluoride washed out plus the amount left in the lungs at the end of washout divided by the alveolar concentration at the end of washin. The amount of sulfur hexafluoride remaining in the lungs was estimated, assuming that the final part of washout was monoexponential. The volume of the tubing between the auxiliary valves, including the bar of the T-piece, was 0.8 ml. All volumes were converted to body temperature and atmospheric pressure, completely saturated with water vapour at body temperature, by multiplying by 1.09. Apparatus dead space was subtracted from the obtained functional residual capacity values. Duplicate measurements were made on each occasion.

Dynamic thorax-lung compliance (C_{dyn}) was calculated as tidal volume/(peak pressure minus PEEP) at mechanical ventilation. Since the air flow at end-inspiration and end- expiration are zero, peak pressure and PEEP were assumed to be the alveolar pressure. The pressure-volume curves were obtained by an occlusion technique that was

originally described for the purpose of measuring static breath-by-breath compliance [15]. The technique was modified for present use and was fully computerized. After switching to the pressure-volume system (Figure 1), which was done during a prolonged inspiratory pause to prevent lung collapse, the lungs were inflated by a syringe to an airway pressure of about 30 cm H_2O. During the following deflation to 0 cm H_2O (equal to functional residual capacity), the expiratory flow was interrupted for 80 msec in a 160 msec cycle by a computer-governed electromagnetic occluder placed close to the tracheal tube. The airway pressure was measured at the end of each occlusion. The computer integrated the flow signal and calculated the volume expired between occlusion. After completion of the manoeuvre, the pressure-volume curve was plotted by an X-Y writer (Hewlett-Packard, Palo Alto, CA) and the data were stored on a computer disk. Total-lung-volume curves were also presented by the computer using the measured functional residual capacity value to calculate the total lung volumes at different airway pressures.

Static compliance ($C_{st26/6}$) was calculated from the pressure-volume curve above functional residual capacity level by dividing the difference between the volumes at the used peak pressure and PEEP. Compliance was also calculated from the pressure-volume curve above functional residual capacity level by dividing the difference between the volumes at pressures 10 ($C_{st10/0}$), 20 ($C_{st20/0}$) and 30 ($C_{st30/0}$) cm H_2O respectively, and 0 cm H_2O. Maximum compliance was defined as the steepest slope of the pressure-volume curve. The specific compliance (C_{spec}) was calculated by dividing static compliance ($C_{st26/6}$) by functional residual capacity. The lung stability index (L), according to Gruenwald [16], was calculated from the pressure-volume deflation curve as $L = (2V_5 + V_{10})/2V_m$, where V_5, V_{10} and V_m are the volumes at 5, 10 and 30 cm H_2O, respectively. Thoracic-lung compliance was also calculated for total lung volumes (inclusive functional residual capacity) at pressures of 10 ($C_{TLV10/0}$), 20 ($C_{TLV20/0}$) and 30 ($C_{TLV30/0}$) cm H_2O.

Statistical analysis. Analysis of variances (ANOVA) was used to assess whether there was an overall difference within or between the two groups. If a difference was found, a post hoc test was used (Student Newman-Keuls multiple comparison procedure). Statistical significance was accepted at $p \leq 0.05$. All data are reported as mean $\pm$ SD.
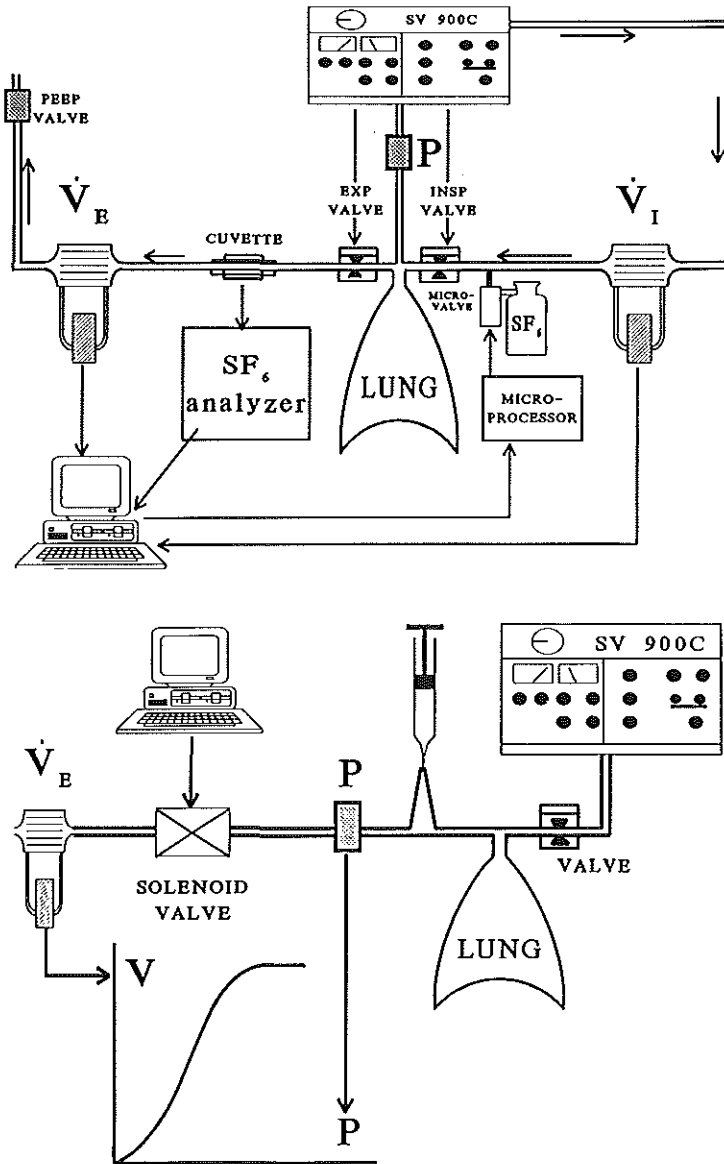
Figure 1. Schema of the functional residual capacity measurement system (*top*) and system for obtaining the pressure-volume curves (*bottum*). V_e, expiratory flow meter; V_i, inspiratory flow meter; P, pressure transducer; SF_6, sulfur hexafluoride; V, volume, *SV 900C*, Servo ventilator 900C.

Results

In healthy animals, before the lavage procedure, dynamic compliance decreased when PEEP was increased to >4 cm H_2O (Table 1). Lung volumes calculated from the pressure-volume curves that were compared with volumes from the functional residual capacity measurements of ≤ 8 cm H_2O, showed a close correlation ($r^2 = 0.99$).

Table 1. Influence of different PEEP levels on PaO_2, $PaCO_2$, functional residual capacity (FRC), and dynamic compliance (C_{dyn}) in healthy animals (n=12) (mean $\pm$ SD).

		PEEP (cm H_2O)			
		0	· 4	6	8
PaO_2	(mmHg)	480±32	506±24	510±36	513±20
	(kPa)	64.0±4.3	67.5±3.2	68.0±4.8	68.4±2.7
$PaCO_2$	(mmHg)	31±6	33±8	34±5	40±7
	(kPa)	4.1±0.8	4.4±1.1	4.5±0.7	5.3±0.9
FRC	(ml/kg bw)	7.6±1.5	14.3±2.5	18.4±2.7	22.5±3.3
C_{dyn}	(ml/kg bw)	2.17±0.47	2.24±0.54	2.02±0.56	1.32±0.18

bw, body weight.
Animals were mechanically ventilated, pressure controlled, at a peak pressure of 10.4±1.7 cm H_2O (above PEEP), a respiratory rate of 20 breaths/min, inspiratory-expiratory ratio of 1:2, and FiO_2 of 1. These ventilatory settings were not altered by changing the PEEP levels.

In all animals, lung lavage induced acute respiratory distress syndrome as demonstrated by a decrease in the mean PaO_2 from 479±32 mmHg (63.9±4.3 kPa) to 61±7 mmHg (8.2±0.9 kPa) (Figure 2). Functional residual capacity decreased by 66% compared with the same PEEP of 6 cm H_2O before lung lavage (Figure 3). Dynamic compliance and maximum compliance decreased by 43% and 47%, respectively, compared with prelavage levels (Tables 2 and 3).
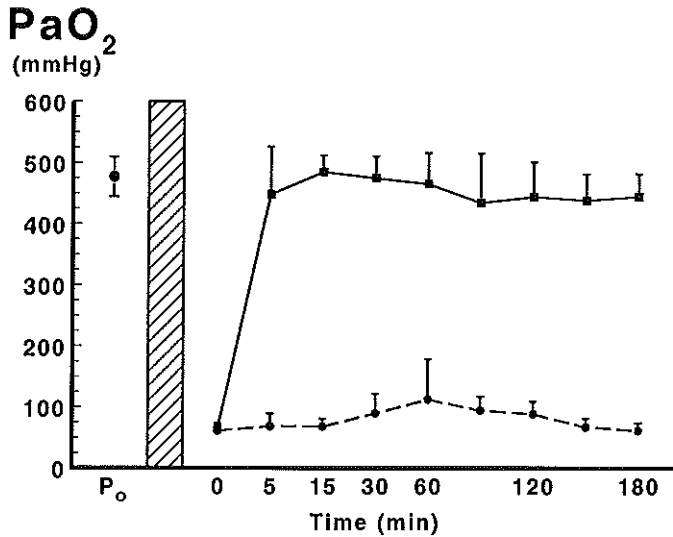
PaO$_2$
(mmHg)

Figure 2. Mean PaO$_2$ before lavage (P_0), postlavage (*time 0*), and after treatment with surfactant (*solid line*, 120 mg/kg) and hypotonic solution (*broken line*). The *bar* indicates the lavage procedure. There was a significant ($p \leq 0.05$) difference between surfactant-treated animals vs. controls. Compared with the postlavage values, there was a significant difference ($p \leq 0.05$) after surfactant instillation. Mean $\pm$ SD values. P_0, positive end-expiratoy pressure of 0 cm H$_2$O.
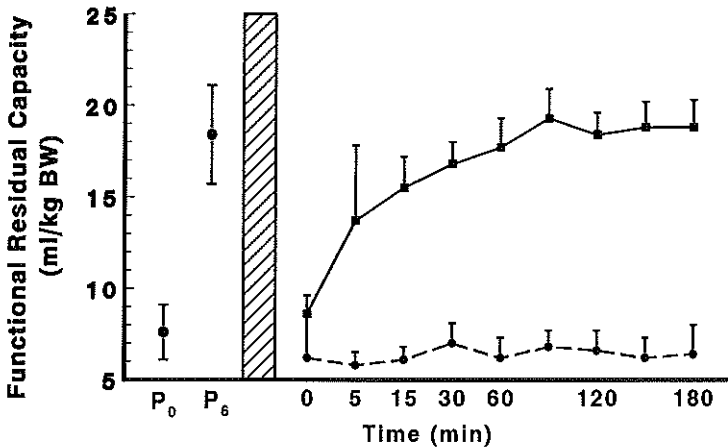
Figure 3. Functional residual capacity before lavage (P_0 and P_6), after lavage (*time 0*), and after treatment with surfactant (*solid line*) and hypotonic solution (*broken line*). The *bar* indicates the lavage procedure. There was a significant ($p \leq 0.05$) difference between surfactant-treated animals vs. controls. Compared with the postlavage values, there was a significant ($p \leq 0.05$) difference at 15 min after surfactant instillation until the end of the experiment. Mean $\pm$ SD values. BW, body weight; P_0, PEEP of 0 cm H$_2$O; P_6, PEEP of 6 cm H$_2$O.

After surfactant instillation, there was an immediate improvement in blood gases (Figure 2) and functional residual capacity (Figure 3) but no significant improvement in dynamic compliance, static compliance ($C_{st26/6}$) and specific compliance (Tables 2 and 3). Static compliance ($C_{st26/6}$) and specific compliance even decreased after surfactant instillation (Table 3). However, the stability index from Gruenwald, calculated from the deflation curve of the pressure-volume curve, static compliance at pressure 10 cm H_2O ($C_{st10/0}$), and maximum compliance, showed significant improvement after surfactant instillation and was also significantly different compared with the values of control animals (Table 3). After surfactant instillation, the pressure-volume curves became steeper over time and the total lung-volume curves were almost restored to the prelavage shape, whereas in control animals, the pressure-volume curves and total lung-volume curves became more flattened over time (Figure 4). Blood pressure was stable during the observation period.
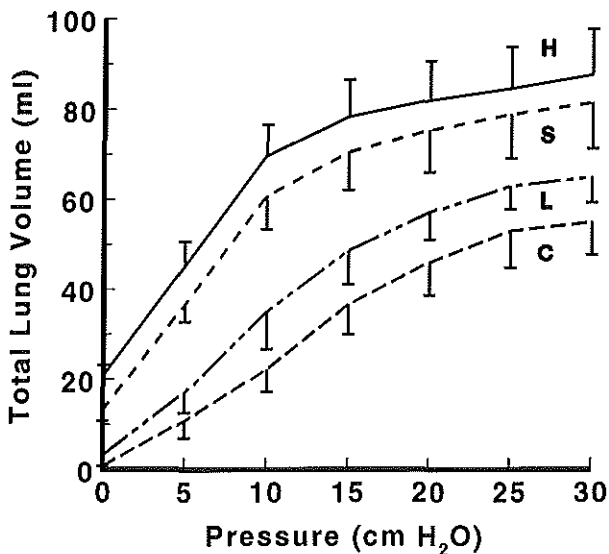
Figure 4. Pressure-volume curves of absolute lung volumes (inclusive functional residual capacity) measured before lavage (*H*), after lavage (*L*), and 60 min after endotracheal instillation of surfactant (*S*) or hypotonic solution (*C*). Mean ± SD values.

In the control group, it was necessary to increase peak pressure and PEEP to maintain sufficient gas exchange, whereas in the surfactant group, no changes in ventilator settings were necessary. In the control group, there was no improvement in any measured or calculated lung mechanic parameter (Figures 2 and 3; Tables 2 and 3).

Table 2. Changes in $PaCO_2$ (mmHg), tidal volume (V_T), and dynamic compliance (C_{dyn}) (mean $\pm$ SD)

	Pre	Group	Lav	Time after treatment (min)						
				5	30	60	90	120	150	180
$PaCO_2$	31.3± 5.6	S	37.8± 2.7	30.5± 3.2[a,b]	30.9± 2.0[a,b]	32.8± 2.3[a,b]	32.8± 2.9[b]	34.7± 3.7[b]	31.7± 3.4[a,b]	33.8± 0.9[a,b]
		C	44.8± 9.8	44.0± 9.9	40.0± 9.4	39.5± 5.2	37.8± 7.7[b]	40.8± 7.6	44.5± 7.9	51.8± 8.6
V_T (ml)	20.2± 3.5	S	21.5± 2.5	25.1± 2.5[b]	24.7± 3.1[b]	24.0± 3.2	23.7± 2.4	24.6± 2.2	24.7± 1.5	24.6± 1.5
		C	24.0± 4.5	23.7± 4.3	25.8± 3.2	27.0± 2.5	24.7± 2.8	24.2± 3.6	22.7± 3.2	21.6± 4.2
C_{dyn}	2.17± 0.42	S	1.11± 0.15[a]	1.29± 0.22	1.29± 0.23	1.27± 0.23	1.27± 0.20	1.16± 0.22	1.25± 0.18	1.22± 0.20
		C	1.21± 0.28	1.26± 0.22	1.22± 0.24	1.20± 0.39	1.20± 0.39	1.18± 0.22	1.16± 0.24	0.98± 0.22

[a], $p < 0.05$, surfactant treated vs. control. [b], $p < 0.05$, vs. postlavage values. Pre, prelavage. Lav, lavage. S, surfactant treated animals. C, control group treated with 0.6% NaCl solution. C_{dyn}, dynamic compliance in ml/cm H_2O.

Variable	Prelavage	Group	Lavage	15	60	120	180
				Time after treatment (min)			
$C_{st26/6}$	5.08 ± 0.68	S	2.53 ± 0.34	1.93 ± 0.29[b]	1.80 ± 0.26[b]	1.72 ± 0.29[b]	1.62 ± 0.42[b]
		C	2.54 ± 0.41	2.15 ± 0.54	1.77 ± 0.42	1.57 ± 0.20[b]	1.31 ± 0.24[b]
C_{spec}	0.24 ± 0.04	S	0.12 ± 0.01	0.05 ± 0.01[a,b]	0.04 ± 0.01[a,b]	0.04 ± 0.01[a,b]	0.04 ± 0.01[b]
		C	0.17 ± 0.09	0.13 ± 0.03	0.10 ± 0.02	0.09 ± 0.01[b]	0.07 ± 0.01[b]
C_{max}	6.85 ± 0.69	S	3.70 ± 0.36	6.82 ± 1.08[a,b]	7.10 ± 1.11[a,b]	7.25 ± 0.91[a,b]	7.37 ± 1.19[a,b]
		C	3.70 ± 0.53	3.48 ± 0.60	3.02 ± 0.44[b]	2.65 ± 0.37[b]	2.43 ± 0.39[b]
$C_{st10/0}$	5.07 ± 0.71	S	2.68 ± 0.80	4.28 ± 0.79[a,b]	4.77 ± 0.69[a,b]	5.05 ± 0.48[a,b]	4.85 ± 0.60[a,b]
		C	2.64 ± 0.45	2.33 ± 0.55	2.31 ± 0.61	2.02 ± 0.34[b]	1.95 ± 0.31[b]
$C_{st20/0}$	3.07 ± 0.43	S	2.90 ± 0.64	2.90 ± 0.46	3.12 ± 0.45[a]	3.18 ± 0.38[a]	3.05 ± 0.51[a]
		C	2.69 ± 0.40	2.59 ± 0.52	2.32 ± 0.37[b]	2.02 ± 0.34[b]	1.45 ± 0.21[b]
$C_{st30/0}$	2.20 ± 0.32	S	2.27 ± 0.38	2.15 ± 0.30	2.28 ± 0.33	2.31 ± 0.27[a]	2.21 ± 0.38[a]
		C	2.36 ± 0.20	2.21 ± 0.37	1.95 ± 0.26[b]	1.64 ± 0.28[b]	1.45 ± 0.21[b]
$C_{TLV10/0}$	7.17 ± 0.76	S	3.37 ± 0.66	5.78 ± 0.55[a,b]	6.12 ± 0.66[a,b]	6.44 ± 0.39[a,b]	6.19 ± 0.67[a,b]
		C	3.35 ± 0.91	2.88 ± 0.88	2.56 ± 0.81[b]	2.29 ± 0.44[b]	2.07 ± 0.47[b]
$C_{TLV20/0}$	4.17 ± 0.50	S	3.11 ± 0.75	3.64 ± 0.36[a,b]	3.79 ± 0.42[a,b]	3.86 ± 0.28[a,b]	3.77 ± 0.55[a,b]
		C	3.01 ± 0.38	2.89 ± 0.58	2.43 ± 0.55[b]	2.19 ± 0.34[b]	2.00 ± 0.34[b]
$C_{TLV30/0}$	2.96 ± 0.36	S	2.54 ± 0.55	2.58 ± 0.32	2.68 ± 0.32[a]	2.68 ± 0.25[a]	2.57 ± 0.38[a]
		C	2.51 ± 0.20	2.31 ± 0.29	2.03 ± 0.24[b]	1.71 ± 0.23[b]	1.58 ± 0.20[b]
Gruenwald-index	0.75 ± 0.06	S	0.40 ± 0.03	0.63 ± 0.05[a,b]	0.69 ± 0.04[a,b]	0.75 ± 0.05[a,b]	0.77 ± 0.08[a,b]
		C	0.35 ± 0.05	0.32 ± 0.07	0.36 ± 0.08	0.40 ± 0.08[b]	0.44 ± 0.09[b]

[a], $p < 0.05$, surfactant treated vs. control. [b], $p < 0.05$, vs. lavage values. S, surfactant treated animals. C, control group treated with 0.6% NaCl solution.

Discussion

It is well known that surfactant function is impaired in respiratory distress syndrome patients, and associated abnormalities may be related to this diminished function [1]. Therefore, exogenous surfactant therapy should result in improved lung expansion, increased functional residual capacity and decreased shunt [17]. Benefits of these changes include: a) enhanced blood oxygenation at lower fractions of inspired oxygen; b) avoidance of toxic levels of oxygen; c) use of lower PEEP with reduced cardio-circulatory depression; and d) lower peak airway pressures with reduced barotrauma [2,17]. The model used in this study indicates a condition similar to respiratory distress syndrome by repeated bilateral whole-lung lavage with saline [9]. This model is widely used for testing different ventilatory settings in respiratory distress syndrome and for surfactant replacement therapy because it remains stable for hours and presents histologic changes similar to those changes in respiratory distress syndrome [18-21]. In the present study, the ventilatory settings were kept constant because we were only interested in the effect of surfactant on lung mechanics; the influence of different ventilatory settings in this model is already known [18].

The method of measuring functional residual capacity gives accurate and reproducible results and does not require alteration of the breathing circuit or any interruption of ventilatory support. Also, due to the small amount of tracer gas needed (1.5% of sulfur hexafluoride), the method can be used at all inspired oxygen concentrations. Modifications of the functional residual capacity measurement system have been used in adults, children and neonates [11-13].

The method employed for measuring pressure-volume curves is adapted from an occlusion technique for static breath-by-breath compliance [14]. Although the occlusion period, 80 msec, appears short, a well-defined pressure plateau at the end of each occlusion was observed. Also, Carlo [22] found that pressure equilibrium was reached within 50-100 msec after an occlusion in a rabbit model of healthy and surfactant-depleted lungs. Another indication of the validity of the method is that increases in lung volume from 0 cm H_2O to the different levels of PEEP calculated from the pressure-volume curves were almost equal to the increases in lung volume obtained by functional residual capacity measurement ($r^2 = 0.99$).

This study demonstrates that treatment with exogenous surfactant immediately reverses hypoxemia and improves functional residual capacity, maximum compliance and static compliance related to total lung volumes (C_{TLV}). However, no significant

improvement in dynamic compliance was found. These results confirm those of other studies [5-8] in which there were significant improvements in oxygenation only, but no improvement in dynamic compliance, directly after surfactant instillation in neonates with respiratory distress syndrome. This discrepancy can be attributed to the increase of functional residual capacity and the change of the shape of the pressure-volume curve directly after surfactant instillation. The pressure-volume curve became steeper over time, the increase of maximum compliance and static compliance at pressure 10 cm H_2O ($C_{st10/0}$) are an expression of this shift (Table 3).

These two changes are best visualized by the total-lung-volume curves (Figure 4), which were almost restored to the healthy state after surfactant instillation. Thus, mechanically ventilating the animals that had been pressure-controlled with a peak pressure of 26 cm H_2O and a PEEP of 6 cm H_2O meant that the animals were ventilated at the upper, flatter part of the pressure-volume curve, resulting in a low value of dynamic compliance. Therefore, if a lower PEEP and tidal volume had been used, the lung would likely have operated on a steeper part of the pressure-volume curve after surfactant therapy and the dynamic compliance would have been increased. This possibility could explain the results of Davis and co-workers [6] who reported that during spontaneous breathing, dynamic compliance increased after surfactant therapy. In their study [6], the infants may not have generated the same transpulmonary pressure as in the mechanically ventilated group and were probably breathing on the steeper part of the pressure-volume curve. This observation remains speculative because no data on pressures (peak pressure, PEEP, continuous positive airway pressure) were presented.

Dynamic compliance was measured within the tidal volume at the used PEEP level. Changes in both tidal volume and PEEP markedly influence the value of dynamic compliance [23,24]. Schaffer and colleagues [24] showed that dynamic compliance values obtained at > 4 cm H_2O of PEEP are significantly less than those values obtained with a lower PEEP. These results are confirmed in the present study (Table 1). Thus, to compare values of dynamic compliance, it is essential to keep tidal volume and PEEP constant. However, since lung function improves after surfactant treatment, PEEP is usually decreased [2]. Therefore, it is difficult to interpret results from studies that have shown an improvement in dynamic compliance 24 h after surfactant therapy in mechanically ventilated neonates [5,7]. In these studies, the ventilatory settings were changed and mean airway pressure was decreased. Moreover, in surfactant-treated vs. nontreated groups, comparisons of dynamic compliance are not appropriate if tidal

volume and PEEP levels are not considered.

We conclude that dynamic compliance and static compliance ($C_{st26/6}$) do not accurately reflect changes in lung function after surfactant replacement therapy in surfactant-depleted, mechanically ventilated rabbits, and that the effects of surfactant therapy on lung mechanics are best characterized by changes in functional residual capacity and maximum compliance obtained from static pressure-volume curves.

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Chapter 3

Comparison of eight different surfactant preparations on improvement of blood gases in lung-lavaged rats

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Summary

A variety of surfactant preparations has been evaluated in different clinical studies but these differ too widely in their study design to allow conclusions about differences of efficacy. Therefore, we compared six natural (Alveofact*, BLES, Curosurf*, Infasurf*, Surfacten*, and Survanta™) and two synthetic (Alec™ and Exosurf*) surfactant preparations in-vivo under standardized conditions. Adult rats were anesthetized, tracheotomized, paralysed and mechanically ventilated. Surfactant deficiency was induced by repeated whole-lung lavage. Ten min later, the animals were randomly divided and received one ml of a surfactant suspension or served as controls. For each surfactant preparation, three different surfactant doses (25, 50 and 100 mg/kg) were tested. After surfactant administration, ventilator settings were not changed for 2 h and blood gases were measured. After 2 h, positive end-expiratory pressure (PEEP) and in parallel peak inspiratory pressure (PIP) were twice reduced by steps of 2 cm H_2O (to PIP/PEEP: 24/4 and 22/2 cm H_2O, respectively) and the effect on blood gases was studied. In the control group, arterial oxygenation did not improve and all animals died within the observation period due to hypoxemia, whereas the induced acute respiratory failure reversed rapidly after all exogenous surfactants. The effect of exogenous surfactant on arterial oxygenation was, in general, dose-dependent. The natural surfactants were more effective in increasing arterial oxygenation to prelavage values than the synthetic surfactants. However, the natural surfactants differed in efficacy to maintain arterial oxygenation at prelavage values, especially when PEEP was reduced.

Introduction

Both natural and synthetic surfactant preparations have been successfully used in clinical trials in neonates suffering from respiratory distress syndrome (RDS), but differences in efficacy were noted [1]. The natural surfactants, which are obtained by organic solvent extraction of bovine or calf lung lavage (Alveofact*, BLES, and Infasurf*) or minced bovine lungs (Surfacten* and Survanta™) or minced pig lungs (Curosurf*), contain 1-2% of the surfactant proteins B and C, whereas the available synthetic surfactants (Alec™ and Exosurf*) are protein free [2] (Table 1). From experimental studies it is known that in contrast to the synthetic surfactants, the natural surfactants are characterized by rapid adsorption to the air-liquid interface, less sensitivity to inactivation by serum proteins, and almost immediate improvement of gas exchange [3-6]. It is assumed that the presence of the surfactant proteins in the natural surfactant preparations accounts for these differences.

Data on direct comparison of surfactants are limited and mostly generated by means of in-vitro tests [2]. It has been established that these in-vitro tests do not accurately predict the performance of a surfactant in in-vivo conditions and, therefore, pharmacological tests of surfactant preparations must be undertaken under well-controlled experimental conditions [7]. In preterm lambs, Cummings and colleagues [4] compared three clinically used surfactants and showed that arterial oxygenation improved immediately after both natural surfactants (Survanta™ vs. Infasurf*) whereas no improvement in gas exchange was observed after synthetic surfactant application (Exosurf*). Based on the different amounts of surfactant given and different application techniques used, this latter study does not, however, allow a firm conclusion about the activity of the tested materials. In a rat model of surfactant deficiency, originally described by Lachmann *et al.* [7,8], Häfner and colleagues [6] compared three clinically used surfactants (Alveofact*, Exosurf* and Survanta™) under standardized ventilator conditions. Furthermore, they kept volume, dose and mode of surfactant administration constant. They confirmed that both natural surfactants (Alveofact* and Survanta™) were more effective in improving gas exchange than the synthetic surfactant (Exosurf*) and demonstrated that Survanta™ was superior to Alveofact* to maintain arterial oxygenation at prelavage values at the used ventilatory settings [6].

In the present study, we used the same surfactant deficiency model under well standardized experimental conditions and evaluated eight surfactant preparations (Alec™, Alveofact*, BLES, Curosurf*, Exosurf*, Infasurf*, Surfacten* and Survanta™)

on the capability to improve gas exchange at a defined peak pressure and positive end-expiratory pressure (PEEP) level.

Materials and methods

Preparation of animals: This study was approved by the local Animal Committee of Erasmus University Rotterdam. Care and handling of the animals were in accord with the European Community guidelines (86/609/EEG). The studies were performed in male Sprague Dawley rats (n=162) with a body weight of 250 ± 30 g (Harlan CPB, Zeist, The Netherlands). After induction of anaesthesia with nitrous oxide, oxygen and halothane (66/33/1-2%), a polyethylene catheter (0.8 mm outer diameter) was inserted into a carotid artery for drawing arterial blood samples. Before tracheotomy, the animals received 40 mg/kg pentobarbital sodium, i.p. (Nembutal[*]; Algin, Maassluis, The Netherlands) and the halothane concentration was decreased to 0.5-1%. After a metal cannula was inserted into the trachea, muscle relaxation was induced by pancuronium bromide 1.2 mg/kg, i.m. (Pavulon[*]; Organon Teknika, Boxtel, The Netherlands) and the animals were immediately connected to the ventilator. Anesthesia was maintained with hourly injections of pentobarbital sodium (40 mg/kg, i.p.) and muscle relaxation was attained with hourly injections of pancuronium bromide (1.2 mg/kg, i.m.). Body temperature was kept within normal range by means of a heating pad.

The animals were mechanically ventilated in parallel, 6 animals simultaneously (Figure 1), with a Servo Ventilator 900 C (Siemens-Elema, Solna, Sweden) in a pressure-control mode, with the following ventilator settings: frequency of 30 breaths/min, peak inspiratory pressure (PIP) of 12 cm H_2O, positive end-expiratory pressure (PEEP) of 2 cm H_2O, inspiratory/expiratory ratio of 1:2, and 100% oxygen. Initially, PIP was increased to 20 cm H_2O for 1 min to recruit atelectatic areas. Next, surfactant deficiency was induced by repeated whole-lung lavage as described by Lachmann *et al.* [8]. 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 4-5 times with 5 min intervals to achieve a $PaO_2 \leq 85$ mmHg (11.3 kPa). Within 10 min after the last lavage, the animals received 1 ml of a surfactant suspension or served as controls. There were two control groups: in one group 1 ml of saline was instilled intratracheally in the same way as instillation of surfactant, whereas the second control group received no treatment apart from

mechanical ventilation. Table 1 shows the different natural and synthetic surfactant preparations used in this study. For each surfactant, three different doses of each surfactant (25, 50 and 100 mg surfactant per kg body weight) were tested in 6 animals each. With Curosurf*, an additional group was tested at the clinically used dose (200 mg/kg). To achieve the required concentrations of surfactant, the surfactant preparations were suspended with warm saline. Administration of the surfactant was performed using a 10 ml syringe which contained 1 ml of a surfactant suspension and 7 ml air. After animals had been disconnected from the ventilator, the syringe was connected to the tracheal cannula via a silicone tube and surfactant suspension was administered first, followed directly by the administration of air, this was immediately followed by re-connection of the animal to the ventilator (ventilator settings were not changed and the supine position of the animals was not changed).
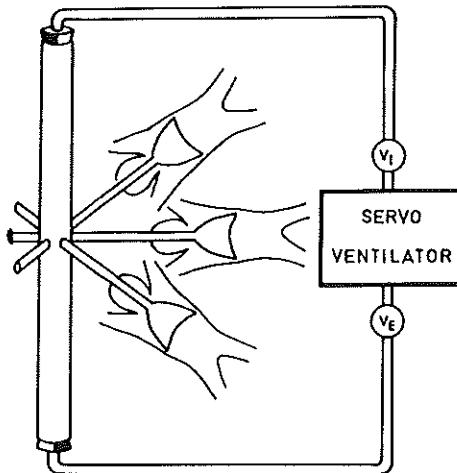
Figure 1. Schema of the device for the simultaneous ventilation of several animals.

In all animals, ventilator settings were not changed for 2 h and arterial blood samples were taken at: 5, 30, 60, 90, 120 min after treatment. After 2 h, PEEP and in parallel PIP were twice reduced by steps of 2 cm H_2O (to PIP/PEEP: 24/4 and 22/2 cm H_2O, respectively) and arterial blood samples were taken at each PIP/PEEP level 5-10 min later. PaO_2, $PaCO_2$ and pH were measured by conventional methods (ABL 505, Radiometer, Copenhagen, Denmark). At the end of each experiment, all animals were killed with an overdose of pentobarbital sodium.

Statistical analysis: All data are expressed as mean ± standard deviation (SD). Statistical analysis of the data was performed with analysis of variance (ANOVA) for repeated time measurements by use of the General Linear Models (GLM) procedure of the SAS statistical package (SAS Users Guide, 1990, SAS Institute Inc., Cary NC). Tests performed were: (1) within a treatment group, the effect of time on changes in PaO_2 and $PaCO_2$, (2) with a surfactant preparation, the effect of doses on PaO_2, (3) the difference in PaO_2 values between groups, using both control groups as negative controls. Tests were performed from t=0' to t=120' to evaluate dose-dependency and overall differences between the groups. Within groups, tests were performed from t=0' to t=5' to evaluate the immediate response of the instilled surfactant, t=5' to t=120' to evaluate stability of PaO_2 over time. To evaluate stability of PaO_2 after PEEP reduction within groups in which PaO_2 kept stable during the first 2 h, additional tests were performed from t=120' to t=130' (=PIP/PEEP of 24/4 cm H_2O) and t=120' to t=140' (=PIP/PEEP of 22/2 cm H_2O). For evaluation of survival rates, the Kaplan-Meier method (product-limit survival estimates) was performed. Statistical significance was accepted at a *p*-value of ≤0.05.

Table 1. Investigated surfactant preparations.

Preparation	Producer	Composition	Phospho-lipids*	Proteins	Clinical doses (mg/kg)
Alec™ (=Pumactant)	Britannia, Redhill, England	Synth. DPPC and PG (7:3)	100%	0 %	100
Alveofact[e] (=SF-RI 1)	Thomae, Biberach, Germany	Lipid extract from bovine lung lavage	88%	1%	100
BLES	F. Possmayer, Univ. Western, Ontario, USA	Lipid extract from calf lung lavage	90%	1%	100
Curosurf[e #]	Chiesi, Parma, Italy	Lipid extract from minced porcine lungs	99%	1%	200
Exosurf[e]	Burroughs-Wellcome, New York, USA	Synth. DPPC, hexadecanol, tyloxapol	84%	0 %	67.5
Infasurf[e] (=CLSE)	Ony Inc., New York, USA	Lipid extract from calf lung lavage	95%	1%	100
Surfacten[e] (=surfactant-TA)	Tokyo Tanabo, Tokyo, Japan	Lipid extract from minced bovine lungs + synth. DPPC	84%	1%	100
Survanta™ (=Beractant)	Abbott, Wiesbaden, Germany	Lipid extract from minced bovine lungs + synth. DPPC	84%	1%	100

DPPC, dipalmitoylphosphatidylcholine. PG, phosphatidylglycerol. Synth., synthetic. *, by weight.
#, Curosurf used in this study was provided by Drs. Curstedt and Robertson, Stockholm, Sweden.

Results

Blood gas data before and directly after repeated lavage were comparable for all groups (Figures 2 A-H and Tables 2, 3). In both control groups, mean PaO_2 values did not improve and all animals died within the 140 min observation period (Table 2). The mortality rates in the control group which received 1 ml of saline was higher than the control group which received no treatment apart from mechanical ventilation (Table 2). Table 3 shows the number of deaths in the different treatment groups. Only in the group treated with Curosurf® at a dose of 25 mg/kg, all animals died within the observation period. In the remaining groups treated with surfactant, only a few animals died during the study period and mostly in the groups which received the lower doses of a surfactant (Table 3).

Table 2. Data on blood gases of the two control groups.

Variable	Group	Pre	Lav	5'	30'	60'	90'	120'	24/4
PaO_2	Saline	539±20	53±17	44±5	48±19 ††	40±8 ††	-††	-	-
(mmHg)	Vent.	543±28	65±14	77±19	76±27 ††	56±13 †	55±12	59±4	-†††
$PaCO_2$	Saline	37±3	73±16	89±19	116±37	129±38	-	-	-
(mmHg)	Vent.	34±3	65±6	65±12	66±11	67±3	73±4	69±8	-

Vent., the control group that received no treatment apart from mechanical ventilation; Pre, before lavage procedure; Lav, lavage; †, death of one animal; 24/4, PIP of 24 cm H_2O and PEEP of 4 cm H_2O. PIP, peak inspiratory pressure; PEEP, positive end-expiratory pressure.

Mean PaO_2 values increased significantly within 5 min after surfactant administration in almost all surfactant-treated groups, but not in the two groups treated with both synthetic surfactants at a dose of 25 mg/kg (Figures 2 A-H). Mean PaO_2 values of all surfactant-treated groups were significantly higher compared with both control groups (Figures 2 A-H and Table 2). The effect of surfactant on PaO_2 was dose-dependent for Alveofact®, BLES, Curosurf®, Exosurf®, Infasurf®, and Surfacten®, but not with the surfactants Alec™ and Survanta™. The natural surfactants behaved differently compared with both synthetic surfactants. With the natural surfactants, mean PaO_2 values increased immediately to prelavage values and remained stable with the highest

used dose and decreased, in general, with the lower doses (25 and 50 mg/kg) during the subsequent 2 h with unchanged ventilator settings (Figure. 2 C-H). With both synthetic surfactants, mean PaO_2 values increased to approximately 50% of the prelavage values within 5 min and remained stable or slowly increased during the subsequent 2 h (Figures 2 A,B).

Clear differences in efficacy were noted between the six natural surfactants tested (Alveofact[*], BLES, Curosurf[*], Infasurf[*], Surfacten[*] and Survanta[TM]). At a dose of 25 mg/kg, mean PaO_2 values increased rapidly to prelavage values for the surfactants BLES, Infasurf[*], Surfacten[*] and Survanta[TM] and remained stable for the subsequent 2 h only in the animals which got Survanta[TM]. For the groups which received the surfactants Alveofact[*], BLES and Curosurf[*] at a dose of 25 mg/kg, mean PaO_2 values decreased even to postlavage values within 2 h (Figures 2 C-E). Comparisons based on a dose of 50 mg/kg showed that mean PaO_2 values increased rapidly to prelavage values for the surfactants Alveofact[*], BLES, Infasurf[*], Surfacten[*] and Survanta[TM], and remained stable with Surfacten[*] and Survanta[TM] but decreased significantly with the other surfactants with unchanged ventilator settings. With the highest used dose (100 mg/kg), mean PaO_2 values remained stable at prelavage values in the subsequent 2 h with the surfactants Alveofact[*], BLES, Infasurf[*], Surfacten[*] and Survanta[TM], whereas dcreased significantly with Curosurf[*]. However, with Curosurf[*], mean PaO_2 values could be maintained at prelavage values for 2 h when the animals received a dose of 200 mg/kg (Figure 2 E). For the groups in which mean PaO_2 values kept stable during the first 2 h, mean PaO_2 values decreased significantly in the group treated with the surfactant BLES at a dose of 100 mg/kg after reduction of the PEEP level to 4 cmH_2O. After further reduction of PEEP level to 2 cm H_2O, mean PaO_2 values decreased significantly in the groups: Curosurf[*] at a dose of 200 mg/kg, Surfacten[*] at a dose of 25 and 50 mg/kg and Survanta[TM] at a dose of 25, 50 and 100 mg/kg (Figures 2 E,G,H).

Mean $PaCO_2$ values of the different surfactant-treated groups are given in Table 3. Directly after the repeated lavage, mean $PaCO_2$ values increased significantly and decreased, in general, after treatment with the exogenous surfactants (Table 3).

A   **Alec**

B   **Exosurf**

Figure 2A,B. Mean change in PaO_2 values ($\pm$SD) over time. Pre, prelavage; Lav, lavage; 24/4 PIP of 24 cm H_2O and PEEP of 4 cm H_2O; 22/2 PIP of 22 cm H_2O and PEEP of 2 cm H_2O; PIP, peak inspiratory pressure; PEEP, positive end-expiratory pressure. *Diagonal striped* bar: dose of 25 mg/kg; *cross-hatched* bar: dose of 50 mg/kg; *horizontal striped* bar: dose of 100 mg/kg; *, $p \leq 0.05$ vs. prelavage values. #, $p \leq 0.05$ vs. lavage values.

C   Alveofact

D   BLES
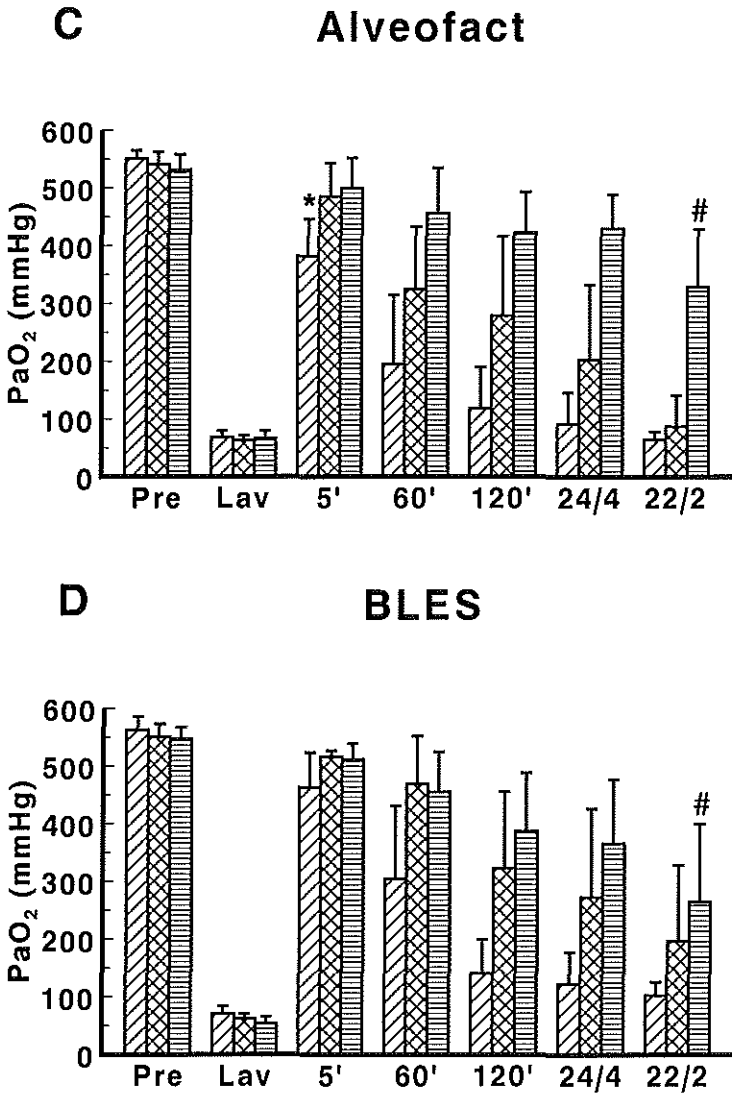
Figure 2C,D. Mean change in PaO$_2$ values ($\pm$SD) over time. Pre, prelavage; Lav, lavage; 24/4 PIP of 24 cm H$_2$O and PEEP of 4 cm H$_2$O; 22/2 PIP of 22 cm H$_2$O and PEEP of 2 cm H$_2$O; PIP, peak inspiratory pressure; PEEP, positive end-expiratory pressure. *Diagonal striped* bar: dose of 25 mg/kg; *cross-hatched* bar: dose of 50 mg/kg; *horizontal striped* bar: dose of 100 mg/kg; *, $p \leq 0.05$ vs. prelavage values. #, $p \leq 0.05$ vs. lavage values.

E   Curosurf

F   Infasurf
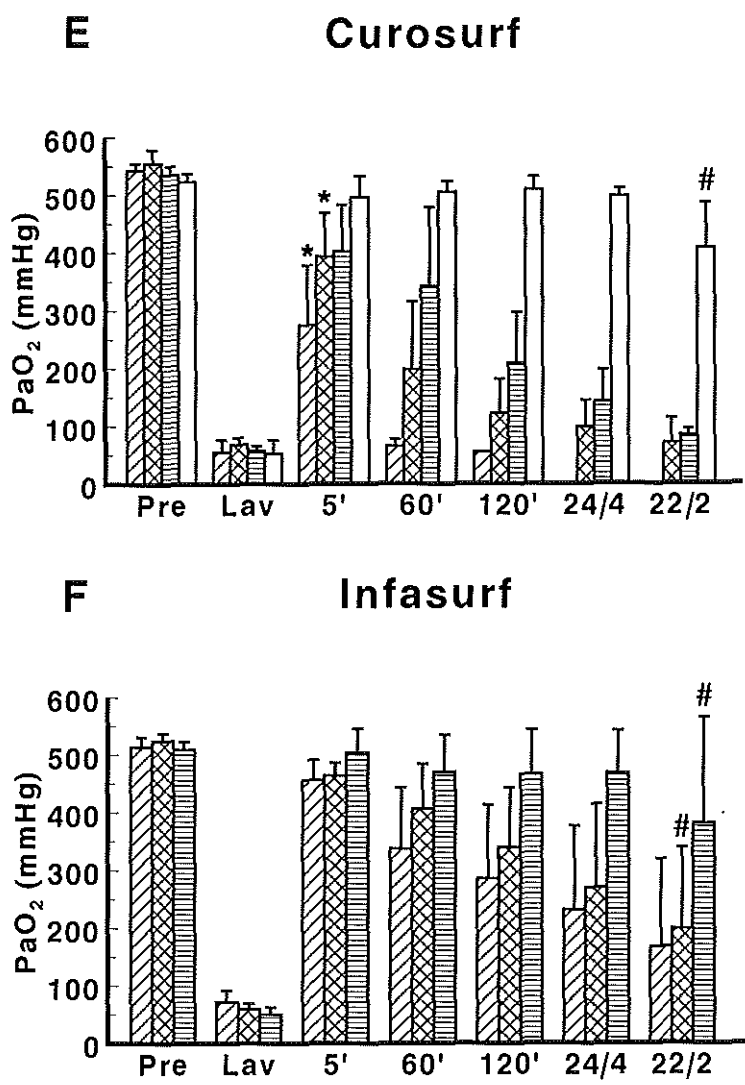
Figure 2E,F. Mean change in PaO_2 values ($\pm$ SD) over time. Pre, prelavage; Lav, lavage; 24/4 PIP of 24 cm H_2O and PEEP of 4 cm H_2O; 22/2 PIP of 22 cm H_2O and PEEP of 2 cm H_2O; PIP, peak inspiratory pressure; PEEP, positive end-expiratory pressure. *Diagonal striped* bar: dose of 25 mg/kg; *cross-hatched* bar: dose of 50 mg/kg; *horizontal striped* bar: dose of 100 mg/kg; *open* bar: dose of 200 mg/kg. *, $p \le 0.05$ vs. prelavage values. #, $p \le 0.05$ vs. lavage values

G   Surfacten

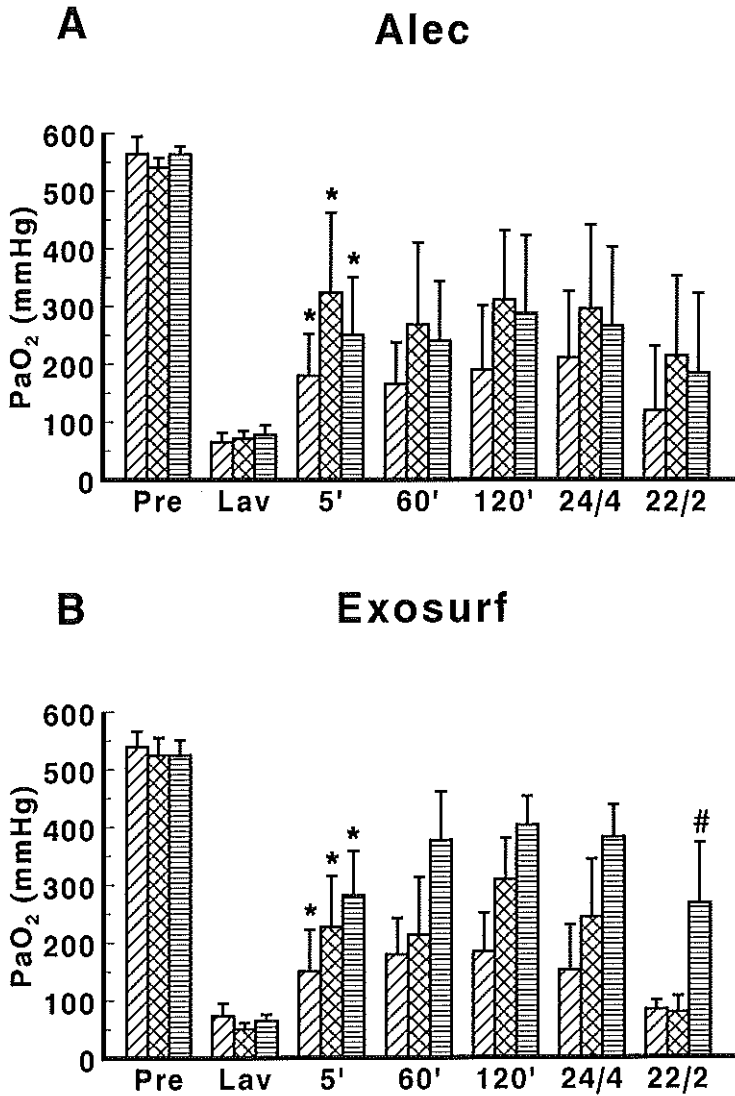
H   Survanta
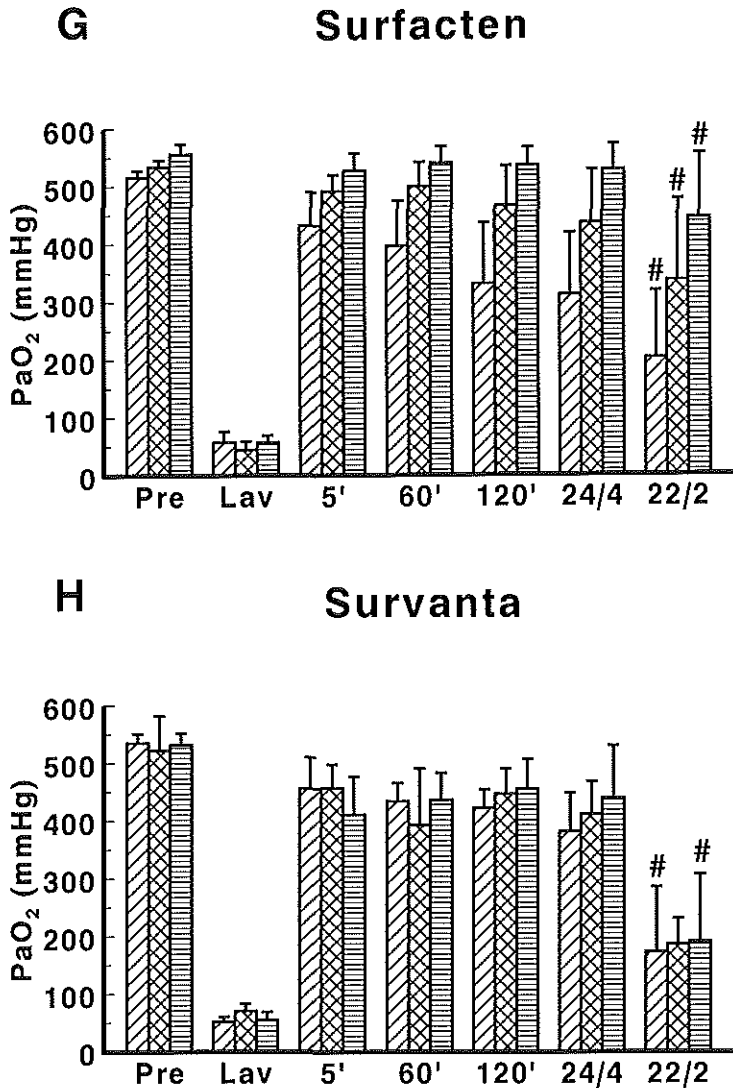
Figure 2G,H. Mean change in PaO_2 values ($\pm$SD) over time. Pre, prelavage; Lav, lavage; 24/4 PIP of 24 cm H_2O and PEEP of 4 cm H_2O; 22/2 PIP of 22 cm H_2O and PEEP of 2 cm H_2O; PIP, peak inspiratory pressure; PEEP, positive end-expiratory pressure. *Diagonal striped* bar: dose of 25 mg/kg; *cross-hatched* bar: dose of 50 mg/kg; *horizontal striped* bar: dose of 100 mg/kg. *, $p \le 0.05$ vs. prelavage values. #, $p \le 0.05$ vs. lavage values.

Table 3. PaCO$_2$ values (mean$\pm$SD) of all surfactant-treated groups.

Surfactant	Dose	Pre	Lav	5'	60'	120'	24/4	22/2
Alec™	25	34±6	72±11	59±6	57±11 †	60±9 †	58±11 †	88±34 †
	50	45±3	85±6	70±5	72±12	67±14 †	68±18	72±24
	100	34±5	70±17	65±19	61±20	63±21	71±34	74±24 †
Alveofact*	25	38±2	66±6	49±3	58±9	73±15	81±15 †††	85±12 †
	50	38±3	66±7	57±9	53±7	46±6	43±7	44±10
	100	39±3	63±10	50±4	46±7	43±3	41±5	44±8
BLES	25	38±4	70±5	55±5	59±8	65±8	67±10 †	74±12 †
	50	39±3	78±8	61±6	55±6	55±9	54±13	62±20
	100	38±2	67±7	48±5	44±4	43±2	40±3	41±4
Curosurf*	25	43±4	81±9	73±8	99±14 †	98±15 ††	95 ††	- †
	50	34±2	66±9	50±6	54±9	56±2 ††	64±6	86±21 †
	100	34±2	70±8	54±6	43±5	54±10	57±9	73±9
	200	40±7	83±17	63±13	47±9	48±8	46±7	56±6
Exosurf*	25	32±5	73±5	70±9	59±12	59±14	66±13	85±17 ††
	50	36±3	67±15	71±15	61±12	56±7	57±7	66±7
	100	35±5	69±12	61±15	58±12	54±11	50±10	53±10
Infasurf*	25	41±7	78±8	68±11	65±8	61±7	62±12	70±31 †
	50	37±4	71±7	54±5	50±5	48±5	45±7	42±4
	100	43±7	69±10	67±9	57±8	56±7	45±7	45±16
Surfacten*	25	43±4	78±5	62±4	57±7	52±7	52±7	59±15
	50	41±4	77±10	64±4	54±3	52±3	50±5	54±8
	100	37±5	74±14	57±13	53±10	49±11	44±10	44±12
Survanta™	25	37±4	65±18	60±21	50±12	44±8	40±7	53±11
	50	38±5	51±8	47±5	46±8	38±7 †	39±8	45±7
	100	41±5	65±12	63±16	53±5	48±7	45±5	58±12

Pre, before lavage; Lav, lavage; 24/4, PIP of 24 cm H$_2$O and PEEP of 4 cm H$_2$O; 22/2, PIP of 22 cm H$_2$O and PEEP of 2 cm H$_2$O; PIP, peak inspiratory pressure; PEEP, positive end-expiratory pressure; Dose, mg surfactant per kg body weight. †, death of one animal.

Discussion

This is the first report of direct comparison of eight clinically used surfactants under standardized conditions with respect to mechanical ventilation and surfactant administration. Most in-vivo evaluations of pulmonary surfactants have been performed in the rabbit fetus model [9]. The reason for using the rat lavage model in the present study and not the immature fetus model needs some considerations. Using the rabbit fetus model, only the survival rate and compliance calculated from the tidal volume, can be studied. We have shown that high values of dynamic compliance can be obtained by a 'pure' detergent in premature rabbits [10]. The detergent had a low static surface tension but was lacking the appropriate properties to stabilize surfactant-deficient alveoli in the expiratory phase. This resulted in a high thorax-lung compliance because the lung volume swings between inspiration and expiration are greater if the alveoli change from the collapsed to the open state [10]. The alveolar stabilisation is, however, one of the main functions of the native pulmonary surfactant system [11]. Normally, this phenomena can only be recognised if lung volume measurements (functional residual capacity) are performed, however, this technique is not yet available for routine clinical use [12]. Another possibility to truly evaluate the efficacy of surfactant preparations is to measure blood gases; they present the global function of the lung. That is why we used the surfactant-deficient animal model in the present study which allows the evaluation of arterial blood gases after exogenous surfactant therapy as an indicator of the quality of surfactant preparations with regard to their capacity to stabilize alveoli in the end-expiratory phase [8,13,14].

The results of this study show significant differences in activity between the used surfactant preparations; the natural surfactants were more effective to improve blood gases to prelavage values than both synthetic surfactants, and between the natural surfactants differences in efficacy were detectable, especially after reduction of the PEEP level. In both control groups, arterial oxygenation did not improve and all animals died within the observation period due to insufficient gas exchange. It has been reported that the combination of saline instillation and mechanical ventilation can harm the lungs [15]. This was confirmed by the results of the present study in which the mortality rate of the control group which received saline was higher compared to the control group which received no treatment apart from mechanical ventilation (Table 2).

Recently, first clinical trials compared a natural with a synthetic surfactant directly and showed a more rapid improvement in oxygenation and ventilation in the

neonates with RDS which received the natural surfactant [16,17]. It has been established from in-vitro studies that surfactant proteins B and/or C are required for a rapid adsorption of the phospholipids to the air-liquid interface [3]. Therefore, it is assumed that the surfactant proteins B and/or C are essential for uniform spreading of exogenous surfactant into the lungs [3]. In the present study, we did not measure surfactant distribution in the lungs, but an indirect proof of uniform alveolar deposition of exogenous surfactant is the rapid improvement of PaO_2 values to prelavage values (Figures 2 C-H). In other words, all existing atelectasis which are responsible for the observed hypoxemia at the used ventilator settings were recruited by exogenous surfactant as indicated by the rapid improvement of arterial oxygenation. We found that mean PaO_2 values increased to prelavage values within 5 min with all natural surfactants, whereas mean PaO_2 values increased to only 50% of the prelavage values with both synthetic surfactants. This means that the increased right-to-left shunt, as a result of the repeated lung lavages, is reduced completely with the natural surfactants at a PEEP of 6 cm H_2O, indicating that the natural surfactants spread more uniformly compared with both synthetic surfactants and that there was no difference in spreading between the different natural surfactants tested.

Between both tested synthetic surfactants (Alec^{TM} vs. Exosurf*) we found no significant difference in blood gases at all three tested doses, despite the presence of a detergent (tyloxapol) and an alcohol (hexadecanol) in Exosurf* used to improve the spreading capacity of the phospholipid (Figures 2 A,B) [2]. In contrast to the results of Cummings and coworkers [4], we showed that arterial oxygenation improved significantly with Exosurf*, but this improvement was significant lower compared with the natural surfactants (Figures 2 B-H).

Between the natural surfactants, marked differences in response pattern were shown. A rapid and sustained improvement of arterial oxygenation to prelavage values was achieved at the clinically used dose which is 200 mg/kg for Curosurf* and 100 mg/kg for the other used natural surfactants [2]. Using the lower doses, the differences in activity became pronounced. Evaluation of the different natural surfactants after administration of a dose of 25 mg/kg showed that mean PaO_2 values remained stable at prelavage values only with Survanta^{TM} and decreased slowly over time with Infasurf* and Surfacten* but dropped back to postlavage values with the surfactants Alveofact*, BLES and Curosurf* within 2 h at unchanged ventilator settings. During the PEEP reduction manoeuvre to 2 cm H_2O, mean PaO_2 values remained stable only in the

groups which got Alveofact*, Infasurf* and Surfacten* at a dose of 100 mg/kg. Based on the overall results, we conclude that Surfacten* was the best surfactant to improve lung function, in particular high blood gas values at low mean airway pressure, in lung-lavaged rats under the applied ventilatory support.

Direct comparison of the surfactants Surfacten* and Survanta™ showed that Surfacten* resulted in significantly higher blood gas values after the reduction of the PEEP level. Survanta™ is, however, a modification of Surfacten* with only small differences in composition [2]. The major difference between both surfactants is that Surfacten* is stored as a freeze-dried, white powder whereas Survanta™ is stored as a ready-to-use suspension. Phospholipids are, however, very sensitive for autooxidation which may lead to possible loss of in-vitro and in-vivo activity [18]. The storage of exogenous surfactant as a powder is, therefore, beneficial over a suspension, but further studies are needed to establish whether this could explain the difference in efficacy between Survanta™ and Surfacten* at decreased level of PEEP.

Our results are consistent with previous studies on direct comparison of exogenous surfactants and indicate that the rat lavage model in combination with the used ventilator protocol can detect clear differences between natural and synthetic surfactant preparations, as well as between the natural surfactant preparations themselves [4,6]. The results of the present study confirm that the efficacy of the natural surfactants is superior to that of synthetic surfactants. Based on the blood gas values obtained 5 min after surfactant administration, we assume that the immediate spreading is homogenous with all natural surfactants whereas not with both synthetic surfactants. Furthermore, there was no difference in immediate spreading between the natural used surfactants. In this study, the various natural surfactants differed in efficacy, especially when PEEP was reduced: Surfacten* was the best natural surfactant to stabilize the lungs during the PEEP reduction manoeuvre.

Speculation

As known, a moderately active exogenous surfactant when combined with artificial ventilation using high level of PEEP, can establish sufficient gas exchange in RDS lungs. However, the optimal surfactant regime should include the use of the lowest possible ventilatory support in order to decrease the risk of ventilator-induced lung injury and therefore strongly supports the use of highly active surfactants. In addition, the search for efficacious surfactant preparation has also an economic dimension and

it is therefore important to explore ways of keeping the amounts of administered surfactant as low as possible (which of course requires administration of surfactant at an early time point in lung injury). Based on the results of this study in lung-lavaged rats, if one could apply these results to the clinical situation, then the ranking in order of preference would be: Surfacten® > Survanta™ > Infasurf® > Alveofact® > BLES > Curosurf® > Exosurf® > Alec™.

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Chapter 4

Prevention of respiratory failure after hydrochloric acid aspiration by intratracheal surfactant instillation in rats

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Summary

Because the surfactant system probably is involved in the pathophysiology of respiratory failure caused by hydrochloric acid (HCl) aspiration, we investigated the effects of different ventilation strategies and intratracheal surfactant instillation at different time intervals on the course of pulmonary gas exchange after HCl aspiration in rats. In this study rats were anesthetized, and mechanically ventilated via a tracheostomy. Respiratory failure was induced by intratracheal instillation of 3 ml/kg 0.1 N HCl. Animals (n=49) were divided into nine groups: Groups 1 and 2 through 9 were ventilated with peak airway pressure/PEEP of 14/2 and 26/6 cm H_2O, respectively; Groups 3 and 4 received surfactant (200 mg/kg) intratracheally, 1 and 10 min after HCl aspiration; Groups 5 and 6 received saline, 1 and 10 min after HCl aspiration; Groups 7 and 8 received surfactant, 60 and 90 min after HCl aspiration; Group 9 received saline instead of HCl. Gas exchange deteriorated in Groups 1, 2, 5, 6, 7 and 8, whereas respiratory failure could be prevented in Groups 3 and 4. After deterioration of gas exchange, surfactant treatment prevented further decrease of PaO_2 values in Group 7, whereas no effect on gas exchange was observed in Group 8; intratracheal instillation of saline had no effect on gas exchange (Group 9). These results suggest that surfactant should be given as early as possible after aspiration of gastric contents to prevent development of respiratory failure.

Introduction

Massive aspiration of gastric contents is one of the most feared complications of general anesthesia and is an important cause of the adult respiratory distress syndrome (ARDS) [1,2]. ARDS caused by aspiration of gastric contents is characterized by deterioration of gas exchange requiring mechanical ventilation with high inspiratory oxygen concentration, high insufflation pressure, and positive end-expiratory pressure (PEEP), and is associated with a mortality rate of over 90% (2). Hydrochloric acid (HCl) causes direct damage to the alveolo-capillary membrane, leading to influx of protein-rich edema fluid into the alveolar space [3-6]. These plasma-derived proteins are known to be potent inhibitors of surfactant function [7-12]. It has also been proposed that HCl directly damages the surfactant system [4,13].

Because the surfactant system probably is involved in the pathophysiology of respiratory failure caused by HCl aspiration, studies have been performed to investigate the effect of surfactant replacement therapy on lung function of animals suffering from respiratory failure due to HCl aspiration. Kobayashi and colleagues [6] demonstrated that surfactant instillation could only partly restore gas exchange in rabbits suffering from respiratory failure due to HCl aspiration after lung edema was removed by bronchoalveolar lavage; surfactant, when given without prior lung lavage, only prevented further deterioration of blood gases. Lamm and colleagues [14] showed improved lung recoil without improvement in gas exchange in rabbits receiving surfactant 5 min after HCl aspiration.

The aim of this study was to investigate the effects of different ventilation strategies and intratracheal surfactant instillation at different time intervals, on the course of pulmonary gas exchange after HCl aspiration in rats.

Materials and methods

Exogenous surfactant: The surfactant used in these experiments is a freeze-dried natural surfactant isolated from bovine lungs in basically the same manner as previously described [15]. It consists of approximately 90% phospholipids, 1% hydrophobic proteins (SP-B and SP-C), the remainder being other lipids such as cholesterol, glyceride and free fatty acids. There is no SP-A (the largest surfactant associated protein, molecular weight 26,000-38,000) in this surfactant preparation as a result of isolation procedures. Surfactant was suspended in saline at a concentration of 50 mg dry weight/ml. This surfactant preparation has proven to be highly effective in

improving gas exchange and lung mechanics in various animal models of respiratory failure of differing etiologies [16-18].

Animal study: The study protocol was approved by the Animal Care Committee of Erasmus University Rotterdam, The Netherlands. The studies were performed in 49 male adult Sprague-Dawley rats (body weight: 300-350 g). After induction of anesthesia with nitrous oxide, oxygen and halothane (65/33/2%) the animals were tracheotomized and a catheter was inserted into the carotid artery. Anesthesia was maintained with pentobarbital sodium (60 mg/kg, intraperitoneally) and muscle relaxation was attained with pancuronium bromide (0.5 mg/kg, intramuscularly). Lungs were ventilated with a Servo Ventilator 900 C (Siemens-Elema, Solna, Sweden) at the following ventilator settings: pressure-controlled ventilation, FiO_2 of 1.0, ventilation frequencyof30/min, peak airway pressure (P_{peak}) of 14 cm H_2O, PEEP of 2 cm H_2O and inspiratory/expiratory ratio of 1:2. After reaching steady state (PaO_2 >500 mmHg), 43 rats received 1.5 ml/kg HCl intratracheally (0.1 N) while lying on their right side, followed by a bolus of air (30 ml/kg). This was followed immediately by instillation of 1.5 ml/kg HCl, while lying on their left side, again followed by a bolus of air. Six rats received saline (2 x 1.5 ml/kg) instead of HCl.

Directly after instillation, P_{peak} was increased to 26 cm H_2O and PEEP to 6 cm H_2O in 38 rats receiving HCl and in the 6 rats receiving saline. P_{peak} was kept at 14 cm H_2O and PEEP at 2 cm H_2O in five rats receiving HCl. In all animals the same ventilator settings were maintained throughout the observation period.

The animals were divided into 9 groups (Table 1): Group 1 (n=5) was ventilated at P_{peak} of 14 cm H_2O and PEEP of 2 cm H_2O (HCl 14/2); Group 2 (n=6) was ventilated at P_{peak} of 26 cm H_2O and PEEP of 6 cm H_2O (HCl 26/6); Group 3 (n=6) received surfactant intratracheally 1 min after HCl (Surf 1'); Group 4 (n=5) received surfactant 10 min after HCl (Surf 10'); Groups 5 and 6 (n=5 and n=5) received saline 1 and 10 min after HCl (Sal 1' and Sal 10', respectively); Group 7 (n=6) received surfactant 60 min after HCl (Surf 60') and Group 8 (n=5) received surfactant after 90 min (Surf 90'); Group 9 (n=6) received saline instead of HCl and served as control (NaCl). Animals from Groups 3, 4, 7 and 8 were treated with surfactant at a dose of 200 mg dry weight/kg body weight.

Blood samples for measurement of PaO_2 and $PaCO_2$ (ABL 330; Radiometer, Copenhagen, Denmark) were taken from the carotid artery of each animal before intratracheal instillation and at 15, 30, 60, 90 and 120 min post-instillation.

At the end of the experiments all animals were sacrificed with an overdose of intra-arterially administered pentobarbital sodium. Lungs of a few animals of Groups 2-6 and 9 underwent bronchoalveolar lavage (BAL) with saline (37 °C; 30 ml/kg).

Bronchoalveolar lavage fluid: To compare all BAL samples with those from healthy controls, six additional male Sprague-Dawley rats (body weight 300-350 g) were lavaged once with saline (37 °C; 30 ml/kg). The BAL fluids of the different groups were prepared as follows: all samples were centrifuged for 15 min at 2000 g to remove cell material. Protein concentration was measured in all samples using a modified Lowry method [19], with bovine serum albumin as standard. Also, surface activity in BAL fluid was measured using 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 500 μl of BAL fluid was applied to the surface of the trough; maximal and minimal surface tensions were measured after three cycles.

Statistical analysis: All data are expressed as mean $\pm$ standard deviation (SD). For the animal study statistical analysis of data was performed using repeated measurements ANOVAs [20], with time as the repeat variable. Three ANOVAs were performed initially: the effect of P_{peak}/PEEP settings against controls, the effect of saline instillation against controls, and finally the effect of surfactant instillation against controls. When significant differences between groups occurred, the difference between these groups was analyzed further. For analysis of BAL parameters, standard ANOVA procedures were performed. Statistical significance was accepted at $p \le 0.05$.

Table 1: Different treatment groups.

Group		n	HCl	P_{peak}/PEEP	Surf/Sal
1	(HCl 14/2)	5	+	14/2	-
2	(HCl 26/6)	6	+	26/6	-
3	(Surf 1')	6	+	26/6	Surf 1'
4	(Surf 10')	5	+	26/6	Surf 10'
5	(Sal 1')	5	+	26/6	Sal 1'
6	(Sal 10')	5	+	26/6	Sal 10'
7	(Surf 60')	6	+	26/6	Surf 60'
8	(Surf 90')	5	+	26/6	Surf 90'
9	(NaCl)	6	- (NaCl)	26/6	-

HCl = hydrochloric acid; Surf 1' = surfactant 1 min after HCl; Surf 10' = surfactant 10 min after HCl; Surf 60' = surfactant 60 min after HCl; Surf 90' = surfactant 90 min after HCl; NaCl = sodium chloride.

Results

Animal study: Figure 1 shows PaO_2 values for each group. Before instillation of HCl or saline, PaO_2 values are high and there is no significant intergroup difference. There is a significant difference in PaO_2 values between rats ventilated at 14/2 or at 26/6 cm H_2O after HCl instillation (HCl 14/2 and HCl 26/6, respectively); saline (NaCl), instead of HCl, at the applied ventilator settings had no influence on PaO_2 values (Figure 1A). There is no intergroup difference in PaO_2 values between rats ventilated at 26/6 cm H_2O, rats treated with saline at 1 min (Sal 1') and rats treated with saline at 10 min (Sal 10') after HCl instillation; for this reason PaO_2 values of these three groups are used (Control) for comparison with other treatment groups. The differences in PaO_2 values between rats treated with surfactant 1 or 10 min after HCl instillation (Surf 1' and Surf 10', respectively) and Control are significant; there is no intergroup difference between PaO_2 values of Surf 1' and Surf 10' (Figure 1B). Although there is a significant difference in PaO_2 values prior to surfactant treatment between groups of rats treated at both 60 and 90 min after HCl instillation (Surf 60' and Surf 90', respectively) and Control, PaO_2 values increase significantly compared to pre-treatment values in Surf 60', whereas there is no increase in PaO_2 values observed in Surf 90' (Figure 1C).

A

B

Figure 1A,B. PaO_2 values (mean ± SD) of the different groups: (A) rats ventilated at $P_{peak}=14$/PEEP$=2$ cm H_2O or $P_{peak}=26$/PEEP$=6$ cm H_2O after HCl aspiration (HCl 14/2 and HCl 26/6, respectively) and rats receiving saline (NaCl) instead of HCl;(B) rats receiving surfactant or saline, 1 or 10 min after HCl aspiration (Surf 1', Surf 10', Sal 1' and Sal 10', respectively); † = one rat died; ↑ = surfactant instillation; PaO_2 = arterial oxygen tension; PEEP = positive end-expiratory pressure.

Figure 1C. PaO$_2$ values (mean ± SD) of the different groups: (C) rats receiving surfactant 60 or 90 min after HCl aspiration (Surf 60' and Surf 90', respectively) and, again, rats ventilated at P$_{peak}$=26/PEEP=6 cm H$_2$O (HCl 26/6); † = one rat died; ↑ = surfactant instillation; PaO$_2$= arterial oxygen tension; PEEP = positive end-expiratory pressure.

Figure 2 shows the PaCO$_2$ values for each group. Before instillation of HCl or saline there are no significant differences in PaCO$_2$ values between the groups. There is a significantly higher increase in PaCO$_2$ values in all animals ventilated at 14/2 cm H$_2$O compared to rats ventilated at 26/6 cm H$_2$O; in rats receiving saline instead of HCl, PaCO$_2$ values remained in physiological range (Figure 2A). Again, there is no intergroup difference in PaCO$_2$ values between rats ventilated at 26/6 cm H$_2$O, rats treated with saline at 1 min and rats treated with saline at 10 min after HCl instillation and thus PaCO$_2$ values of these three groups are used (Control) for comparison with other treatment groups. Although PaCO$_2$ values of rats treated with surfactant at 1 or 10 min after HCl instillation appear to be lower compared to Control, these differences are not significant (Figure 2B). There also is no significant difference in PaCO$_2$ values between rats receiving surfactant after 60 min and 90 min and Control (Figure 2C).
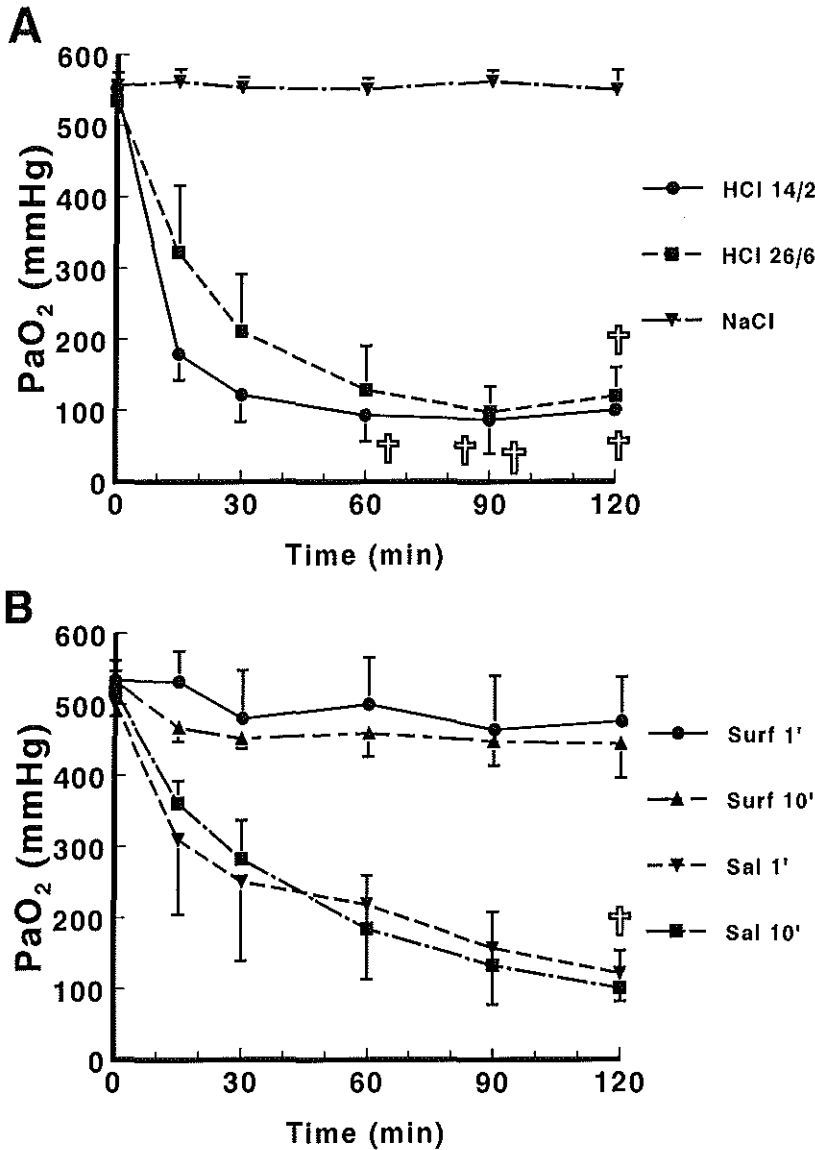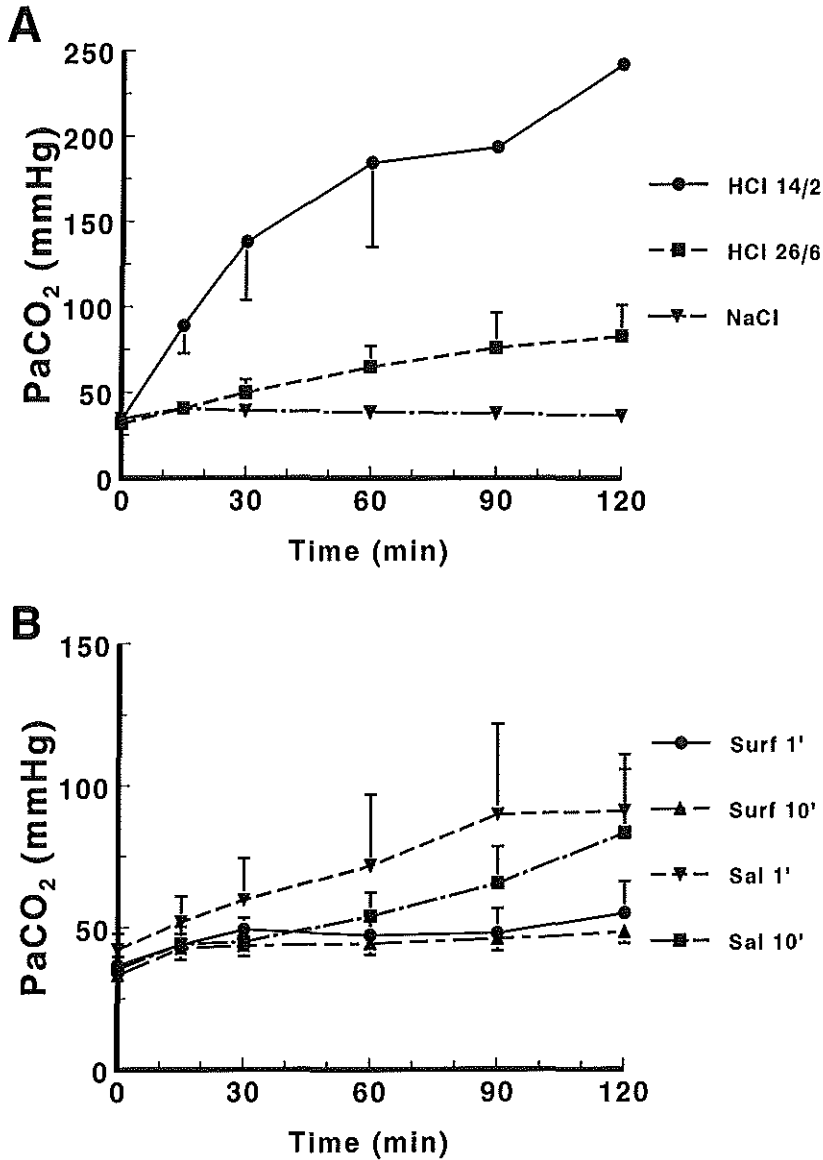
Figure 2A,B. PaCO$_2$ values (mean ± SD) of the different groups; (A) rats ventilated at P$_{peak}$ = 14/PEEP = 2 cm H$_2$O or P$_{peak}$ = 26/PEEP = 6 cm H$_2$O after HCl aspiration (HCl 14/2 and HCl 26/6, respectively) and rats receiving saline (NaCl) instead of HCl; (B) rats receiving surfactant or saline, 1 or 10 min after HCl aspiration (Surf 1', Surf 10', Sal 1' and Sal 10', respectively); † = one rat died; PEEP = positive end-expiratory pressure; PaCO$_2$ = arterial carbon dioxide tension.

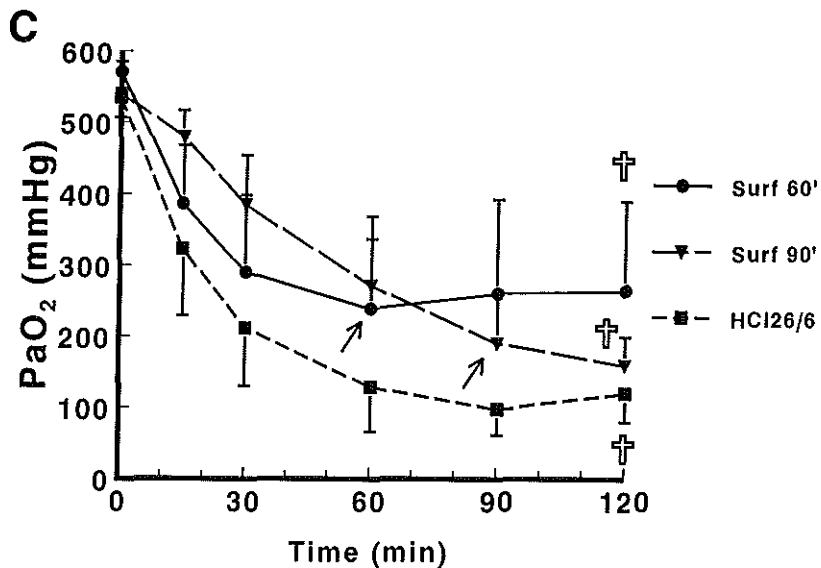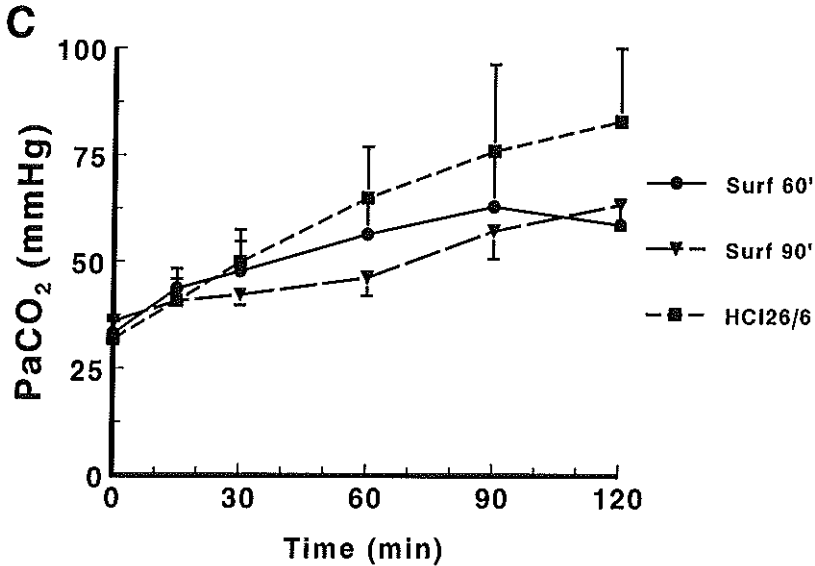**Figure 2C.** PaCO$_2$ values (mean $\pm$ SD) of the different groups; (C) rats receiving surfactant 60 or 90 min after HCl aspiration (Surf 60' and Surf 90', respectively) and, again, rats ventilated at P$_{peak}$=26/PEEP=6 cm H$_2$O (HCl 26/6); $\dagger$ = one rat died; PEEP = positive end-expiratory pressure; PaCO$_2$= arterial carbon dioxide tension.

Bronchoalveolar lavage fluid: Table 2 shows the recovery percentage, protein concentration and surface tension properties of BAL fluid samples. For statistical reasons the BAL parameters of rats receiving saline 1 and 10 min after HCl instillation were regarded to be comparable (as evidenced by a small SD), as were BAL parameters of rats receiving surfactant 1 and 10 min after HCl instillation.

The protein concentration in BAL fluid of animals receiving HCl is significantly higher than that of BAL fluid of controls and rats receiving saline instead of HCl; there is no significant difference in protein concentration between groups receiving HCl. Surface tension measurements demonstrate decrease in surface activity in BAL fluid from rats after HCl aspiration, not receiving surfactant treatment. BAL fluid from rats receiving surfactant 1 and 10 min after HCl aspiration demonstrate favorable surface activity (for detailed statistical differences between groups, see Table 2).

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Table 2: Bronchoalveolar lavage (BAL) variables.

Sample	n	Recovery [%]	Protein [mg/ml]	Surface tension [mN/m]	
				maximal	minimal
Control	6	95.1 ± 5.23	0.24* ± 0.06	64.5 ± 5.83	24.2@ ± 3.99
Saline	5	93.8 ± 5.45	0.41* ± 0.42	62.9 ± 3.11	25.1§ ± 2.51
HCl (26/6)	5	98.1 ± 5.56	7.45 ± 1.33	60.5 ± 3.24	31.2 ± 2.33
HCl (Sal 1' + 10')	6	100.7 ± 5.62	8.07 ± 1.16	64.6 ± 4.56	34.3 ± 4.42
HCl (Surf 1' + 10')	7	95.5 ± 4.33	6.98 ± 1.31	46.8# ± 6.52	19.6¶ ± 4.11

All data are mean ± SD; Control = BAL fluid of healthy contol rats; Saline = BAL fluid of rats receiving saline instead of HCl, intratracheally; (26/6) = rats ventilated at P_{peak} = 26 and PEEP = 6 cm H_2O; (Sal 1' + 10') and (Surf 1' + 10') = rats receiving saline or surfactant, respectively, 1 or 10 min after HCl aspiration.
*, significant difference between Control/Saline and other groups;
#, significant difference between HCl (Surf 1' + 10') and other groups;
@, significant difference between Control and both HCl (26/6) and HCl (Sal 1' + 10');
§, significant difference between Saline and all HCl groups;
¶, significant difference between HCl (Surf 1' + 10') and the two other HCl groups.

Discussion

This study demonstrates that surfactant, when given within 10 min, prevents deterioration of gas exchange after massive aspiration of HCl (pH = 1.0), but cannot prevent accumulation of plasma-derived proteins into the alveolar space. It is also shown that respiratory failure cannot be prevented by increasing insufflation pressure and PEEP to levels used in this study, although more animals survived during the whole observation period (HCl 14/2 versus HCl 26/6). Similar results have been reported by others; namely, that lungs of rabbits receiving surfactant (100 mg/kg body weight) 5 min after HCl aspiration (pH = 1.0, 2 ml/kg) showed improved lung recoil compared to lungs which did not receive surfactant; however, this study did not demonstrate

higher PaO_2 values in rabbits treated with surfactant, although it must be noted that low PEEP levels were used [14].

The mechanism of respiratory failure in HCl aspiration can be explained by (a) damage to the alveolar-capillary membrane [4], leading to increased permeability [5,21,22] and thus influx of protein-rich edema fluid into the alveolar space; (b) direct damage to the surfactant system [4,13]; and (c) secondary inhibition of remaining surfactant activity caused by plasma-derived proteins present in the edema fluid [7-12]. As a result of (functional) surfactant deficiency, suction force across the alveolo-capillary membrane increases, leading to further outpouring of edema fluid into the alveolar space; this way a vicious circle exists. Also, as a result of diminished surfactant function, surface tension on the alveolar wall increases, leading to increased retractive forces across the alveolar-capillary membrane, with subsequent formation of atelectasis and mismatch of the ventilation-perfusion ratio, leading to hypoxemia.

Surfactant is inhibited by plasma-derived proteins in a dose-dependent manner [7-12]. Thus, with a high surfactant/inhibitor (S/I) ratio there is no inhibition of surfactant function, whereas with a low S/I ratio most surfactant function is abolished. Although the exact mechanism of inhibition remains unclear, it seems that competition between surfactant and inhibitors at the air-liquid interphase of the alveolar wall plays an important role [9,12]. When surfactant is given before deterioration of gas exchange (i.e., before the remaining surfactant function is inhibited) the S/I ratio is increased, thus preventing the pathophysiological mechanisms that lead to respiratory failure. It is emphasized that the initial damage to lung tissue by HCl cannot be prevented, as HCl is neutralized within 30 seconds [21] and, thus, lung structure is already damaged. This is confirmed in the present study, where protein concentration in BAL fluid of rats receiving surfactant within 10 min is almost as high as protein concentration of rats receiving saline, or ventilated only. Comparable findings were also reported in the study mentioned earlier [14], namely increased wet/dry weight ratio in lungs of rabbits which received surfactant 5 min after HCl aspiration as well as in lungs of rabbits not receiving surfactant. However, surface tension properties of BAL fluid of rats receiving surfactant demonstrated low minimal and low maximal surface tensions compared to BAL fluid of rats receiving saline. Thus, due to the lower surface tension of the "intrapulmonary fluid" the applied ventilator settings (26/6 cm H_2O) were sufficient for maintaining (normal) gas exchange.

Earlier studies have demonstrated that intratracheal surfactant instillation fully

restores lung function in animals suffering from respiratory failure due to pneumonia [16-18]. Also, gas exchange in rats suffering from respiratory failure after bilateral whole lung lavage could be fully restored after surfactant treatment [23]. In the present study surfactant treatment, 60 min after HCl instillation, showed slight improvement of gas exchange; surfactant treatment 90 min after HCl instillation did not prevent further deterioration of gas exchange during the observation period. A possible explanation could be that after HCl aspiration lung structure is more severely damaged compared with animals suffering from pneumonia, or after whole lung lavage. This would lead to increased permeability of the alveolo-capillary membrane and more severe intra-alveolar edema accumulation. After deterioration of gas exchange due to HCl aspiration, S/I ratio (before surfactant instillation) is low and lung structure is damaged. When surfactant is given in these conditions, little or no effect is seen on gas exchange. A possible explanation is that surfactant, at a concentration used in this study (200 mg/kg), is inhibited directly by high concentration of inhibitors. Another explanation could be that the edema fluid and atelectatic areas form a mechanical barrier, preventing surfactant from entering the alveolar spaces. However, there are studies in which gas exchange could be improved either by giving a large amount of surfactant (280-350 mg/kg body weight) [24], or by lavaging the lungs with saline before surfactant instillation [6], as mentioned in the introduction. With both methods an improved S/I ratio could be achieved.

Similar results have also been reported in animals suffering from respiratory failure due to hyperoxic lung injury. Surfactant substitution (60 mg/kg body weight) can prevent development of respiratory insufficiency in rabbits exposed to 100% oxygen for 64 hours: PaO_2 values of surfactant-treated rabbits, during mechanical ventilation with 100% oxygen, remained at high levels compared with saline-treated animals [25]. Also, wet/dry ratio in animals receiving surfactant was high compared to lungs of healthy controls. However, in guinea pigs suffering already from respiratory failure due to prolonged exposure to 100% oxygen, surfactant treatment (200 mg/kg body weight) was not able to restore gas exchange [7].

In the present study a "point of no return" mechanism seems to be applicable to the situation: after deterioration of gas exchange, little or no beneficial effect can be seen after surfactant instillation at a concentration used in this study (200 mg/kg body weight), whereas gas exchange is preserved when surfactant is given within 10 min. Although it is difficult to extrapolate from this animal study to the clinical situation, our

results suggest that when massive aspiration of gastric contents is suspected, surfactant should be given as soon as possible to prevent development of respiratory failure. An important consideration which favors direct treatment with surfactant is that ARDS, caused by aspiration of gastric contents, has a mortality rate of over 90% [2].

In conclusion, increasing insufflation pressure and PEEP alone to levels used in this study has no beneficial effect on the course of hypoxemia after HCl aspiration in rats. Surfactant given within 10 min after HCl aspiration does prevent development of respiratory failure but does not prevent damage to lung structure, as evidenced by high protein concentrations in BAL fluid. Surfactant, at the dose used in this study, given after deterioration of gas exchange, can only prevent further decrease in PaO_2 values.

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Chapter 5

In-vivo evaluation of the inhibitory capacity of human plasma on exogenous surfactant function

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Summary

Objective: The adult respiratory distress syndrome (ARDS) and neonatal respiratory distress syndrome (RDS) are characterized by high permeability pulmonary edema which contains plasma-derived proteins inhibiting pulmonary surfactant function. Currently, discussion continues as to what dose of surfactant is required for treatment of these syndromes.

Design: The purpose of this study was to investigate the amount of exogenous surfactant needed to overcome the inhibitory components in human plasma. Male adult rats suffering from respiratory failure due to surfactant depletion after whole-lung lavage received human plasma (4 ml/kg body weight) mixed with surfactant at different concentrations, intratracheally. Rats receiving surfactant only at different concentrations served as controls. Blood gas analysis was performed.

Measurements and results: It was demonstrated that plasma (4 ml/kg ≈ 273 mg plasma proteins/kg) mixed with surfactant at 300 mg/kg was able to increase and maintain PaO_2 at normal values. Plasma mixed with surfactant at 100 mg/kg, after initial restoration of blood gases, showed deterioration of PaO_2 values. Plasma mixed with surfactant at a dose of 50 mg/kg did not improve PaO_2, whereas surfactant at 50 mg/kg, without plasma, restored blood gases to prelavage values.

Conclusion: It is concluded that approximately 1 mg surfactant phospholipids is required to overcome the inhibitory effect of approximately 1 mg plasma proteins. For clinical practice this means that an excess of surfactant should be given, or repeatedly be substituted ('titrated') at low concentrations, until blood gases improve.

Introduction

The adult respiratory distress syndrome (ARDS) and neonatal respiratory distress syndrome (RDS) are both characterized by respiratory failure and require therapy consisting of intubation and mechanical ventilation with high oxygen concentrations. RDS is characterized by primary surfactant deficiency due to immaturity of the lungs, while in ARDS high permeability pulmonary edema exists, containing plasma-derived proteins which inhibit pulmonary surfactant function [1-7]. Due to impaired surfactant function, surface tension at the air-liquid interphase on the alveolar walls is increased, leading to increased suction force across the alveolo-capillary membrane, favouring further accumulation of protein-rich edema fluid into the alveolar space [8].

Several clinical trials have reported successful treatment with intratracheal surfactant instillation in prematures suffering from RDS [9-14]. In these studies, however, some infants did not respond to a single dose of surfactant and some infants had an only transient improvement of lung function [10,11]. One reason for this could be that surfactant function is inhibited by plasma-derived proteins present in intra-alveolar edema fluid.

A few reports on ARDS patients treated with surfactant have been published. Although the results from these studies are not consistent, the best results were seen in those patients treated with higher surfactant concentrations [15-19].

Currently, discussion continues as to what dose of surfactant should be used in prematures with established RDS and what dose should be used for treatment of ARDS. To investigate this, a study was performed in an animal model of respiratory failure induced by bronchoalveolar lavage (BAL). This model has proven useful for a variety of experimental purposes, including e.g. testing of different surfactant preparations and demonstrating that exogenous surfactant restores blood gases to normal [20-22]. After respiratory failure was established, animals received plasma mixed with surfactant at different concentrations. Plasma was used to simulate protein-rich edema, an established characteristic of ARDS. Instead of lung mechanics, blood gas measurements were measured, since these have proven to be more sensitive to therapeutic interventions in this model [23].

Materials and methods

Materials. The surfactant used in these experiments was a freeze-dried natural surfactant isolated from bovine lungs in basically the same manner as previously

described [24]. It consists of approximately 90% phospholipids, 1% hydrophobic proteins (SP-B and SP-C), the remainder being other lipids such as cholesterol, glyceride and free fatty acids. There is no SP-A in this surfactant preparation. This surfactant preparation has proven to be highly effective in improving gas exchange and lung mechanics in various animal models of respiratory failure of differing etiologies [25-27] and in newborn babies suffering from respiratory failure due to congenital diaphragmatic hernia [28].

Pooled citrated plasma was collected from healthy volunteers and prepared according standard techniques. Protein concentration was measured using a modified Lowry method [29], with bovine serum albumin as standard. Protein concentration of the pooled plasma was 68.3 mg/ml.

Animal study. The protocol was approved by the Animal Care and Use Committee of the Erasmus University Rotterdam. The studies were performed in 45 male adult Sprague-Dawley rats (body weight 300-350 g). After induction of anesthesia with nitrous oxide, oxygen and halothane (65/33/2%) the animals were tracheotomized and a catheter was inserted into the carotid artery. Anesthesia was maintained with pentobarbital sodium (60 mg/kg/h, i.p.) and muscle relaxation was attained with pancuronium bromide (0.5 mg/kg/h, i.m.). The rats were ventilated with a Servo Ventilator (900C, Siemens-Elema, Solna, Sweden) at the following ventilator settings: pressure-controlled ventilation, FiO_2 of 1.0, ventilation frequency of 30 cycles/min, peak airway pressure of 14 cm H_2O, positive end-expiratory pressure (PEEP) of 2 cm H_2O, and inspiratory/expiratory ratio of 1:2.

After reaching steady state (PaO_2 > 500 mmHg) respiratory failure was induced by BAL as described by Lachmann *et al.* [20]. In brief: lungs were lavaged 6-7 times with warm saline (37 °C; 30 ml/kg) to produce a PaO_2 < 80 mmHg at peak pressure of 26 cm H_2O and PEEP of 6 cm H_2O. These ventilator settings were unchanged throughout the entire observation period.

Approximately 5 min after PaO_2 < 80 mmHg animals were randomly divided into seven groups: groups I, II, and III (n=6, n=7 and n=7, respectively) immediately received intratracheally the undiluted plasma (4 ml/kg≈273 mg plasma proteins/kg) mixed with surfactant at concentrations of 50, 100 and 300 mg phospholipids/kg body weight, respectively; groups IV, V and VI (n=6, n=6 and n=7, respectively) received surfactant suspended in saline at concentrations of 25, 50 and 100 mg phospholipids/kg body weight, respectively (total amount of surfactant suspension was 4 ml/kg); group

VII (n=6) received undiluted plasma (4 ml/kg), intratracheally. In all groups intratracheal instillation was followed by a bolus of air (32 ml/kg), and mechanical ventilation was continued. Before intratracheal instillation, surfactant-plasma mixtures were incubated for 30 min at 37 °C.

Blood samples for measurement of PaO_2 and $PaCO_2$ were taken from the carotid artery before BAL and 5 min after the last lavage (directly followed by treatment) and at 5, 30, 60, 90, 120, 150 and 180 min post-treatment (ABL 330; Radiometer, Copenhagen, Denmark). The animals were then sacrificed with an overdose of intra-arterially administered pentobarbital sodium.

Statistical analysis. All data are expressed as mean $\pm$ standard deviation (SD). Statistical analysis of data was performed using ANOVA for repeated measurements, with time as the repeat variable. When differences between and/or within groups occurred, these differences were further analysed with a test that compensated for multiple comparison (Student-Newman-Keuls test). Statistical significance was accepted at $p \leq 0.05$.

Results

Figures 1A and 1B show PaO_2 values for all groups. The intergroup differences both before and after lavage are not statistically different. After treatment, the PaO_2 values of groups receiving surfactant only at 50 and 100 mg/kg (groups V and VI, respectively) increase to prelavage values and remain high during the whole observation period and the differences between these groups are not significant; for this reason PaO_2 values of these two groups are used for statistical comparison with other groups. Surfactant only at 25 mg/kg (group IV), after initial improvement to prelavage values, did not stabilize PaO_2 values over the whole observation period; the difference with groups V and VI was significant after 60 min. PaO_2 values of rats receiving plasma (4 ml/kg) mixed with surfactant at 100 and 300 mg/kg (groups II and III, respectively) also increase to normal values. Immediately after treatment there is no significant difference in PaO_2 values between groups II and III and groups V and VI. However, PaO_2 values of group II decrease significantly 60 min after treatment compared to group III. PaO_2 values of group III remain at high levels throughout the observation period and are not significantly different from groups V and VI. PaO_2 values of rats receiving plasma mixed with surfactant at 50 mg/kg (group I) did not increase significantly. Rats receiving plasma only (group VII) died within 60 min.
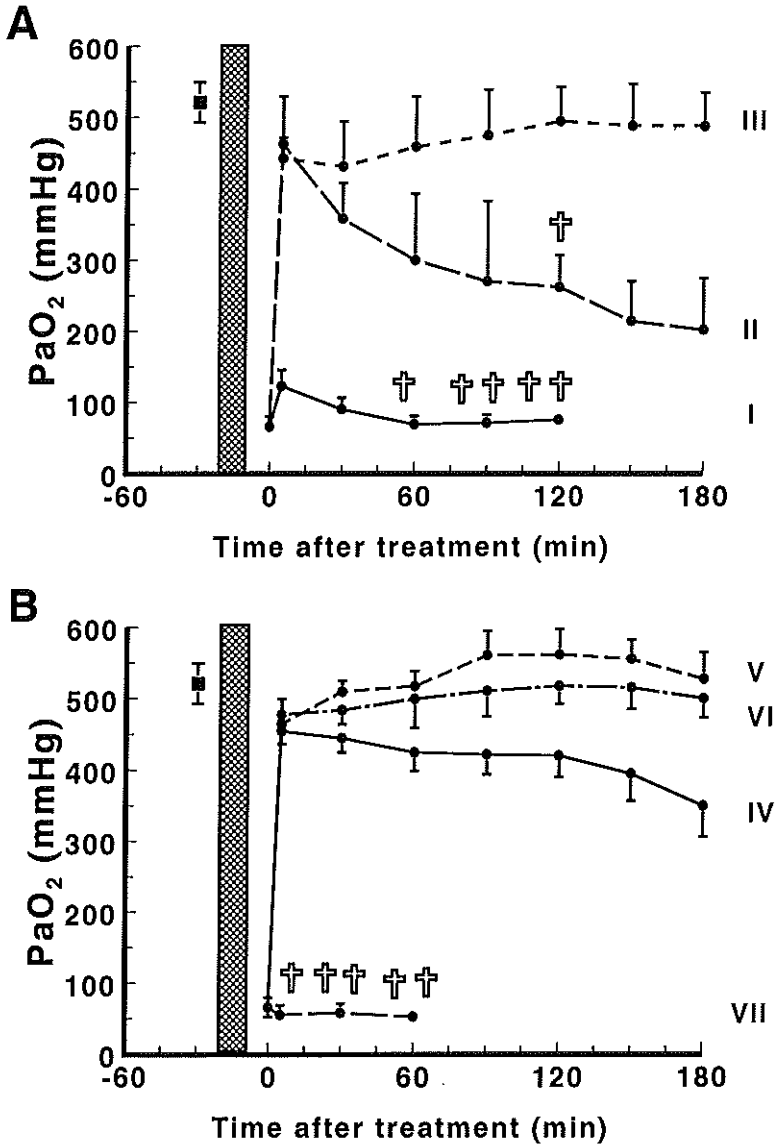
Figures 1 A and B. PaO_2 values (mmHg; mean $\pm$ SD) before (*square*) and after BAL (=grey bar) in different treatment groups: (A) groups I, II, and III received plasma (4 ml/kg) mixed with surfactant at 50, 100 and 300 mg/kg, respectively; (B) groups IV, V and VI received surfactant only at 25, 50 and 100 mg/kg, respectively and group VII received plasma only (4 ml/kg); t=0 indicates the PaO_2 values 5 min after BAL, immediately followed by treatment; † = one rat died; PaO_2 = arterial oxygen tension.

Figures 2A and 2B show the $PaCO_2$ values for all groups. The intergroup differences both before and after lavage are not statistically different. $PaCO_2$ values of rats receiving surfactant only at 50 or 100 mg/kg do not differ significantly, and are used for comparison with other groups. $PaCO_2$ values of groups I, II and VII differ significantly from groups V and VI. There is no significant difference in $PaCO_2$ values between group III and groups V and VI. There are no significant differences in $PaCO_2$ values between groups II and III.

Figure 2 A. $PaCO_2$ values (mmHg; mean $\pm$ SD) before (*square*) and after BAL (=grey bar) in different treatment groups: groups I, II, and III received plasma (4 ml/kg) mixed with surfactant at 50, 100 and 300 mg/kg, respectively; t=0 indicates the $PaCO_2$ values 5 min after BAL, immediately followed by treatment; $PaCO_2$ = arterial carbon dioxide tension.
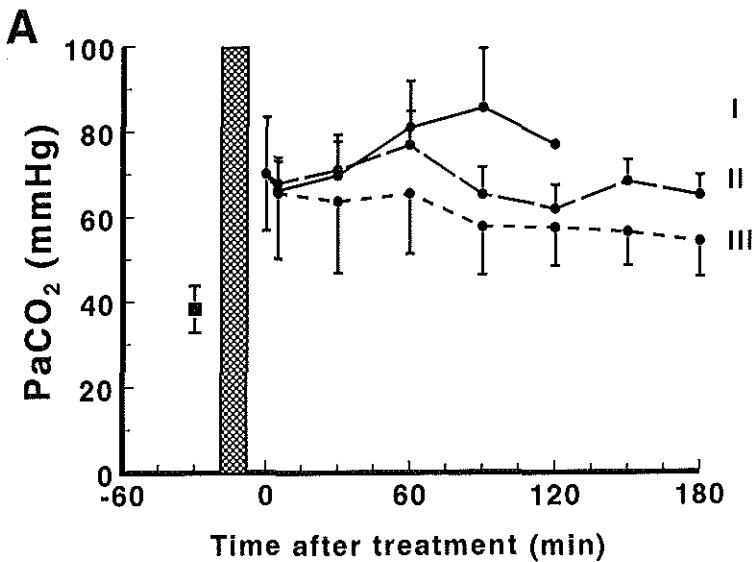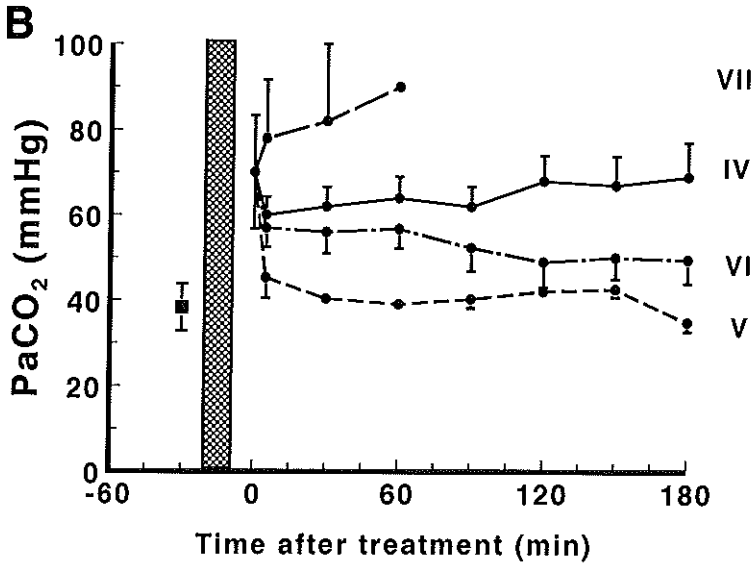
Figure 2 B. $PaCO_2$ values (mmHg; mean $\pm$ SD) before (*square*) and after BAL (=grey bar) in different treatment groups: groups IV, V and VI received surfactant only at 25, 50 and 100 mg/kg, respectively and group VII received plasma only (4 ml/kg); t=0 indicates the $PaCO_2$ values 5 min after BAL, immediately followed by treatment; $PaCO_2$ = arterial carbon dioxide tension.

Discussion

It has been established that in both ARDS and RDS the alveolo-capillary membrane is highly permeable to plasma proteins, leaking from the circulation into the alveolar space, resulting in edema formation [2,30,31]. BAL material of patients suffering from these syndromes demonstrates quantitative and qualitative changes in phospholipid composition of surfactant, and contains high protein concentrations [2,31-35]. Saline extracts of lung minces from infants who died from RDS and from adults with ARDS contained less phospholipids and had higher minimal surface tension than similar extracts from lungs of patients who died from other causes [15,36]. Several clinical studies have been performed treating infants suffering from RDS with intratracheal surfactant instillation [9-14]. The surfactant preparations used varied from natural surfactant (e.g. CLSE, Curosurf, Surfactant TA and human surfactant) to synthetic surfactant preparations (ALEC, Exosurf). The doses used in these clinical trials varied from low (60 mg/kg) to high (200 mg/kg). It has been reported that some infants did not respond to, or had an only transient improvement after a single treatment of low-

dose surfactant [10,11]. It has been suggested that in these patients increased permeability of the alveolo-capillary membrane leads to accumulation of proteins present in the alveolar space, causing inhibition of the surfactant preparation. This hypothesis could be confirmed in other clinical studies in which better clinical outcome was seen in infants treated with either a higher dose of surfactant [11] or by treatment with multiple doses [14]. Only a few patients with ARDS have been treated with surfactant. Although the results from these studies are not consistent, the best results were seen in patients treated with higher surfactant concentrations [15-19].

After treatment of RDS and ARDS with a low dose of surfactant the reason for lack of response, or only transient improvement, is attributed to the alveolar space in these patients being filled with protein-rich edema fluid. Clinical and animal studies have established that these plasma-derived proteins inhibit surfactant [1-7,30,31,37-41]. Surface tension studies have shown that several plasma proteins inhibit surfactant in a dose-dependent manner [7,38,39,42-47]. It is also established that some surfactant preparations, especially synthetic surfactants, are more sensitive to the inhibitory effect of plasma-derived proteins than other preparations [48-50]. However, in recent studies it was demonstrated that addition of surfactant-associated proteins increases resistance of synthetic surfactants to inhibition of plasma proteins [51,52]. In physiological studies using excised lungs it was shown that intratracheal instillation of hemoglobin, albumin or membrane lipids decreased lung compliance, which could be reversed by intratracheal surfactant instillation [44].

The mechanism by which plasma derived proteins inhibit surfactant function is not clear. Balis and colleagues [53] have postulated two different types of surfactant inhibition. They demonstrated that inhibition of surfactant by serum could be reversed by centrifugating the surfactant-serum mixture: the sediment revealed normal surfactant function. Mixing surfactant with plasma allowed clot formation to occur; this 'coagulative type' of inhibition could not be reversed by centrifugation. Seeger and colleagues [7] reported that fibrin monomers are potent inhibitors of surfactant; inhibition of surfactant could partially be reversed by adding plasmin to the fibrin-surfactant mixture. Other investigators have hypothesized that inhibition of surfactant by plasma-derived proteins is due to competition for space at the air-liquid interphase. Holm and colleagues [44,45] reported that at high surfactant concentrations the inhibitory effect of high concentrations of plasma protein or membrane lipids is abolished. Other studies confirm the probable existence of a competition mechanism:

after centrifugation of surfactant-inhibitor mixtures derived from BAL fluid of animals treated with surfactant or after centrifugation of surfactant-protein mixtures, the sediments containing surfactant revealed normal surface tension characteristics compared with before centrifugation [2,37,40,45,53]. Also, when these BAL fluids or the surfactant-protein mixtures were investigated for surface tension properties, it could be demonstrated that after a certain number of cycles the minimal surface tension decreased from initially high levels to low levels [7,38,41,42]. These findings probably indicate that, if there is any chemical interaction between proteins and surfactant, this interaction is not strong.

If the mechanism of competition between surfactant and plasma proteins is correct, then this would be of great importance for treatment of ARDS and neonatal RDS. Namely, whether the lungs are filled with plasma-derived proteins or not, intratracheal instillation of sufficiently high doses of surfactant should restore lung function. Surfactant treatment of animals with enormous amounts of pulmonary edema caused by prolonged exposure of 100% oxygen or hydrochloric acid (HCl) aspiration does not seem to have any positive influence on restoring gas exchange at the concentrations used [1,4]. Kobayashi and colleagues [4] have demonstrated that surfactant treatment was only able to restore gas exchange in rabbits suffering from respiratory failure after HCl aspiration after lungs had been lavaged, this way removing proteins from the alveolar space. This strongly suggests that a more favourable surfactant-inhibitor ratio was established after lung lavage followed by surfactant treatment. In another study [54], surfactant instillation at very high doses (280-350 mg/kg) was able to restore gas exchange in guinea pigs suffering from severe respiratory failure due to high permeability pulmonary edema after intravenous instillation of anti-lung serum; thus a more favourable surfactant-inhibitor ratio was obtained by giving a large amount of surfactant. Recently, Kobayashi and colleagues [5] investigated the capability of surfactant mixed with edema fluid at several ratios to restore lung function in immature rabbit fetuses, as measured by tidal volume at preset insufflation pressures. It was demonstrated that surfactant (25 mg/ml) mixed with edema fluid at a protein to lipid ratio (P/L ratio) of 2.2 was capable of restoring lung function, whereas surfactant mixed with edema fluid at P/L ratio of 11.2, was not. Surface tension properties of these mixtures demonstrated high minimal surface tensions at P/L$\geq$3.4 and low surface tensions at P/L$\leq$1.8. In the present study a P/L ratio of 2.7 (group II) there was only transient improvement. At P/L ratio of 5.5 (group

I), no improvement was seen. Our results are in accordance with the observations reported by Kobayashi and colleagues [5], although a different model looking at different parameters and a different surfactant preparation were used. The results confirm the hypothesis of the competition mechanism between surfactant and proteins, although it cannot be ruled out that some direct interaction between surfactant and proteins occurs.

The lung lavage model, used in the present study, has proven useful for a variety of experimental purposes, including e.g. testing of different surfactant preparations and demonstrating that exogenous surfactant restores blood gases to normal [20-22]. In this model, the concentration of the surfactant preparation (which is more or less comparable with all other natural surfactant preparations) needed to restore blood gases over a 3 hour observation period was 50 mg/kg (Figure 1B). To get the same results when surfactant was mixed with human plasma (4 ml/kg ≈ 273 mg plasma proteins/kg), six times as much surfactant (300 mg/kg) was necessary to restore and stabilize gas exchange (Figure 1A). This means that approximately 1 mg surfactant phospholipids is needed to overcome the inhibitory effect of 1 mg plasma proteins. Surfactant at 100 mg/kg initially restored gas exchange, however appeared not be enough to overcome the additional inhibition by plasma proteins leaking into the alveolar space. Because of this, gas exchange deteriorated after initial improvement. In these experiments citrated plasma was used to obtain full inhibitory capacity, including fibrinogen. However, the inhibitory components in diseased lungs probably consist not only of products derived from blood but also consist of, for example, specific proteases, inflammatory mediators, bacteria, membranes, etc. Plasma-derived proteins, however, may be the most important compounds of high-permeability edema fluid and thus, from this point of view, it makes sense to 'titrate' the amount of surfactant for replacement therapy by mixing it with full plasma.

Our results favour the competition hypothesis: when mixed with plasma, surfactant at 50 mg/kg shows no improvement, at 100 mg/kg there is only transient improvement, and at 300 mg/kg there is sustained improvement of blood gas values. These results imply that for treatment of ARDS or established RDS a high concentration of surfactant is required to overcome the inhibitory effect of plasma-derived proteins. If after surfactant instillation there is no, or only transient, improvement of blood gas values in patients with either ARDS or RDS (fibrotic lungs excluded) this does not mean that surfactant treatment does not work. It only means that

the concentration of the surfactant preparation used is too low in relation to the amount of surfactant inhibitors in the lungs. This raises the question of how to exclude the existence of fibrotic lungs. In our clinical practice we investigate this as follows: we increase the mean airway pressure in mechanically ventilated patients and observe blood gases. If blood gases improve this would mean that there are still recruitable lung parts which could be stabilized by exogenous surfactant treatment. On the other hand, if blood gases remain stable or even deteriorate after increasing mean airway pressure due to further mismatch of the ventilation-perfusion ratio, surfactant instillation may worsen the clinical situation by filling up the remaining areas participating in gas exchange, which is typical for fibrotic lungs. Thus, before giving surfactant, especially after a long period of artificial ventilation (2-3 weeks), one should always examine if there is still some lung tissue left which can be recruited by high ventilation pressures. As a consequence, in treatment of ARDS or established RDS: 1) surfactant should be given as early as possible and 2) an excess of surfactant should be given, or repeatedly be substituted ('titrated') at low concentrations until blood gases improve.

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Chapter 6

Bronchoalveolar lavage with a diluted surfactant suspension prior to surfactant instillation improves the effectiveness of surfactant therapy in experimental acute respiratory distress syndrome (ARDS)

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Summary

Objective: To assess whether bronchoalveolar lavage (BAL) with a diluted surfactant suspension prior to surfactant instillation prevents the only transient improvement in lung function as reported after surfactant instillation in severe acute respiratory distress syndrome (ARDS).

Design: Randomized, prospective, experimental study.

Setting: Laboratory and animal facility of a large university.

Materials: Adult male Sprague-Dawley rats (280 ± 30 g).

Interventions: All animals underwent repetitive whole lung saline lavage to induce acute lung injury. Then, animals were randomly divided into seven study groups: the first group received surfactant (150 mg/kg) within 10 min after the last lavage (early treatment), whereas in the other six groups mechanical ventilation was continued for 3 h before treatment (late treatment). Treatment consisted of: surfactant instillation at a dose of 150 mg/kg; at a dose of 250 mg/kg; BAL with saline; BAL with a diluted surfactant suspension (2.5 mg/ml); BAL with saline, immediately followed by surfactant instillation (150 mg/kg); and BAL with a diluted surfactant suspension (2.5 mg/kg), immediately followed by surfactant instillation (150 mg/kg).

Measurements and results: Blood gases were measured for 6 h and then BAL was performed to measure protein concentration and surface tension properties. Mean PaO_2 values increased immediately after surfactant instillation to pre-lavage values and remained stable only in the group that received surfactant immediately after lavage procedure and the group that underwent BAL with a diluted surfactant suspension prior to surfactant instillation.

Conclusion: BAL with a diluted surfactant suspension prior to surfactant instillation at a later time point in lung injury resulted in a stable improvement of lung function. This improvement is comparable with the results seen after surfactant instillation immediately after lung lavage.

Introduction

In worldwide clinical trials, surfactant therapy has been successfully used in newborns with respiratory distress syndrome (RDS) and mortality has been decreased by 30-40% [1]. Despite substantial evidence for surfactant dysfunction in acute respiratory distress syndrome (ARDS), only a few case reports and results of limited clinical studies are available in which adult patients with ARDS were treated with exogenous surfactant [2]. Although these reports showed that some patients did not respond to, or had only a transient improvement after, a single dose of surfactant, better results were seen when large or multiple doses were instilled (up to 400-500 mg of a natural surfactant per kg body weight) [2-5]. It has been suggested that the increased permeability of the alveolo-capillary membrane in ARDS leads to accumulation of plasma proteins in the alveolar space, causing inhibition of the instilled surfactant in a dose-dependent way [6]. Thus, for treatment of ARDS, high concentration of surfactant is required to overcome the inhibitory effect of the plasma proteins. However, the prohibitive price and the non-availability of large amounts of surfactants make surfactant therapy infeasible in adults at present [2].

Experience in neonates has taught us that exogenous surfactant therapy is more effective when administration takes place in the early stages of RDS [1]. Therefore, it is expected that early treatment of ARDS may thus require smaller amounts of exogenous surfactant due to smaller amounts of surfactant inhibitors. Indeed, experimental studies have demonstrated that administration of exogenous surfactant at an early time point in lung injury resulted in stable improvement of lung function, whereas transient improvement of lung function was found with the later treatments [7,8]. Further, Kobayashi and colleagues [9] have demonstrated, in a model of acid aspiration, that removal of surfactant inhibitors by bronchoalveolar lavage (BAL) with saline prior to administration of surfactant was necessary to improve arterial oxygenation. Lung lavage with saline, however, can damage pulmonary function [10]. Therefore, we performed BAL with a diluted surfactant suspension and found that this approach resulted in a superior response compared to exogenous surfactant administration with or without previous BAL with saline [11]. In that study, however, the effect of the different surfactant regimes on lung function was studied for only 1 hour and there was no group which received a combination of BAL with a diluted surfactant suspension and exogenous surfactant [11]. This study was therefore designed to evaluate the effect of different surfactant treatment regimes on lung function at a

later time point in lung injury compared with administration of surfactant directly after the repeated lung lavage; the combination of BAL with a diluted surfactant suspension, immediately followed by exogenous surfactant administration was of particular interest.

Materials and methods

This study was approved by the Institutional Animal Committee at the Erasmus University Rotterdam; care and handling of the animals were in accord with the European Community guidelines (86/609/EEG). The studies were performed in male Sprague-Dawley rats (n=52) with a bodyweight of 280 ± 30 g (Harlan CPB, Zeist, The Netherlands). After induction of anaesthesia with nitrous oxide, oxygen and halothane (66/33/1-2%), a polyethylene catheter (0.8-mm outer diameter) was inserted into a carotid artery for drawing arterial blood samples. Before tracheotomy, the animals received pentobarbital sodium (Nembutal*; 60 mg/kg, i.p.). After a metal cannula was inserted into the trachea, muscle relaxation was given with pancuronium bromide (Pavulon*; 2 mg/kg, i.m.) and the animals were immediately connected to the ventilator. The animals were mechanically ventilated with a Servo Ventilator 900 C (Siemens-Elema AB, Solna, Sweden) in parallel (6 animals simultaneously) in a pressure-control mode with the following ventilator settings: frequency of 30 breaths/min, positive end-expiratory pressure (PEEP) of 2 cm H_2O, inspiratory /expiratory ratio of 1:2, FiO_2 of 1.0, and a peak inspiratory pressure of 10-12 cm H_2O to keep $PaCO_2$ between 30 to 40 mmHg. Anesthesia was maintained with hourly injection of pentobarbital sodium (Nembutal*; 60 mg/kg/h); muscle relaxation was achieved with hourly injection of pancuronium bromide (Pavulon*; 2 mg/kg/h). Body temperature was kept within normal range by means of a heating pad.

Initially, the peak pressure was increased to 20 cm H_2O for 1 min to open up atelectatic regions in the lungs and after reaching steady state, blood gases were measured. Next, respiratory failure was induced by repeated bronchoalveolar lavage (BAL) according to Lachmann *et al.* [10]. Each lavage was performed with saline (32 ml/kg) heated to 37 °C. Just before the first lavage, peak inspiratory pressure and PEEP were elevated to 26 and 6 cm H_2O, respectively. Lung lavage was repeated 4-5 times with 5 min intervals to achieve a PaO_2 ≤ 125 mmHg. After the blood gases had reached a steady state, they were measured and this was defined as time zero (t=0). Then, animals were randomly divided into seven groups of six animals: the first group received exogenous surfactant (150 mg/kg) within 10 min (early treatment), whereas

in the other six groups mechanical ventilation was continued for 3 h before treatment (late treatment; $t=3$ h). Treatment consisted of: exogenous surfactant (150 mg/kg); exogenous surfactant (250 mg/kg); BAL with saline; BAL with a diluted surfactant suspension (2.5 mg surfactant per ml saline); BAL with saline, immediately followed by exogenous surfactant (150 mg/kg); and BAL with a diluted surfactant suspension (2.5 mg surfactant per ml, from which 0.7 mg/ml could be detected in the recovered BAL fluid), immediately followed by exogenous surfactant (150 mg/kg; in total 210 mg/kg). The amount of saline used in BAL was 32 ml/kg at 37 °C. The phospholipid content of the diluted surfactant suspension was determined by phosphorus analysis before and after one lavage [12].

A freeze-dried natural surfactant was used, isolated from pig lungs as previously described [13]. It consists of approximately 90-95% phospholipids, 1% hydrophobic proteins (surfactant-proteins B and C) and 1% free fatty acids, the remainder being neutral lipids; there is no surfactant-protein A in this surfactant preparation. The surfactant powder was suspended in warm saline (37 °C) at a concentration of 40 mg/ml. For instillation of the surfactant, animals had been disconnected from the ventilator and received 1-2 ml of the surfactant suspension directly into the endotracheal tube followed by insufflation of 28 ml/kg of air via a syringe. After surfactant instillation, animals were immediately reconnected to the ventilator (ventilator settings were not changed).

During the observation period, arterial blood samples were collected at the following times: 5 min and 1, 2, 3 h after the lavage procedure; and 15 min after treatment (late treatment) and every hour for 3 h. Blood samples of 0.20 ml were taken and replaced by heparinized saline (5 IE/ml). Of each blood sample PaO_2, $PaCO_2$, and pH were measured by conventional methods (ABL™ 505, Radiometer A/S, Copenhagen, Denmark).

At the end of the observation period, all animals were killed with an overdose of pentobarbital sodium and then BAL was performed for measurements of total protein concentration and surface tension properties. The lungs were lavaged once with saline (32 ml/kg) and cell material was removed by centrifugation (10 min at 300 g). The lungs of those animals which died after late treatment were lavaged one time immediately after death to get material for protein and surface tension measurements. The protein concentration was determined using the modified Lowry method [14], with bovine serum albumin as standard. The minimal surface tension of the BAL fluid was

measured using a modified Wilhelmy balance (E. Biegler GmbH, Mauerbach, Austria) by applying 100 μL of BAL fluid to the surface of a saline-filled trough [15]. The surface area was compressed and expanded with a cycling time of 3 min per cycle and maximum and minimum surface areas of 64 and 12.8 cm^2, respectively (100% and 20%). The minimal surface tension was measured after 3 cycles at 20% surface area, and is expressed as milli Newton/metre (mN/m).

Analysis of variance (ANOVA) was used to assess whether there was an overall difference within or between the groups. If a difference was found, a post hoc test was used (Student-Newman-Keuls' multiple comparison procedure). Statistical significance was accepted at $p \le 0.05$. All data are reported as mean $\pm$ standard deviation (SD).

Results

Blood gases before and after the repeated saline lavage were comparable in all animals (Figure 1 and Table 1). Before late treatment (t=3 h), 10 animals died due to insufficient gas exchange and these animals were excluded from the study and not included in the statistical analyses. The total protein concentration and the minimal surface tension of the BAL fluid obtained 3 h after the repeated saline lavage were significantly increased compared to the first BAL fluid of the repeated saline lavage which served as controls (Table 2).

Mean PaO$_2$ values increased rapidly to pre-lavage values in the group which received exogenous surfactant (150 mg/kg) within 10 min after the last lavage (early treatment) and remained stable during the subsequent 6 h study period (Figure 1). In the four groups which received a bolus of surfactant (with or without previous BAL) 3 h after the repeated saline lavage (late treatment), mean PaO$_2$ values increased immediately to pre-lavage values but remained stable only in the group that underwent BAL with a diluted surfactant suspension prior to administration of surfactant. In the other three groups (surfactant 150 mg/kg; surfactant 250 mg/kg; BAL with saline and surfactant 150 mg/kg), mean PaO$_2$ values decreased over time and were significantly lower compared to the group that underwent BAL with a diluted surfactant suspension prior to administration of surfactant at the end of the observation period (Figure 1). In addition, there were no significant differences in mean PaO$_2$ values between these 3 groups (surfactant (150 mg/kg); surfactant (250 mg/kg); and BAL with saline together with surfactant) during the subsequent 3 h. Further, in the group in which the lungs were lavaged once with a diluted surfactant suspension, mean PaO$_2$ values initially

increased to pre-lavage values but deteriorated over time, whereas mean PaO_2 values did not improve at all in the group which underwent BAL with saline (Figure 1).
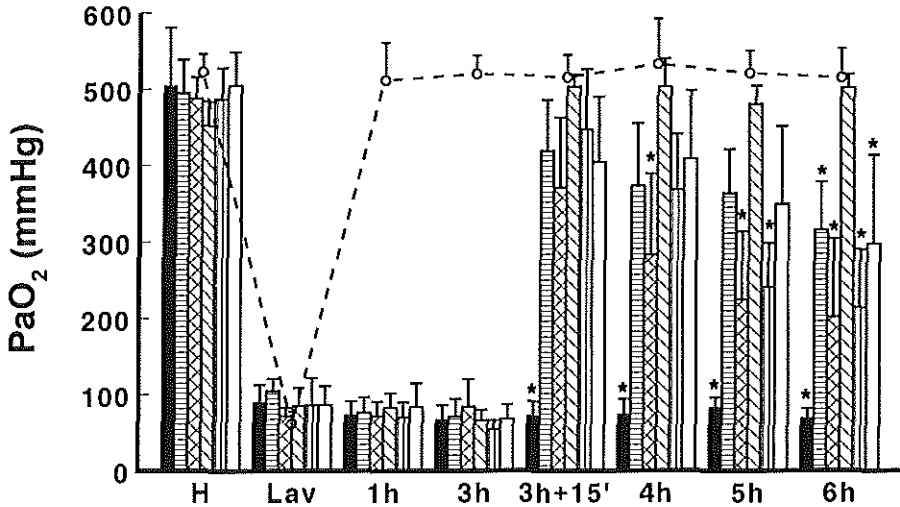
Figure 1. Change in mean arterial oxygenation (PaO_2) ($\pm$SD) of the 7 study groups before lung lavage (H), after lavage (Lav), and during the 6 h observation period. The *dashed line*: animals (n=6) that received a bolus of exogenous surfactant (150 mg/kg) intratracheally within 10 min after the repeated lavage (early treatment). The bars indicate the 6 study groups which received treatment 3 hours after the repeated lavage (late treatment). *Solid bar*: animals (n=6) which underwent BAL with saline only; *horizontal-striped bar*: animals (n=6) which underwent BAL with saline immediately followed by a bolus of surfactant (150 mg/kg); *hatched bar*: animals (n=6) which underwent BAL with a diluted surfactant suspension (2.5 mg/ml); *diagonal-striped bar*: animals (n=6) which underwent BAL with a diluted surfactant suspension (2.5 mg/ml) immediately followed by a bolus of surfactant (150 mg/kg); *vertical-striped bar*: animals (n=6) which received a bolus of surfactant (150 mg/kg); *open bar*: animals (n=6) which received a bolus of surfactant (250 mg/kg). *H*, healthy. *Lav*, repeated saline lavage in order to induce acute lung injury. *, $p \leq 0.05$ vs group which was lavaged with a diluted surfactant suspension immediately followed by a bolus of exogenous surfactant (150 mg/kg). To convert mmHg to kPa, divide the value by 7.5.

Mean $PaCO_2$ values were comparable in the groups which were treated at the late time point in lung injury and were higher compared to the group which received exogenous surfactant at the early time point (Table 1). Data on recovery of BAL fluid,

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minimal surface tension and total protein concentration of BAL samples are given in Table 2. The protein concentration of BAL fluid of healthy controls (obtained from the first BAL of the repeated saline lavage) was much lower compared with the BAL samples of all six study groups. Compared to BAL samples taken at time 3 h, protein concentration was decreased only in the group which underwent BAL with surfactant suspension, immediately followed by exogenous surfactant (Table 2). The minimal surface tension of BAL fluid was significantly decreased in the groups which received a bolus of exogenous surfactant with or without previous BAL with saline or surfactant in comparison with BAL sample performed 3 h after surfactant depletion (Table 2).

Table 1. $PaCO_2$ (mmHg) data of the different study groups. Values are given as mean $\pm$ SD.

Group:	Time point:	H	Lav	1 h	3 h	4 h	5 h	6 h
Surf. 150	early	34±5	57±12	43±4	38±3	35±3	33±6	34±5
BAL	late	31±3	47±5	52±10	42±13	39±9	35±3[††]	39±4[†]
BAL+Surf.	late	37±3	48±13	49±16	51±10	40±8[†]	44±6	47±4[†]
BAL with S.	late	32±4	51±7	50±10	52±8	47±5	38±7	42±15[†]
Surf. 150	late	37±6	58±7	51±9	48±6	43±8	45±6	44±7
BAL with S.+Surf.	late	32±5	49±9	49±8	51±11	46±4	40±10	41±3[†]
Surf. 250	late	29±4	51±13	45±9	53±10	56±10	51±11	45±13

H, healthy. Lav, the repeated saline lavage in order to induce the lung injury. BAL, bronchoalveolar lavage with saline (32 ml/kg). BAL with S., bronchoalveolar lavage with a surfactant supension (2.5 mg/ml). Surf., administration of a bolus of surfactant at a dose of 150 mg/kg (150) or 250 mg/kg (250). †, death of one animal.

Table 1 shows the numbers of deaths in the different groups. In the group in which the lungs were lavaged with saline, three animals died during the next 3 h due to severe hypoxemia. In the group which received the combination of BAL with saline and bolus of surfactant (150 mg/kg) two animals died during the observation period; of these, one animal died within one hour after treatment as a result of hyperventilation ($PaCO_2$ value <18 mmHg) while the PaO_2 value was at prelavage value. At the end

of the observation period, one animal died in the surfactant lavage group due to hypoxemia. In the group surfactant lavage together with bolus of surfactant, one animal died as a result of hyperventilation with high PaO_2 value.

Table 2. Data on recovery, protein concentration and minimal surface tension of BAL fluid of the different study groups. Values are given as mean ± SD.

Group:	Time point:	Time:	Recovery (%)	Protein (mg/ml)	Minimal surface tension (mN/m)
		T0	90±3	0.6±0.3	21.1±2.4
		T3	99±7	4.9±0.6	36.1±4.9#
Surf. 150	early	T6	93±3	2.2±0.6*	22.3±1.3*
BAL	late	T6	98±3	6.3±1.3	34.5±2.4#
BAL + Surf. 150	late	T6	93±3	3.7±0.8	27.5±3.5
BAL with S.	late	T6	94±2	3.9±0.7	28.9±5.3
Surf. 150	late	T6	96±2	4.8±1.1	25.3±2.8*
Surf. 250	late	T6	95±4	4.2±1.4	23.1±4.2*
BAL with S. + Surf.	late	T6	91±2	3.0±0.9*	21.5±2.2*

T0, time zero. T3, time 3 hours after the repeated lavage (without treatment). T6, time 6 hours after the repeated lavage which was the end of the observation period. BAL, bronchoalveolar lavage with saline (32 ml/kg). BAL with S., bronchoalveolar lavage with a surfactant supension (2.5 mg/ml). Surf., administration of a bolus of surfactant at a dose of 150 mg/kg (150) or 250 mg/kg (250). *, $p < 0.05$ vs. T3. #, $p < 0.05$ vs. T0.

Discussion

The results of this study demonstrate that lung lavage with a diluted surfactant suspension together with instillation of exogenous surfactant resulted in a sustained improvement of lung function in lung lavaged rats treated 3 h after the repeated lavage (late treatment), whereas lung function deteriorated during the subsequent 3 h after instillation of a large dose of exogenous surfactant (250 mg/kg). The concentration of surfactant recovered after lavage with the diluted surfactant suspension was only 28±1.5%. This indicates that 72% of the applied surfactant remained in the lungs.

BAL was performed with 32 ml per kg and the surfactant content of the surfactant lavage was 2.5 mg/ml. Probably, a dose of approximately 60 mg/kg of surfactant has been additionally applied by the lavage procedure. In other words, the group receiving BAL with the diluted surfactant suspension and a bolus of surfactant (150 mg/kg) has received in total a dose of 210 mg/kg. Thus, despite that the latter group received 15-20% less surfactant, the improvement of arterial oxygenation at the end of the observation period was significantly better compared to the 250 mg/kg bolus group.

Instillation of exogenous surfactant (150 mg/kg) directly after the repeated saline lavage (early treatment) resulted in stable improvement of lung function during the 6 h observation period. These data correspond to previous investigations in which the therapeutic effects of exogenous surfactant improved when administration of surfactant takes place in the early stages of lung injury [7,8]. It has been established that the transient effect after surfactant therapy is due to inactivation of the instilled surfactant by plasma-proteins and inflammatory mediators such as oxygen radicals, proteases and phopholipases [for review see (6)]. The purpose of BAL prior to exogenous surfactant therapy is the removal of these potent surfactant inhibitors. It is known, however, that BAL with saline can harm the lungs and this was confirmed by the results of this study which showed that three of the six animals in the group that underwent BAL with saline died within the observation period due to insufficient gas exchange (Table 1).

The improvement of arterial oxygenation after BAL with a diluted surfactant suspension was only transient because the amount of surfactant remaining in the lungs was low (about 60 mg/kg) and it has been shown that additional surfactant substitution is needed to maintain blood gases at pre-lavage values (Figure 1). In contrast to our results, Balaraman *et al.* [16] demonstrated that administration of surfactant by lavage is more effective in improving lung function compared to normal bolus instillation in lung lavaged piglets. In that study, however, an artificial surfactant was used, which is protein-free, whereas in the present study a natural surfactant was used, containing 1-2% of the specific surfactant proteins B and C. In lung lavaged rats, we have demonstrated that mean PaO_2 values increased to prelavage values within 5 min with all available natural surfactants whereas mean PaO_2 values increased to only 50% of the prelavage values with the synthetic surfactants [17]. Using the same model, Häfner and colleagues further showed that mean PaO_2 values increased rapidly to prelavage values after the addition of surfactant protein C to synthetic surfactant [18]. In addition, it has been established in in-vitro studies that surfactant proteins B and/or C are

required for a rapid adsorption of the phospholipids to the air-liquid interface (for review see [19]). It has therefore been concluded that the surfactant proteins B and/or C are essential for uniform spreading of exogenous surfactant to the lungs. This was confirmed by the results of two recently published clinical trials that directly compared a natural with an artificial surfactant preparation and demonstrated that the natural surfactant produced a much more rapid improvement in lung function in neonates with RDS [20,21].

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]. Repeated whole-lung lavage with saline produces an acute quantitative surfactant deficiency and, together with conventional mechanical ventilation, leads to severe lung injury with impaired gas exchange, decreased lung compliance and functional residual capacity, increased permeability changes of the alveolo-capillary membrane with edema, and sustained pulmonary hypertension [10,13,22]. It has been postulated that, in the acute phase, this model reflects more a primary surfactant deficiency, as seen in neonatal RDS [1]. Therefore, in the present study the animals were first ventilated for 3 h after induction of respiratory failure, before surfactant application to induce a more severe lung injury, as demonstrated by the increase of protein concentration in the BAL fluid (Table 2). Despite the fact that the lung injury in this study is not exactly representative of the pathology as seen in humans with ARDS, this model is ideal for testing various therapeutic interventions that may be considered as therapy of acute lung injury [10,13,23,24].

In adults with ARDS, increased alveolo-capillary permeability combined with inflammation is known to inactivate the functional alveolar surfactant, resulting in failure of the lung as a gas exchange organ [6]. The value of surfactant therapy is that the functional impairment of active surfactant can be reversed by instillation of an excess of exogenous surfactant [2,25]. Anzueto and colleagues [3], however, showed that administration of aerosolized artificial surfactant (without surfactant proteins) had no effect on mortality and lung function in 725 patients with sepsis-induced ARDS. The authors speculated that less than 25 mg of the surfactant per kilogram body weight was actually delivered into the lungs over a time period of 5 days [3]. We have demonstrated that intratracheal application of only large quantities of natural surfactant (dose of ≥200 mg/kg) resulted in an immediate improvement of blood gases and lung mechanics in animal models of ARDS, such as acid aspiration and widespread

pneumonia [11,26,27]. This was recently confirmed by two clinical reports [4,5]. In adults with ARDS, Gregory *et al.* [5] studied four different dosing strategies and the results showed that maximum improvement in oxygenation, minimum ventilatory requirements, and the lowest mortality rate were obtained by using four doses of 100 mg/kg of a natural surfactant (total amount of 400 mg/kg). Walmrath and colleagues [4] reported an impressive acute improvement of arterial oxygenation in response to bronchoscopic application of a large quantity of natural surfactant (300 mg/kg) in 10 adult patients with severe ARDS and sepsis. In half of their patients, however, a second dose (200 mg/kg) was required within 24 h to achieve a prolonged effect on gas exchange. The authors conclude that the bronchoscopic technique offers a feasible and safe approach for the delivery of surfactant to the distal airways in severe ARDS [4]. We speculate that, if the lungs were lavaged prior to administration of the surfactant in order to remove the potent surfactant inhibitors, the second dose had probably not been necessary to achieve a stable improvement in lung function in such severe states of respiratory failure.

With a flexible fiberoptic bronchoscope, a lung lobe can easily be lavaged after the bronchoscope has been wedged into an airway. Lavage with fluids with a high surface tension, such as saline, requires high pressures for passage through the small airways [28]. Enhorning and colleagues [29] have demonstrated that the resistance of the rat lungs' conducting airways increased with saline lavage and reduced again after flushing with surfactant suspension (3 mg/ml). Therefore, we suggest adding a small amount of exogenous surfactant to the BAL fluid and speculate that BAL with a diluted surfactant suspension will also reach the more peripheral located alveoli, leading to the removal of more surfactant inhibitors located in these parts of the lung.

We conclude that BAL with a diluted surfactant suspension prior to administration of exogenous surfactant improves the effectiveness of surfactant therapy in the ARDS type of lung failure. In two newborns with massive meconium aspiration, BAL with saline, followed by surfactant administration, was used successfully to reduce airway obstruction and prevent non-homogeneous distribution of surfactant [30]. In contrast to BAL with saline, we speculate that lavage with a diluted surfactant suspension is less harmful for the lungs, removes more of the potent surfactant inhibitors and contributes to a more even surfactant distribution over the lungs.

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Chapter 7

Surfactant therapy in combination with high-frequency oscillatory ventilation is not superior to conventional mechanical ventilation in reducing lung injury in lung-lavaged rabbits

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Summary

The aim of this study was to compare high-frequency oscillatory ventilation (HFOV) to conventional mechanical ventilation (CMV) with and without surfactant in the treatment of acute lung injury caused by normal saline lavage in adult rabbits. The efficacy of each therapy was assessed by evaluating gas exchange, lung deflation stability and lung histopathology. After 4 hours of assigned therapy, we evaluated lung deflation stability by decreasing mean airway pressure (MAwP) from 12 to 9 to 6 cm H_2O at 10 min intervals. After each change of MAwP blood gases were measured. Following assessment of deflation stability, all 4 groups of animals were restarted on CMV at the same settings as used during lung lavage and gas exchange was again assessed. To evaluate lung injury, we assessed lung histopathology. The lungs were fixed at PEEP + 1 cm H_2O. Lungs of 3 additional animals were removed directly after the lavage procedure and served as controls.

Arterial oxygenation in both HFOV groups (with or without surfactant) was kept above 350 mmHg (according the high-lung volume strategy) during the first 4 h by using appropriate MAwP. In the CMV group with surfactant, PaO_2 increased from 62 ± 16 to 400 ± 80 mmHg within 5 min and remained stable during the subsequent 4 h. In the CMV group without surfactant, PaO_2 did not improve and all animals died within the observation period. In both HFOV groups, a MAwP of 18-20 cm H_2O had to be applied initially to open up the lungs, that could be decreased during the subsequent 4 h while PaO_2 remained stable. During deflation stability, mean PaO_2 values decreased to post-lavage values in the group that received HFOV alone after MAwP was decreased to ≤ 9 cm H_2O but not in both groups that received surfactant (HFOV and CMV). The HFOV group without surfactant showed more cellular infiltration and epithelial damage compared with the lavaged only animals and both surfactant-treated groups (HFOV and CMV). In addition, the surfactant-treated animals (HFOV and CMV) showed no additional structural lung damage compared to the lavaged only animals.

We conclude that the use of surfactant therapy in combination with HFOV is not superior to CMV in improving gas exchange, lung deflation stability and in the prevention of lung injury.

Introduction

High-frequency oscillatory ventilation (HFOV) and exogenous surfactant therapy are two therapies to treat neonatal respiratory distress syndrome (RDS) but deal with the pathophysiology of this disease in different ways. HFOV is intended to counterbalance the increased tendency for collapse by applying a constant distending pressure ('mean airway pressure') [1]. Exogenous surfactant therapy aims to decrease the alveolar surface tension by application of a surface active material [2]. In contrast to the limited success of the early HFOV trials [3], exogenous surfactant therapy has been used successfully in worldwide clinical trials and has decreased mortality by 30-40% [2]. Therefore, exogenous surfactant therapy with conventional mechanical ventilation (CMV) is now routinely used in most neonatal ICUs to prevent and treat neonatal RDS [2].

In the first HFOV trial, a low distending airway pressure was used in order to minimize the risk of barotrauma [3]. Experimental studies have, however, shown that alveoli should be actively opened and that relatively high airway pressure has to be used to stay above the closing pressure to avoid hypoxemia and lung injury [4,5]. This is called the high-lung volume strategy; results of recent pilot studies in neonates with RDS applying this strategy are encouraging [6-8].

Until now, only a few studies have been published on the combined use of surfactant and HFOV in animals or humans [6, 8-11]. It was shown that after surfactant therapy HFOV was superior to CMV in improving pulmonary function and reducing lung injury [6, 8-11]. In these studies, however, HFOV was used in combination with the high-lung volume strategy whereas CMV was not. Recently, Froese and colleagues [10] 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) after surfactant therapy with the use of CMV [10]. 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 hours [12-14]. Therefore, the purpose of the present study was to compare the use of HFOV to CMV with and without surfactant therapy in the management of acute lung injury caused by lung lavage in adult rabbits. During the first 4 h, target PaO$_2$ ranges were above 350 mmHg according to the high-lung volume

strategy [5]. To evaluate whether lung function was improved after 4 h of ventilation, MAwP was decreased three times in succession and blood gases were measured. We used a high PaO_2 value at low MAwP as an indicator for deflation stability conferred by optimal surfactant function. Further, we performed histopathology of the lungs in order to evaluate whether HFOV is superior to CMV, with or without surfactant, in the prevention of additional lung injury secondary to mechanical ventilation.

Materials and methods

This study was approved by the local Animal Committee of the Erasmus University Rotterdam; care and handling of the animals were in accord with the European Community guidelines (86/609/EEG). A total of 27 adult New Zealand White rabbits (IFFA-Credo, Brussels, Belgium) with a mean body weight of 2.7 ± 0.3 kg were anaesthetized with pentobarbital sodium (50 mg/kg) via an auricular vein and then placed in a supine position. An endotracheal tube (i.d. 3.5 mm) was inserted via tracheostomy and mechanical ventilation was initiated with a Servo Ventilator 900C (Siemens-Elema AB, Solna, Sweden) in a pressure-control mode, indicating time-cycled ventilation with decelerating flow, with the following ventilator settings: FiO_2 of 1.0, positive end-expiratory pressure (PEEP) of 2 cm H_2O, frequency of 30 breaths/min, inspiratory/expiratory ratio of 1:2 and a peak inspiratory pressure of 10-14 cm H_2O to keep $PaCO_2$ within normal range. An infusion of 2.5% glucose was continuously administered via the auricular vein as a maintenance fluid (5 ml/kg/h). Anaesthesia was maintained by hourly injection of pentobarbital sodium (5 mg/kg/h, i.v.); muscle paralysis was achieved by hourly injection of pancuronium bromide (0.1 mg/kg/h, i.m.).

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

In all animals, respiratory insufficiency was induced by repeated whole-lung lavage according to Lachmann *et al.* [15]. Each lavage was preformed with saline (30 ml/kg) heated to 37 °C. Lung lavage was repeated 5-8 times at 2-5 min intervals to achieve a PaO_2 <85 mmHg at a peak pressure and PEEP of 26 and 6 cm H_2O,

respectively (other ventilator settings were not changed). After reaching steady state, 24 animals were divided randomly into four groups of six animals. In the first group, animals received a bolus of surfactant intratracheally (Alveofact*; 100 mg/kg) and immediately followed by connection to a high-frequency oscillator (type OHF-1, S.A. Dufour, Villeneuve d'Ascq, France). During HFOV, a frequency of 10 Hz was used and mean airway pressure (MAwP) was increased until PaO_2 was above 350 mmHg to guarantee the high-lung volume strategy (i.e. open lungs) [4,5]. When PaO_2 was above 550 mmHg during the study period, MAwP was decreased by steps of 1 cm H_2O. Pressure amplitude was initiated with 30 cm H_2O and was altered as necessary to keep $PaCO_2$ within normal range (35-45 mmHg). The second group received exogenous surfactant intratracheally (Alveofact*; 100 mg/kg) and conventional mechanical ventilation (CMV) with the Servo Ventilator 900C was continued (ventilator settings were not changed). For instillation of surfactant, the animals had been disconnected from the ventilator and received the surfactant suspension (25 mg/ml) directly into the endotracheal tube via a syringe; immediately followed by re-connection to the ventilator. The other two groups served as controls and received HFOV or CMV alone in the same way as described above.

After 4 h, MAwP was decreased to 12 cm H_2O for 10 min in those animals that received a MAwP of ≥12 cm H_2O and then MAwP was decreased to 9 and 6 cm H_2O, respectively. Finally, all study groups were restarted on CMV at the same settings as used during lung lavage, indicating a MAwP of ±12 cm H_2O. After 10 min, blood samples were taken and the lungs were intrathoracally fixated and subsequently removed for histopathologic examination.

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

At the end of the observation period, lungs were ventilated with air (other ventilator settings were not changed), the abdomen of the rabbit was opened and the diaphragm was inspected for evidence of pneumothorax. The inferior vena cava was cannulated and a perfusion solution, consisting of saline saturated with 95% O_2 and 5%

CO_2, 2.2 mM $CaCl_2$, 0.5% procaine and 1% heparin, was then infused at a rate of 3 l/h. The abdominal aorta was cut and the infusion was stopped when clear fluid flowed from the aorta. Thereafter, the peak pressure above PEEP level was lowered to 1 cm H_2O, that was maintained while the lungs were fixated by infusing ± 100 ml of a fixation solution, consisting of 3.6% formaldehyde and 0.25% glutaraldehyde, via the inferior vena cava. After fixation, the trachea was clamped at a pressure of 6 cm H_2O, the thorax was opened and the lungs were removed en bloc and stored in the fixation solution. The lungs were numbered and histopathologic examinations of the lungs were performed blindly. The lungs of the three remaining animals were fixed 10 min after the lavage procedure as described above and histopathologic examinations were performed. These animals were used to study the influence of the lavage procedure itself on morphological changes.

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

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

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

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

Mean PaO_2 values in both HFOV groups (with or without surfactant) were kept above 350 mmHg in the first 4 h by using appropriate MAwP (Figure 1, Table 1). In the CMV group with surfactant, PaO_2 increased from 62 ± 16 to 400 ± 80 mmHg within 5 min and remained stable during the subsequent 4 h without changing the ventilator settings (Figure 1).
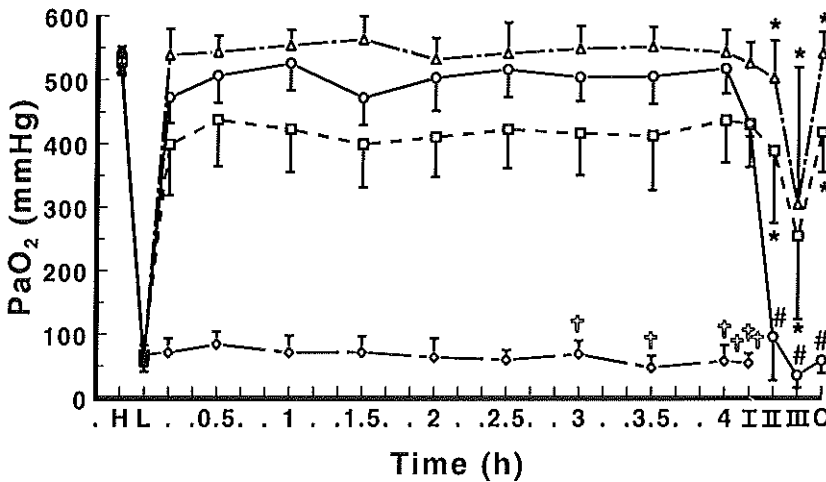
Figure 1. Change in mean arterial oxygenation (PaO_2) ($\pm$SD) of the four study groups before lung lavage (**H**), after lavage (**L**), and during the subsequent 4 h and 40 min observation period. O, animals (n=6) that received HFOV without surfactant; ◊, animals (n=6) that received CMV without surfactant; □, animals (n=6) that received CMV and surfactant (100 mg/kg); ▵, animals (n=6) that received HFOV and surfactant (100 mg/kg). H, healthy. L, 10 min after the last lavage. I, II and III are the 3 reduction steps of MAwP to: **I** = 12 cm H_2O; **II** = 9 cm H_2O; **III** = 6 cm H_2O. C, pressure-controlled ventilation with the same settings as during the lavage procedure, indicating a MAwP of $\pm$12 cm H_2O. *, $p \leq 0.05$ versus HFOV group. #, $p \leq 0.05$ versus PaO_2 values at 4 h. To convert mmHg to kPa, divide the value by 7.5.

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

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

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

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

The mean $PaCO_2$ values gradually increased in the group CMV without surfactant (Table 1). In the other three groups, mean $PaCO_2$ values were kept between 35 and 45 mmHg and were comparable (Table 1). MAwP data are shown in Table 1. In both groups that received HFOV (with or without surfactant), MAwP was initially increased to 18-20 cm H_2O and could be decreased significantly during the subsequent 4 h while PaO_2 remained stable. At time point 4 h, MAwP of the group HFOV with surfactant was significantly lower compared to the HFOV group without surfactant (Table 1).

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

Figure 2 shows the lung injury score of the different groups. The groups that received surfactant combined with HFOV or CMV had significantly less lung injury than both groups without surfactant (HFOV and CMV) (Figure 2). The extent of lung injury of both surfactant-treated groups was comparable with that of the animals that were lavaged only. Representative photomicrographs are shown in Figures 3 A,B and 4 A,B. More detailed quantitative comparisons between both surfactant treated groups (HFOV and CMV) were not made.
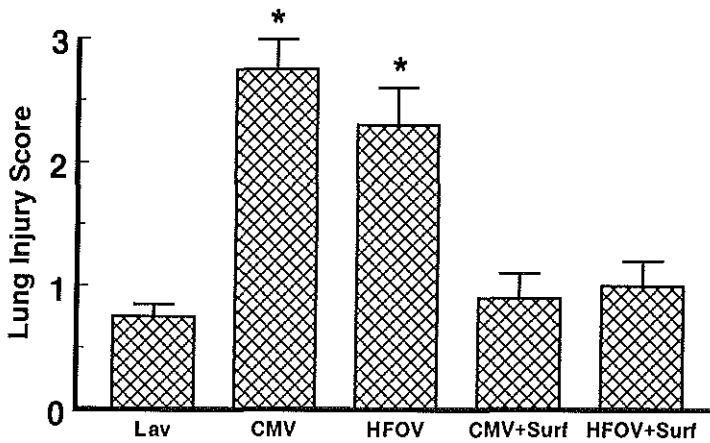
Figure 2. Semi-quantitative lung injury score for all groups. Lav, animals that were lavaged only. Surf, surfactant. *, $p \leq 0.05$ vs. animals that were lavaged only.
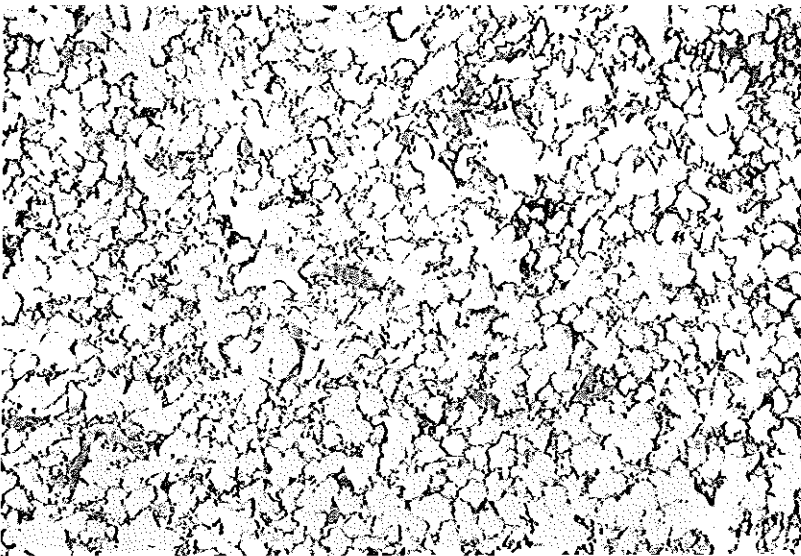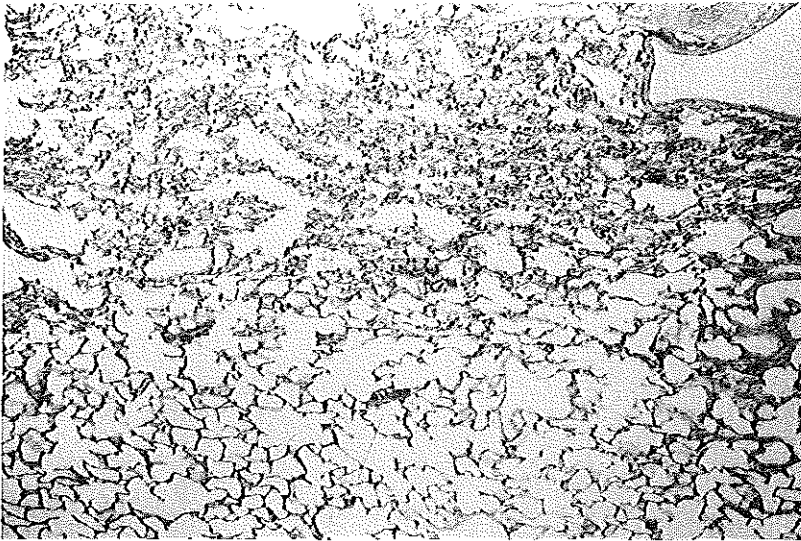
Figure 3A,B. (A) Half of the lung tissue is atelectatic in the group that were lavaged only. No cellular reaction exists and the septa are structured regularly. (B) Aerated lung tissue with focal atelectasis (group HFOV+Surfactant). Collapse of the alveoli is accompanied by a slight interstitial and intra alveolar infiltrate. Light microscopy, haematoxylin eosin stain, original magnification x 40.
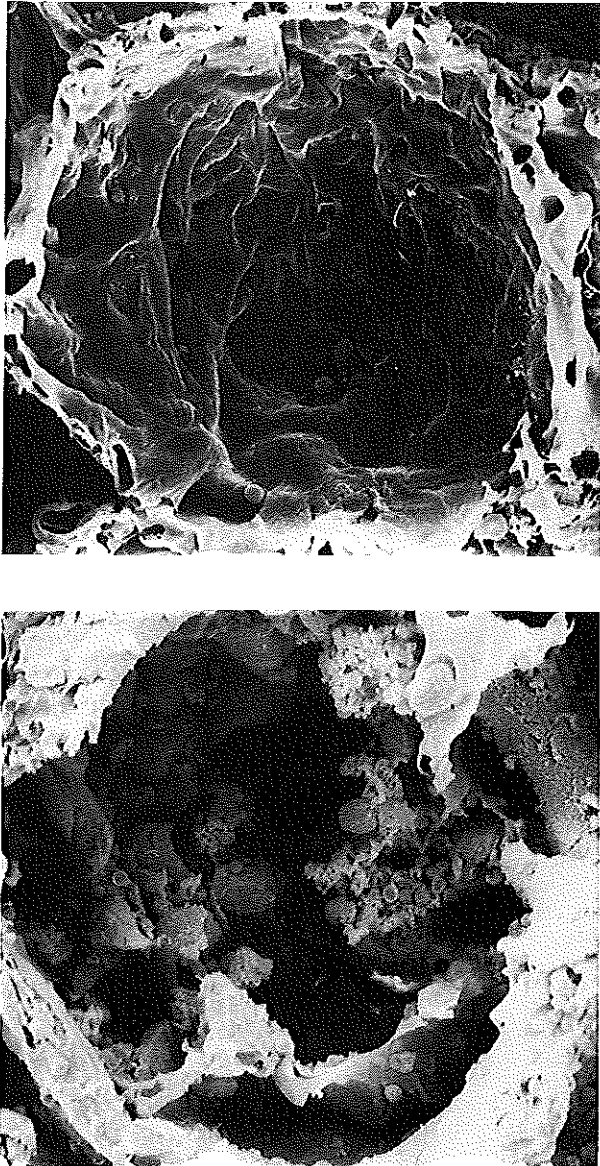
Figure 4A,B. (A) Representative alveoli of the group that received CMV for only 10 min after the lavage procedure. (B) the group that received HFOV without surfactant for 4.5 h. The alveolus of the HFOV group contain fibrin, leucocytes, erythrocytes, and macrophages (B). Scanning electron microscopy, magnification x 750.

Discussion

In the present study we used the lung lavage model that has proved to be a consistent and convenient model of acute lung injury [12-15]. Despite the use of adult animals, it has been postulated that this model reflects in the acute phase a more primary surfactant deficiency, as seen in neonatal RDS [2]. Several investigators have confirmed the direct relationship in this model between arterial oxygenation and lung volume [4,5,13,16]. It has been demonstrated that arterial oxygenation increased with increasing lung volume as alveoli re-expanded and shunt flow decreased [16]. In the present study, we therefore used arterial oxygenation as a reflection of lung volume.

The results of this study demonstrate that after surfactant therapy there is no difference between the use of HFOV and CMV in improving gas exchange, lung deflation stability and prevention of lung injury in lung-lavaged rabbits. These results are in contrast to the results of Froese *et al.* [10] who demonstrated that the effect of exogenous surfactant on arterial oxygenation remained stable with HFOV, whereas it decreased significantly during the 4 h study period with CMV at high-lung volume. In their study, the high-lung volume strategy with CMV was performed by a gradual increase of PIP and PEEP but without an active volume recruitment manoeuvre as used with HFOV [10]. Furthermore, CMV was used with high tidal volumes (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 alveolar level [17,18]. In the present study, we did not perform an active volume recruitment manoeuvre with CMV after surfactant therapy, but arterial oxygenation increased to above 350 mmHg within 5 min (without change of ventilator settings) and kept stable during the subsequent 4 h. In contrast to the study of Froese *et al.* [10], we used normal tidal volumes (10 ml/kg) and installed the surfactant at a higher concentration (100 mg/kg) and as one bolus, that is known to improve the surfactant distribution and its efficacy [14].

Surfactant metabolism and turnover is known to be strongly influenced by ventilation and some authors suggested that secretion of surfactant is increased with HFOV [19-21]. In the present study, we confirmed that in lung-lavaged rabbits optimal gas exchange can be obtained with HFOV without surfactant, by using the high-lung volume strategy. However, lung function does not improve over time, as shown by the results that mean PaO_2 values at the end of the observation period were comparable with the post-lavage values at the same ventilatory support (Figure 1). This indicates

that the reduced end-expiratory stability, due to the repeated lung lavages (i.e. surfactant deficiency), was apparently not improved by HFOV. This confirms the results by Meredith *et al.* [22], who showed excellent gas exchange using HFOV with the high-lung volume strategy in premature baboons, but no beneficial effect on lung volume at zero pressure (functional residual capacity) determined at 24 h. This indicates that optimization of alveolar expansion with HFOV markedly improves oxygenation but does not influence alveolar stability as long as the underlying cause, i.e. surfactant deficiency, is not reversed. This will occur by gradual synthesis of endogenous surfactant over time, or after exogenous surfactant instillation [2].

Morphologic changes were more pronounced in the animals that received CMV or HFOV alone compared to the animals that were lavaged only (Figure 2). In surfactant-deficient lungs, high shear forces between open and closed alveoli are, to a great extent, held responsible for the damage caused by artificial ventilation [9]. Therefore, alveoli should be actively opened and kept open during the entire respiratory cycle in order to minimize additional lung damage [4,5]. Studies in lung-lavaged rabbits demonstrated that HFOV had beneficial effects on preventing development of lung injury due to mechanical ventilation when arterial oxygenation was kept above 350 mmHg (indicating alveolar expansion) and not when arterial oxygen tensions were kept between 70 and 100 mmHg [4,5]. This may explain the higher degree of lung injury as seen in the present study in the group HFOV without surfactant. In that group, mean PaO_2 values dropped to below 100 mmHg for only 30 min, after MAwP was decreased to ≤ 9 cm H_2O at the end of the observation period (Figure 1). In contrast, mean PaO_2 values remained above 350 mmHg almost for the entire observation period, despite the reduction of the MAwP, in both surfactant-treated groups (HFOV and CMV). Also, histopathology examinations of both groups that received surfactant (HFOV and CMV) showed no additional structural lung damage in comparison with the animals that were lavaged only (Figure 2). This result is supported by earlier experimental studies in which surfactant therapy has shown to improve uniform alveolar expansion and end-expiratory alveolar stability and thereby effectively prevents the progression of ventilator-induced lung injury [9,23].

In contrast to Jackson *et al.* [11], we found no difference in the prevention of lung damage between HFOV and CMV after surfactant therapy (Figure 2). Further, it was shown that the HFOV group without surfactant showed more cellular infiltration and epithelial damage than the HFOV group with surfactant (Figure 2). This indicates

that achieving and maintaining alveolar expansion (i.e. open lung) is of more importance than the type of mechanical ventilation (HFOV vs. CMV). The importance of an open lung strategy is supported by the results of Amato and colleagues [24] who recently demonstrated in adults with ARDS that CMV with an open-lung approach had, for the first time, a significant impact on survival and barotrauma. Therefore, we concluded that surfactant therapy with CMV is equally effective to prevent ventilator-induced lung injury as HFOV combined with surfactant, as long as alveoli are opened and kept open to avoid high shear stress. This can be achieved by the use of PEEP level that sufficiently counterbalances the retractive forces or by higher and/or repeated doses of exogenous surfactant to reduce the retractive forces.

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

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Chapter 8

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

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Summary

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

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

Setting: Research laboratory.

Subjects: Thirty male Sprague-Dawley rats.

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

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

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

Introduction

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

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

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

Materials and methods

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

The non-blinded studies were performed in male Sprague-Dawley rats (n=24) with a body weight of 260-330 g (Harlan CPB, Zeist, The Netherlands). After induction of anesthesia with nitrous oxide, oxygen and ethrane (66/33/3%), a polyethylene catheter (0.8-mm outer diameter) was inserted into the right carotid artery for drawing arterial blood samples. Before tracheotomy, the animals received pentobarbital sodium 60 mg/kg body weight, i.p. (Nembutal*; Algin BV, Maassluis,

The Netherlands) and the inhalation of ethrane was decreased by 50%. After a metal cannula was inserted into the trachea, muscle relaxation was given with pancuronium bromide 2.0 mg/kg, i.m. (Pavulon[*]; Organon Technika, Boxtel, The Netherlands) and the animals were connected to a ventilator. Anesthesia was maintained with pentobarbital sodium, i.p. (Nembutal[*]; 60 mg/kg/h) and muscle relaxation was attained with pancuronium bromide, i.m. (Pavulon[*]; 2 mg/kg/h). Body temperature was kept within normal range by means of a heating pad.

The animals were mechanically ventilated in parallel (6 animals simultaneously) with a Servo Ventilator 300 (Siemens-Elema AB, Solna, Sweden) at the following ventilator settings: pressure constant time cycled mode, frequency of 30 breaths/min, peak inspiratory pressure (PIP) of 12 cm H_2O, PEEP of 2 cm H_2O, I/E ratio of 1:2, and an FiO_2 of 1.0. Initially, PIP was increased to 20 cm H_2O for 1 min to open up atelectatic regions in the lungs. After this opening up procedure, the ventilator settings were returned to the previous ones and a 0.15 ml blood sample was taken and replaced by heparinized (10 IE/ml) saline (0.9% NaCl). PaO_2, $PaCO_2$, and pH were measured by conventional methods (ABL 505, Radiometer, Copenhagen, Denmark). Next, respiratory failure was induced by repeated whole-lung lavage as described by Lachmann *et al.* [9]. Each lavage was performed with saline (32 ml/kg) heated to 37 °C. Just before the first lavage, PIP and PEEP were elevated to 26 and 6 cm H_2O, respectively. Lung lavage was repeated 5-8 times with 5 min intervals to achieve a $PaO_2 < 85$ mmHg (11.3 kPa). Five minutes after the last lavage, blood gases were measured and within 10 min each animal received 1.2 ml of a surfactant suspension (25 mg/ml) at a dose of 100 mg/kg. The surfactant used in this experiment is a natural surfactant isolated from pig lungs as previously described [10].

Five minutes after surfactant replacement the animals were randomized in groups of six to be pressure-constant time-cycled ventilated with an FiO_2 of 1.0 at different PIP, PEEP, I/E ratio and frequency settings. One group (n=6) was ventilated with a peak pressure of 26 cm H_2O, a PEEP of 2 cm H_2O, an I/E ratio of 1:2 and a frequency of 30 breaths/min (group 26/2); a second group (n=6) was ventilated with a PIP of 26 cm H_2O, a PEEP of 6 cm H_2O, an I/E ratio of 1:2 and a frequency of 30 breaths/min (group 26/6); a third group (n=6) was ventilated at a PIP of 20 cm H_2O and a PEEP of 10 cm H_2O, an I/E ratio of 1:2 and a frequency of 30 breaths/min (group 20/10-static). A fourth group (n=6) was ventilated at a PIP of 20 cm H_2O, a PEEP of 6 cm H_2O, an I/E ratio of 7:3 and a frequency of 130 breaths/minute necessary to create an

auto-PEEP of 4 cm H_2O (group 20/10-auto). The total level of PEEP was recorded with a tip catheter pressure transducer (Raychem EO 2A 121, USA) in combination with a Siemens Sirecust 1280 monitor (Siemens, Danvers, Mass., USA) from a Y-connection piece with the tip located in one lumen of the Y-connection piece proximal to the tracheal tube in each animal. It was verified that end-expiratory flow was zero in the groups ventilated with static PEEP by recording time-flow curves (Servo Screen, Siemens-Elema, Solna, Sweden).

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

After killing, the lungs were lavaged five times with saline/1.5 mM $CaCl_2$ (32 ml/kg) [11]. The percentage of lung lavage fluid recovered was calculated. The active component of surfactant in the bronchoalveolar lavage was separated from the non-active surfactant component by differential centrifugation [11] followed by subsequent phosphorus analysis [12] and the ratio of inactive to active surfactant was calculated. To get an indication of the concentration of plasma-derived inhibitory proteins in the lavage fluid, protein concentration in the supernatant of the 40,000 g centrifugation was determined [13] using a photospectrometer (Beckman DU 7400, Fullerton CA, USA) at 595 nm with bovine serum albumin (Sigma St Louis, MO, USA) as a standard.

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

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

Results

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

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

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

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

Table 1. Effect of increasing frequency (bpm) on $PaCO_2$ and PaO_2 in lung lavaged rats given exogenous surfactant (100 mg/kg) and ventilated at PIP of 20 cm H_2O, static-PEEP of 10 cm H_2O and I/E ratio of 1:2. Values are mean $\pm$ SD.

	$PaCO_2$ in mmHg (kPa)	PaO_2 in mmHg (kPa)
Before lavage	27 ± 1 (3.6 ± 0.1)	535 ± 19.3 (71.4 ± 2.6)
After lavage	49 ± 2 (6.5 ± 0.2)	55 ± 4.0 (7.3 ± 0.5)
After surfactant	27 ± 5 (3.6 ± 0.6)	528 ± 37 (70.4 ± 4.9)
Freq. of 30	64 ± 10 (8.5 ± 1.3)	546 ± 71 (72.8 ± 9.4)
Freq. of 60	82 ± 24 (10.9 ± 3.2)	580 ± 67 (77.3 ± 9.0)
Freq. of 90	77 ± 26 (10.3 ± 3.4)	560 ± 53 (74.7 ± 7.1)
Freq. of 130	80 ± 30 (10.7 ± 4.1)	570 ± 62 (76.0 ± 8.3)
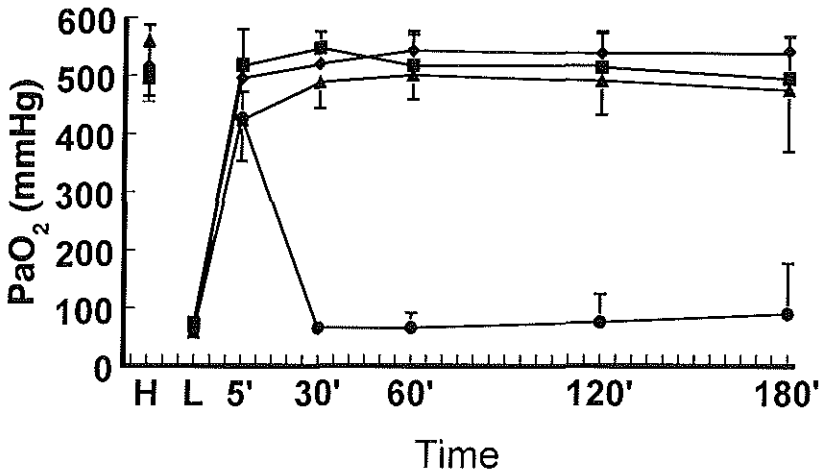
Figure 1. PaO$_2$ values in mmHg (mean ± SD) of group 26/2 (●), group 26/6 (▲), group 20/10-static (■) and group 20/10-auto (♦) over time. H = healthy, L = after lavage. Time 5, 30, 60, 120 and 180 min indicate PaO$_2$ values 5, 30, 60, 120 and 180 min after exogenous surfactant treatment. Statistical significant differences have been indicated in the text.
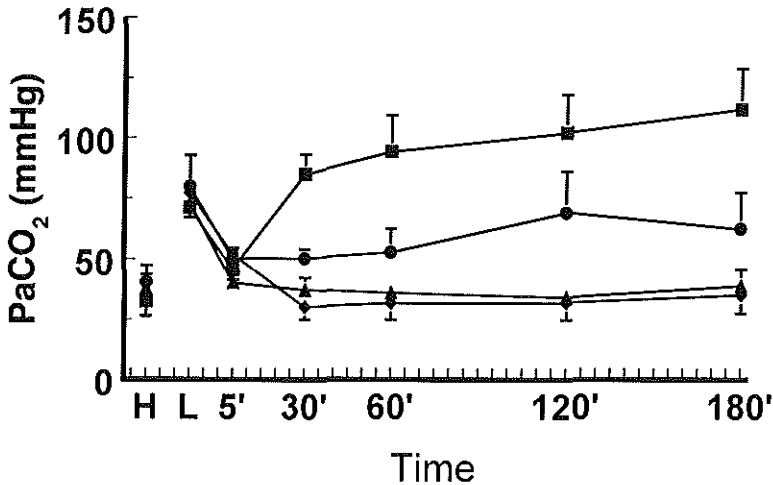
Figure 2. PaCO$_2$ values in mmHg (mean ± SD) of group 26/2 (●), group 26/6 (▲), group 20/10-static (■) and group 20/10-auto (♦) over time. H = healthy, L = after lavage. Time 5, 30, 60, 90, 120 and 180 min indicate PaCO$_2$ values 5, 30, 60, 120 and 180 min after exogenous surfactant treatment. Statistical significant differences have been indicated in the text.
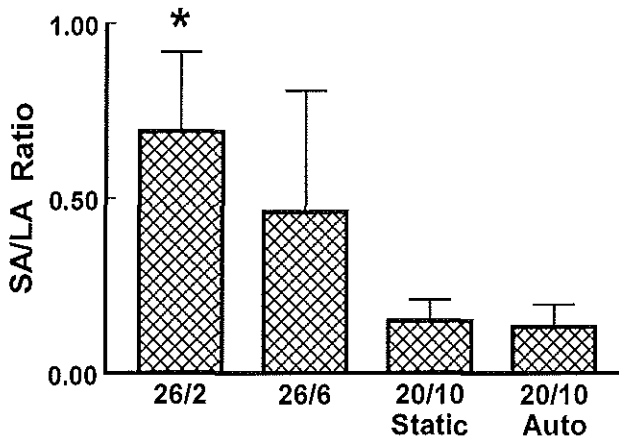
Figure 3. Surfactant small (SA) to surfactant large aggregate (LA) ratio. Group 26/2 showed a significant conversion from surface active large aggregates into non-surface active small aggregates during the ventilation period (values are mean ± SD; * $p \leq 0.05$ versus groups 20/10-static and 20/10-auto).
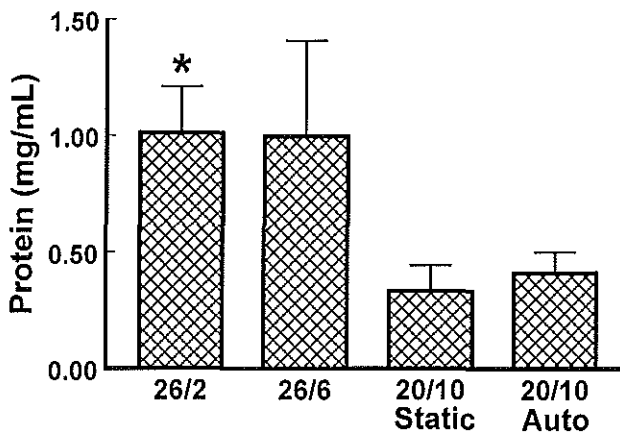
Figure 4. Protein concentration (mg/ml) of the 40,000 g supernatant fraction. Group 26/2 had a significant increase in protein concentration compared to the other three groups (values are mean ± SD; * $p \leq 0.05$ compared to groups 20/10-static and 20/10-auto).

Discussion

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

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

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

More recent studies by Froese and colleagues [5] in surfactant treated (80 mg/kg) lung-lavaged rabbits have clearly demonstrated that HFOV with small volume cycles at high rates and high end-expiratory lung volumes is most beneficial in exogenous surfactant treatment and leads to a constant improvement of PaO_2 with a low alveolar protein influx and a high amount of active surfactant at the end of a 4 hour study period. However, HFOV may not be routinely available in most neonatal intensive care units. The improvements in oxygenation after exogenous surfactant therapy with conventional volume-constant ventilation in the same study showed a decline over time; analysis of surfactant composition at the end of the study period

demonstrated a greater conversion of active into non-active surfactant with CMV than with HFO [5]. Such differences in conversion were explained by differences in volume cycles, which were ten-fold higher during CMV than during HFOV [5]. Gross *et al.* were the first to show that conversion of active into non-active surfactant subfractions is dependent on cyclic changes in surface area in vitro [17]. To maintain an adequate pool of functional surfactant subfraction in the air spaces in vivo, it is necessary to maintain a balance between secretion, uptake and clearance of the active and non-active surfactant subfractions [18]. Recent in vivo studies by Veldhuizen *et al.* in rabbits attribute the surfactant conversion to a change in alveolar surface area associated with mechanical ventilation [6, 8]. They found that changing the respiratory rate did not affect the rate of conversion but that conversion of surfactant subfractions is dependent on tidal volume and time [8].

It may be reasoned that also conventional modes of mechanical ventilation that combine small pressure amplitudes with high end-expiratory lung volumes have a beneficial effect on exogenous surfactant composition and function. The low surfactant conversion rate and the adequate oxygenation in group 20/10-static show that this is indeed the case. However, the $PaCO_2$ level indicates that carbon dioxide could not be effectively removed with these settings of the ventilator. Increasing the ventilation frequency in the static-PEEP group to the same level as in the auto-PEEP group had no influence on $PaCO_2$ (Table 1), which indicates that alveolar ventilation could not be increased by increasing ventilation frequency. The more effective carbon dioxide removal is therefore not explained by the ventilatory frequency, but rather by the differences in driving pressures, which is 14 cm H_2O (20 cm H_2O PIP - 6 cm H_2O static PEEP at the start of expiration in the auto-PEEP group, whereas it is only 10 cm H_2O in the static-PEEP group. High levels of $PaCO_2$ may lead to pathophysiological changes in the cardiovascular system and central nervous system [19]. Therefore, although CMV with small pressure amplitudes and high end-expiratory pressure levels preserves the active exogenous surfactant subfraction, the resulting high levels of $PaCO_2$ associated with such ventilator settings (at any of the set ventilatory frequencies) may not be desirable in certain categories of patients [19].

If at pressure-constant ventilation one either increases the I/E ratio at a constant frequency, or increases the frequency at a constant I/E ratio (or both) to establish an expiratory time which will be too short to allow emptying of the lung to the ambient pressure, an auto-PEEP will be created [20-24]. This mode of mechanical ventilation

can only be applied during pressure-constant time-cycled mechanical ventilation, and not during volume-constant ventilation, where there is the risk of dangerous lung overinflation. Our data show that when applying the same level of total-PEEP with such a ventilation mode (by 4 cm H_2O of auto-PEEP and 6 cm H_2O static-PEEP), effective oxygenation and carbon dioxide elimination can be achieved with the same level of preservation of the active surfactant subfraction as with static PEEP only. Our findings on carbon dioxide elimination confirm previous results with this type of mechanical ventilation in patients with ARDS [23].

It has been established that (plasma-derived) proteins inhibit surfactant dose-dependently [16]. Therefore, to establish an optimal function of exogenous surfactant, it is necessary to maintain an optimal ratio between surfactant phospholipids and such inhibitory proteins. Next to higher levels of active surfactant, CMV with small pressure amplitudes and high end-expiratory lung volumes, both with static and auto PEEP, also resulted in a lower intra-alveolar protein influx than in animals ventilated with higher pressure amplitudes and low PEEP levels; such findings will also have influenced PaO_2 levels.

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

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Chapter 9

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

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Summary

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

Design: Experimental, comparative study.

Setting: Research laboratory of a large university.

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

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

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

Conclusions: These data indicate that inhaled nitric oxide is able to improve arterial oxygenation after alveolar recruitment by means of a low dose of exogenous surfactant and not by elevation of PEEP from 6 to 10 cm H_2O, in lung-lavaged rabbits.

Introduction

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

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

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

Materials and methods

This study was approved by the local Animal Committee of the Erasmus University Rotterdam. Care and handling of the animals were in accord with the European Community guidelines (86/609/EEG). A total of 28 adult New Zealand white rabbits (IFFA-Credo, Brussels, Belgium) with a mean body weight of 2.7 ± 0.3 kg were anesthetized with intravenous pentobarbital sodium (50 mg/kg) via an auricular vein. Tracheostomy was performed, and an uncuffed endotracheal tube was introduced into the trachea. Mechanical ventilation with an FiO_2 of 1.0 was performed using a ventilator (Servo 300, Siemens-Elema, Solna, Sweden) with volume-controlled mode, tidal volume of 10 ml/kg, positive end-expiratory pressure (PEEP) of 2 cm H_2O,

frequency of 30 breaths/min, inspiration time of 25% and a pause time of 10%. An infusion of 2.5% glucose was continuously administered via the auricular vein as a maintenance fluid (5 ml/kg/h). Anesthesia was maintained with intermittent injection of pentobarbital sodium (5 mg/kg/h, i.v.); muscle paralysis was achieved with pancuronium bromide (0.1 mg/kg/h, i.m.). A femoral artery was cannulated with a polyethylene catheter for continuous blood pressure measurements and for intermittent blood sampling. Arterial samples were analysed for blood gases, hemoglobin and methemoglobin saturation using conventional methods (ABL-505 and Osm-3; Radiometer, Copenhagen, Denmark). Core temperature was measured using an esophageal thermistor (Elektrolaboratoriet, Copenhagen, Denmark) and maintained at 37 ± 0.5 °C by a heating pad.

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

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

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

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

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

Results

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

In the two groups receiving exogenous surfactant (25 mg/kg), mean PaO_2 values increased from 58 ± 11 mmHg (7.7 ± 1.5 kPa) to 283 ± 64 mmHg (37.6 ± 8.5 kPa) in 30 min (Figure 1). After additional nitric oxide inhalations, PaO_2 further increased and decreased when nitric oxide was switched off. The mean PaO_2 values of the group receiving the combination of surfactant and nitric oxide were significantly higher compared with the group that received surfactant only (Figure 1). In this last group,

PaO_2 was maximal at 30 min after surfactant instillation and decreased over time (Figure 1). $PaCO_2$ and peak airway pressure decreased directly after surfactant instillation but slowly increased during the following observation period (Table 1).

In the two groups in that PEEP was increased from 6 to 10 cm H_2O, mean PaO_2 values increased from 62 ± 15 mmHg (8.2 ± 2.0 kPa) to 239 ± 48 mmHg (31.8 ± 6.4 kPa) in 30 min (Figure 1). After inhaling nitric oxide in one of these groups, no significant difference in mean PaO_2 values was seen compared with the group that received a PEEP of 10 cm H_2O only (Figure 1). In the group receiving the combination of PEEP of 10 cm H_2O and inhaled nitric oxide, mean PaO_2 values improved over time but did not decrease when nitric oxide was switched off. Peak airway pressure and mean airway pressure were significantly higher in the two groups that received a PEEP of 10 cm H_2O as compared with the two surfactant treated groups; half of the animals in both PEEP groups developed a pneumothorax during the observation period (Table 1).
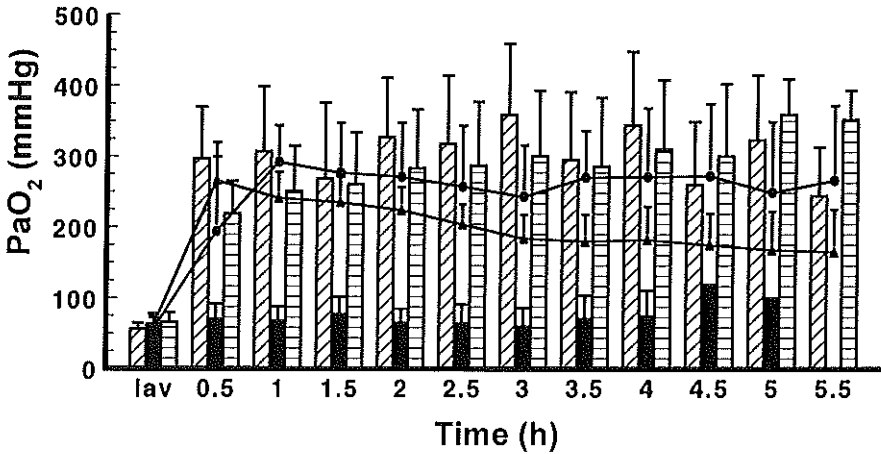
Figure 1. Mean PaO_2 values ($\pm$SD) of all 5 groups. *Diagonal-striped bar*: animals (n=6) treated with a low dose of exogenous surfactant (25 mg/kg) and inhaled nitric oxide; *horizontal-striped bar*: animals (n=6) treated with a PEEP of 10 cm H_2O and inhaled nitric oxide; *solid bar*: animals (n=6) treated with inhaled nitric oxide alone. The *solid lines* are two control groups: *triangles*, animals (n=5) treated with a low dose of exogenous surfactant (25 mg/kg) alone; *circles*, animals (n=5) treated with a PEEP of 10 cm H_2O. *Lav*, 45 min after the last lavage. To covert mmHg to kPa multiply the value by 0.1333.

There was no change in mean PaO_2 values after inhalation of nitric oxide in the group that received nitric oxide only (Figure 1). In this group, $PaCO_2$ and peak airway pressure increased over time and 4 of the 6 animals did not survive the observation period (Table 1). For the three groups that received nitric oxide, the mean changes of PaO_2 per nitric oxide concentration are given in Figure 2. The PaO_2 response was higher in the group receiving the combination of surfactant and nitric oxide for each nitric oxide concentration but there was no difference in PaO_2 response to the different nitric oxide concentrations used (Figure 2). Non-response, which was defined as an increase of PaO_2 of less than 10% above the baseline PaO_2 value, was 27% in group surfactant and nitric oxide; 93% in group PEEP and nitric oxide; and 58% in the group that received nitric oxide only.
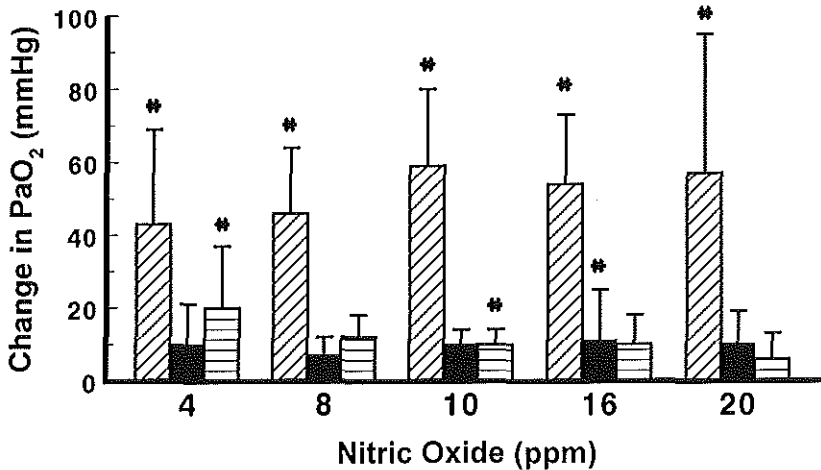
Figure 2. Mean PaO_2 values ($\pm$SD) per nitric oxide concentration for the three different groups that inhaled nitric oxide. *Diagonal-striped bar*: animals (n=6) treated with a low dose of exogenous surfactant (25 mg/kg) and inhaled nitric oxide; *horizontal-striped bar*: animals (n=6) treated with a PEEP of 10 cm H_2O and inhaled nitric oxide; *solid bar*: animals (n=6) treated with inhaled nitric oxide alone. * = significant improvement of PaO_2 due to inhaled nitric oxide (evaluated with a paired *t*-test by comparing the mean PaO_2 value when nitric oxide was switched on for 30 min with the baseline PaO_2 value). The baseline blood gas value was defined as the mean PaO_2 value of both wash out periods before and after nitric oxide was switched on. To convert mmHg to kPa, multiply the value by 0.1333.

Table 1. PaCO$_2$ (mmHg), MAP (mmHg), mean airway pressure (MAwP) and peak pressure (cm H$_2$O) in the five study groups (mean ± SD).

	Prelavag	Group	Lavage	30'	60'	90'	120'	150'	180'	210'	240'	270'	300'	330'
PaCO$_2$	36±3	SURF+NO	58±10	46±6	45±3	48±6	48±7	48±7	48±8	48±9	49±10	50±11	50±11	50±10
	37±3	PEEP+NO	57±7	54±6	57±6	58±4	60±5	58±5	57±7	55±6	55±4	55±5§§§	53±2	51±1
	34±5	NO	52±6	51±10	54±12	55±14	57±13	60±14	60±18†	55±19†	53±17†	54±2†	54±2	
	32±6	SURF	55±6	48±3	49±6	49±2	48±3	50±3	50±5	51±5	51±6	51±6	52±7	52±6
	32±4	PEEP	46±4	45±4	46±7	47±3	48±4	49±6	53±3§	53±3	55±3§	54±2	57±1§	54±4
MAP	97±9	SURF+NO	90±10	82±11	81±7	81±11	81±7	83±7	87±9	88±6	90±10	89±9	91±10	89±9
	108±11	PEEP+NO	101±14	87±9	86±12	89±13	86±14	82±12	78±10	78±11	78±9	77±8§§§	76±6	70±15
	99±8	NO	88±13	82±17	83±14	85±10	88±10	89±15	74±18	77±23†	82±9†	87±3†	85±4	
	108±7	SURF	91±9	90±14	89±13	90±14	89±12	89±14	94±13	88±13	91±17	86±15	83±25	80±19
	104±11	PEEP	94±10	89±5	91±11	89±5	85±5	91±9	90±9§	88±1	85±5§	84±5	90±4§	76±16
MAwP	5±3	SURF+NO	11±1	10±1	10±1	10±1	10±1	10±1	10±1	10±1	10±1	11±1	11±1	11±1
	6±1	PEEP+NO	12±1	17±2	17±2	17±2	17±2	17±2	17±2	17±2	17±2	17±2§§§	18±2	18±2
	5±0	NO	11±1	12±1	12±1	12±1	12±1	12±1	12±1	12±1†	12±2†	12±2†	12±2	
	5±0	SURF	11±1	10±1	11±1	11±1	11±1	11±1	11±1	11±1	11±1	11±1	11±1	11±1
	6±1	PEEP	12±2	16±1	16±1	16±1	16±1	16±1	16±1§	16±1	16±2§	16±2	15±1§	15±1
Ppeak	11±1	SURF+NO	24±2	22±2	22±2	22±3	23±3	23±3	23±3	23±3	23±2	23±2	23±2	24±2
	13±2	PEEP+NO	24±2	28±1	27±1	27±1	28±1	28±1	28±1	28±1	28±2	28±1§§§	27±1	27±1
	13±2	NO	25±1	26±2	26±2	28±2	28±1	29±2	29±2	30±1†	30±1†	30±1†	30±1	
	12±1	SURF	25±2	24±2	24±2	24±2	25±2	25±3	25±3	25±3	25±3	25±3	25±3	25±3
	12±1	PEEP	26±2	29±2	30±2	29±1	29±1	30±1	31±1§	31±1	32±1§	32±1	32±1§	31±1

SURF+NO, represents the animals treated with surfactant and nitric oxide; PEEP+NO, represents the animals treated with a PEEP of 10 cmH$_2$O and nitric oxide; NO, represents the animals treated with nitric oxide only; SURF, represents the animals treated with surfactant only; PEEP, represents the animals treated with PEEP of 10 cm H$_2$O only; MAP, mean arterial pressure; MAwP, mean airway pressure; Ppeak, peak inspiratory pressure; †, death of one animal; §, pneumothorax of one animal.

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

Table 2. Expiratory nitric oxide (NO) and nitric dioxide (NO$_2$) concentrations measured with an on-line electrochemical sensor (mean ± SD).

NO (ppm)	NO-expired (ppm)	NO$_2$-expired (ppm)
4	3.7±0.6	0.25±0.04
8	7.2±0.6	0.34±0.05
10	8.8±0.8	0.38±0.06
16	14.3±0.7	0.60±0.05
20	17.7±0.7	0.78±0.07

Discussion

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

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

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

PEEP can also improve the efficacy of inhaled nitric oxide. However, we speculate that in the present study, the airway pressures used were too high, leading to high intrathoracic pressures that made vasodilation of the pulmonary vasculature impossible due to inhaled nitric oxide. Therefore, we suggest that alveolar recruitment induced by exogenous surfactant is more beneficial than increased PEEP for improving arterial oxygenation due to inhaled nitric oxide because of the use of lower airway pressures.

The results of previous clinical observations are controversial concerning the dose-dependency of inhaled nitric oxide [5,6,19,20]. In the present study, there was no dose-dependent effect of the different used nitric oxide concentrations (4 to 20 ppm) (Figure 2). Most of the animals had a different nitric oxide concentration by which the change in arterial oxygenation was maximal. It appears that each lung has its own 'optimal' nitric oxide concentration that probably depends of the severity of the disease process (i.e., atelectasis, edema). In the present study, a low doses of nitric oxide (< 20 ppm) were used because of the demonstrated efficacy of low doses in animal experiments and patients, and to minimize toxicity [21]. In the presence of oxygen, nitric oxide is rapidly oxidized to nitrite (NO_2), or nitrates (NO_3) that can induce tissue damage [22]. Also, nitric oxide reacts with superoxide anions (O_2^-) to produce peroxynitrite ($ONOO^-$) [22]. Haddad and colleagues [23,24] demonstrated in-vitro that peroxynitrite inhibits pulmonary surfactant function by lipid peroxidation and damaging surfactant proteins. In the present study, there was no evidence that the surfactant function was more decreased after inhalation of nitric oxide when compared with the control group that only received surfactant (Figure 1). Furthermore, severe methemoglobinemia due to inhalation of nitric oxide has been reported [25]. However, in the present study, no changes in methemoglobin concentration were observed with the used nitric oxide concentrations that were used during the 30 min inhalation periods.

In the present study, we used the lung lavage model which has proved to be a consistent and convenient model of acute lung injury [11]. Repeated whole-lung lavage produces an acute quantitative surfactant deficiency. This deficiency, together with conventional mechanical ventilation, leads to severe lung injury with impaired gas exchange, decreased lung compliance and functional residual capacity, increased permeability changes of the alveolo-capillary membrane with edema, and sustained pulmonary hypertension [11,12,26]. In the present study, animals were first ventilated for 45 min after the last lavage before treatment was started to induce a more severe

lung injury. Although an untreated control group was not used in this study, we [27] have previously demonstrated with the same model that following the lavage procedure, there is no spontaneous improvement in oxygenation or lung mechanics over a 6 h period. Despite the fact that the lung injury in this study is not representative of the pathology as seen in humans with ARDS, this model is ideal for testing various therapeutic interventions that may be considered as therapy of acute lung injury [12,27-32].

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

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Summary and conclusions

Pulmonary surfactant is synthesized by pneumocytes type II cells in the lung and consists of phospholipids, neutral lipids and at least four specific surfactant proteins. Its primary function is to reduce the surface tension of the air-liquid interface, which promotes alveolar expansion and prevents end-expiratory collapse (see *Chapter 1*). Pulmonary surfactant is therefore a prerequisite for normal breathing, as demonstrated by the respiratory failure in premature infants suffering from respiratory distress syndrome (RDS) in whom surfactant production has not yet occurred. Sine 1980, exogenous surfactant therapy has been successfully used in worldwide trials in neonates with RDS, almost without any side effects, and this treatment is now routinely used in most neonatal intensive care units.

Chapter 1 describes diseases other than the neonatal RDS in which abnormalities of the pulmonary surfactant system are at least involved, such as: Acute Respiratory Distress Syndrome (ARDS), infectious lung disease, etc. Despite the diversity of the etiologies, the common pathological characteristic is increased alveolo-capillary permeability associated with damage of the epithelium. The permeability changes, together with the inflammatory response, result in the influx of proteins and inflammatory cells into the airspace with disturbance of the function of the surfactant system. As a logical consequence, the missing or inactivated surfactant should be replaced by exogenous surfactant; case-reports and first pilot studies of surfactant therapy are presented in *Chapter 1*. Although the results from these studies are not unequivocal, the best results were seen in those patients treated with high concentrations, or multiple doses, of surfactant. Besides the concentration of surfactant used, the reason for lack, or only transient, response after surfactant therapy is further attributed to the type of surfactant preparation, timing of instillation, method of administration and ventilatory support. The literature concerning these issues is briefly reviewed in last part of *Chapter 1*, and some of these aspects have been studied in this work.

Improved oxygenation, decreased ventilator requirements, and increased survival have been extensively demonstrated in worldwide clinical trials of surfactant therapy in neonates with RDS. However, little attention has been paid to the effect of exogenous surfactant on lung mechanics. *Chapter 2* presents a study that demonstrates that, in lung-lavaged rabbits, treatment with a bolus of exogenous surfactant immediately reversed hypoxemia and improved functional residual capacity (FRC) and

static compliance related to total lung volumes. However, no significant improvement in dynamic compliance (calculated from tidal volumes) was found. It was shown that this discrepancy can be attributed to the increase of FRC and the change of the shape of the pulmonary pressure-volume (P-V) curve; the increase of maximum compliance was an expression of this shift of the P-V curve. Thus, the limited ability to detect changes in pulmonary compliance during mechanical ventilation after surfactant administration is attributed to the fact that mechanical breathing operates at the 'flattened' upper portion of the P-V curves, resulting in a low value of dynamic compliance. It was concluded that the effects of surfactant therapy on lung mechanics are best characterized by changes in functional residual capacity and maximum compliance obtained from static pressure-volume curves.

Various surfactant preparations are already available on the market for the treatment of neonatal RDS but data on direct comparison of surfactants are limited. In the rat lavage model, we compared eight preparations (six natural and two synthetic) of surfactant on their capacity to improve gas exchange under standardized conditions with respect to concentration of surfactant, method of administration and ventilator conditions (see *Chapter 3*). It was shown that the effect of surfactant was, in general, dose-dependent. The naturally-derived surfactants were superior to both synthetic surfactants in improving blood gases and it is assumed that the difference lies in the presence of the surfactant proteins B and C in the natural surfactant preparations. Further, clear differences in efficacy were found between the six natural surfactants tested, especially after reduction of the PEEP level. In addition, the rat lavage model in combination with the used ventilator protocol proved to be very useful for testing the efficacy of a surfactant batch under in-vivo conditions.

In a model of acid aspiration, we have shown that respiratory failure can be prevented when exogenous surfactant was given before deterioration of lung function (i.e. within 10 min after acid aspiration), whereas after development of respiratory failure exogenous surfactant served only to prevent further deterioration of lung function but did not restore gas exchange (see *Chapter 4*). From these results it was concluded that surfactant should be given as soon as possible to prevent development of respiratory failure. In addition, in lung-lavaged rats we demonstrated that exogenous surfactant given at an early stage of lung injury resulted in a sustained improvement of lung function, whereas lung function deteriorated when a bolus of surfactant was given at a relative late time point in lung injury, probably due to an increased amount of

proteins (see *Chapter 6*). Therefore, it is expected that early treatment of patients with ARDS may require smaller amounts of exogenous surfactant and the outcome results will probably be better.

The surfactant deficiency that is likely to develop when ARDS is becoming manifest could be treated by reducing the protein concentration and/or by increasing the content of pulmonary surfactant. Plasma-derived proteins are known to be potent inhibitors of surfactant function, in a dose-dependent way. *Chapter 5* presents a study we designed to investigate the amount of exogenous surfactant needed to overcome the inhibitory components in human plasma. Lung lavaged rats received intratracheally human plasma mixed with surfactant at different concentrations. It was found that when mixed with plasma, surfactant at a concentration of 50 mg/kg showed no improvement, at 100 mg/kg there was only a transient improvement, and at 300 mg/kg there was a sustained improvement of arterial oxygenation during the 3 hour study period. From these results, it was concluded that approximately 1 mg surfactant phospholipids is required to overcome the inhibitory effect of 1 mg plasma proteins. For clinical practice this means that an excess of surfactant should be given, or repeatedly be substituted ('titrated') at low concentrations, until blood gases improve. *Chapter 6* focuses on the therapeutic role of bronchoalveolar lavage (BAL). To evaluate this, prior to surfactant therapy BAL was performed to lower the intrapulmonary protein content thereby increasing the surfactant-inhibitor ratio. In this study, the rat lavage model was modified: lung-lavaged animals were ventilated for 3 hours, before surfactant treatment, to induce a more severe lung injury. Different surfactant regimes were evaluated: bolus of surfactant with or without prior lung lavage with saline or with a diluted surfactant suspension. It was shown that lung lavage with a diluted surfactant suspension together with instillation of exogenous surfactant resulted in a sustained improvement of lung function, whereas lung function deteriorated during the subsequent 3 hours with the other regimes. In contrast to lung lavage with saline, we suggest that lavage with a diluted surfactant suspension is less harmful for the lungs, removes more of the potent surfactant inhibitors, and contributes to a more even surfactant distribution over the lungs. Bronchoscopic application of a large quantity of exogenous surfactant has been used successfully in patients with ARDS. A lung lobe can easily be lavaged with a flexible fiberoptic bronchoscope, and we therefore suggest to lavage the lungs with a diluted surfactant suspension prior to surfactant instillation in order to save exogenous surfactant in the treatment of severe ARDS.

Chapters 7 and 8 adress the effect of different ventilator pattern on surfactant function. In *Chapter 7*, the effect of high-frequency oscillatory ventilation (HFOV) was compared with conventional mechanical ventilation (CMV) on lung function and histopathology after surfactant instillation in lung-lavaged rabbits. HFOV was used in combination with the so-called 'high-lung volume strategy' in order to minimize ventilator induced lung injury. It was shown that surfactant combined with CMV resulted in the same sustained improvement of gas exchange as seen with HFOV and surfactant. Also, histopathologic examinations showed no difference in lung injury between HFOV and CMV after surfactant therapy. From these results, it was concluded that surfactant therapy with CMV is equally effective to prevent ventilator-induced lung injury as HFOV combined with surfactant, as long as alveoli are opened and kept open to avoid shear stress. In another study, we investigated the effect of different ventilator settings with CMV on exogenous surfactant therapy with respect to gas exchange, protein influx and conversion of active into non-active surfactant in lung-lavaged rats (see *Chapter 8*). It was shown that PEEP had a major impact on the effect of exogenous surfactant therapy on arterial oxygenation and settings that combine small pressure amplitudes with high levels of PEEP best preserve the large aggregate, surface-active component of exogenous surfactant and resulted in lower protein concentration of the BAL fluid. Further, it was shown that CMV with a mode of ventilation that creates auto-PEEP, compared to a mode that applies the same level of PEEP by static PEEP alone, was more effective to remove carbon dioxide.

Chapter 9 describes the use of inhaled nitric oxide in combination with exogenous surfactant. In lung-lavaged rabbits, blood gases were first increased either by instillation of a low dose of exogenous surfactant (25 mg/kg) or by increasing the PEEP from 6 to 10 cm H_2O, before inhalation of nitric oxide was started. Additional increases of oxygenation were seen after inhalation of nitric oxide but only in the animals that received a low dose of surfactant. The control group, that received inhaled nitric oxide only, showed no significant change in oxygenation. From these results, we hypothesized that progressive atelectasis decrease effective delivery of this inhalational agent to its site of action in the terminal lung units, and that surfactant increased the ventilated area in the lung by recruiting previously collapsed alveoli; inhaled nitric oxide selectively improves the perfusion of these ventilated areas, causing the additional effect of exogenous surfactant on the effect of nitric oxide inhalation on arterial oxygenation. In this study, we suggest that the alveolar recruitment induced by

exogenous surfactant is more beneficial than increased PEEP for improving arterial oxygenation due to inhaled nitric oxide because of the use of lower airway pressures.

The work presented in this thesis demonstrates that the response of exogenous surfactant can be improved by different aspects, such as; the use of a highly active natural surfactant, early administration in the disease stage, removal of inhibitors by lung lavage with a diluted surfactant suspension, mechanical ventilation with sufficient level of PEEP and small pressure amplitudes, and in combination with inhaled nitic oxide. It is expected that the improved responsiveness may require smaller amounts of exogenous surfactant and the clinical outcome results will probably be better. The prohibitive price and the non-availability of large amounts of surfactant, make surfactant therapy not yet feasible for routine application in adults; it is therfore important to explore ways of keeping the administered surfactant as small as possible in the treatment of ARDS and ARDS-like syndromes.

Samenvatting en conclusies:

Het surfactant in de longen wordt gemaakt door de type II pneumocyten en bestaat uit fosfolipiden, neutrale lipiden en uit ten minste vier specifieke surfactant eiwitten. Zijn primaire funktie is het verlagen van oppervlakte spanning op de lucht-vloeistof overgang, wat de alveolaire expansie bevordert en eind-expiratoire collaps voorkomt (zie *Hoofdstuk 1*). Longsurfactant is daarom een eerste vereiste voor een normale ademhaling zoals is aangetoond bij longfalen in te vroeg geboren kinderen, het zgn. 'Respiratory Distress Syndrome' (RDS), waar produktie van surfactant nog niet plaatsvindt. Sinds 1980 is wereldwijd met succes exogeen surfactant gebruikt in studies bij neonaten met RDS. Bijwerkingen traden hierbij nauwelijks op en de behandeling wordt nu routinematig gebruikt op de meeste neonatale intensive care's.

Hoofdstuk 1 beschrijft naast de neonatale vorm van RDS andere ziektebeelden waarin stoornissen in het pulmonair surfactant systeem een rol spelen, zoals: 'Acute Respiratory Distress Syndrome' (ARDS), infectieuse longaandoeningen, etc. Ondanks de verscheidenheid in oorzaken is de gemeenschappelijke pathologische basis een toename in de permeabiliteit van capillairen met epitheel beschadiging. Permeabiliteits-veranderingen in combinatie met een onstekingsreaktie gaan gepaard met instroom van eiwitten en onstekingscellen in de luchtwegen, waarbij het surfactant systeem verstoord raakt. Als logisch gevolg zou het verdwenen of geïnactiveerde surfactant vervangen moeten worden door exogeen surfactant; 'case reports' en de eerste oriënterende klinische studies met surfactant therapie worden gepresenteerd in *Hoofdstuk 1*. Alhoewel de resultaten van deze studies niet eenduidig zijn, werden de beste resultaten gezien in die patienten behandeld met hoge concentraties, of na herhaalde toediening van surfactant. Naast de concentratie van het surfactant, wordt de reden van geen, of alleen een tijdelijk effect na surfactant behandeling, verder toegeschreven aan het type surfactant, tijdstip van toediening, methode van toediening en methode van beademingsondersteuning. De literatuur betreffende deze onderwerpen wordt in grote lijnen besproken in het laatste deel van *Hoofdstuk 1* en een aantal van deze zaken wordt bestudeerd in deze dissertatie.

In wereldwijde klinische studies naar surfactant behandeling in neonaten met RDS zijn een verbeterde oxygenatie, verminderde beademingsbehoefte, en een toegenomen overleving duidelijk aangetoond. Weinig aandacht is er echter besteed aan de effecten van exogeen surfactant op de longmechanica. In *Hoofdstuk 2* wordt aangetoond dat in konijnen, na longlavage, behandeling met een bolus van exogeen

surfactant onmiddellijk de bestaande hypoxie opheft en de functionele residuale capaciteit (FRC) en de statische compliantie gerelateerd aan het totaal long volume verbetert. Een significante verbetering van de dynamische compliantie (berekend uit het ademvolume) werd echter niet gevonden. Aangetoond werd dat deze discrepantie kan worden toegeschreven aan de toename van de FRC en de verandering van vorm van de druk-volume curve; verschuiving van de druk-volume curve heeft een toename van de maximale compliantie tot gevolg. Dus, de beperkte mogelijkheid om veranderingen in pulmonaire compliantie te detecteren gedurende mechanische beademing na surfactant toediening wordt toegeschreven aan het feit dat mechanische ademhaling opereert op het 'vlakke' bovenste gedeelte van de druk-volume curve, resulterend in een lage waarde van de dynamische compliantie. Er wordt geconcludeerd dat de effecten van surfactantbehandeling op de longmechanica het beste beschreven kunnen worden aan de hand van veranderingen in de functionele residuale capaciteit en veranderingen in de maximale compliantie zoals verkregen uit statische druk-volume curves.

Verschillende surfactant preparaten voor de behandeling van neonataal RDS zijn op de markt, maar er zijn slechts beperkte gegevens over direkte vergelijking tussen surfactants beschikbaar. In het rat-lavage model hebben we van acht surfactant preparaten (zes natuurlijke en twee synthetische) de capaciteit om de gasuitwisseling te verbeteren vergeleken, onder gestandaardiseerde condities met betrekking tot concentratie van surfactant, methode van toediening en beademingscondities (zie *Hoofdstuk 3*). Aangetoond wordt dat het effect van surfactant in het algemeen dosisafhankelijk is. De natuurlijk verkregen surfactants waren superieur ten opzichte van beide synthetische surfactants in de verbetering van bloedgaswaarden en aangenomen werd dat de aanwezigheid van de surfactant eiwitten B en C in de natuurlijke surfactants verantwoordelijk is voor dit verschil. Verder werden duidelijke verschillen in werkzaamheid gevonden tussen de zes geteste natuurlijke surfactants, vooral na vermindering van het PEEP niveau. Daarnaast bewees het rat-lavage model in combinatie met het gebruikte beademingsprotocol zeer bruikbaar te zijn om de werkzaamheid van een surfactant batch onder in-vivo condities te testen.

In een zuur-aspiratie model werd aangetoond dat respiratoir falen kan worden voorkomen wanneer exogeen surfactant werd toegediend vóórdat verslechtering van de longfunktie optrad (dat betekent binnen 10 minuten na het aspireren van zuur). Ná de ontwikkeling van respiratoir falen diende exogeen surfactant alleen nog ter voorkoming van verdere verslechtering van de longfunktie, de gasuitwisseling herstelde niet (zie

Hoofdstuk 4). Aan de hand van deze resultaten werd geconcludeerd dat surfactant zo snel mogelijk moet worden toegediend om de ontwikkeling van respiratoir falen te voorkomen. Daarnaast hebben we in long-gelaveerde ratten aangetoond dat exogeen surfactant toegediend in een vroeg stadium van longschade resulteerde in een blijvende verbetering van de longfunktie, terwijl de longfunktie verslechterde wanneer een bolus surfactant was toegediend op een relatief laat tijdstip in het proces van longschade, waarschijnlijk als gevolg van de toegenomen hoeveelheid eiwitten in de alveolus (zie *Hoofdstuk 6*). Daarom is het te verwachten dat er bij een vroege behandeling van patiënten met ARDS kleinere hoeveelheden van exogeen surfactant nodig zullen zijn met waarschijnlijk een beter resultaat.

Het surfactant tekort dat zich naar verwachtiging ontwikkelt bij een manifest ARDS beeld, kan worden behandeld door middel van vermindering van de eiwit concentratie en/of verhoging van de hoeveelheid alveolair surfactant. Eiwitten afkomstig uit plasma zijn bekende, krachtige inhibitoren van de surfactant funktie, met een dosis-afhankelijke werking. In de studie beschreven in *Hoofdstuk 5* onderzochten we hoeveel exogeen surfactant nodig is om de remming door humane plasma componenten op te heffen. Long-gelaveerde ratten kregen intratracheaal humaan plasma gemengd met surfactant in wisselende concentraties toegediend. Gevonden werd dat surfactant, wanneer vermengd met plasma, in een concentratie van 50 mg/kg geen verbetering liet zien. Bij 100 mg/kg was er slechts een voorbijgaande verbetering en bij 300 mg/kg was er een aanhoudende verbetering van de arteriële oxygenatie tijdens de studieperiode van 3 uur. Uit deze resultaten werd geconcludeerd dat ongeveer 1 mg surfactant fosfolipiden nodig is om het inhibitoire effect van 1 mg plasma eiwitten op te heffen. Voor de kliniek betekent dit dat een overmaat aan surfactant gegeven zou moeten worden of dat herhaaldelijk surfactant gesubstitueerd moet worden ('getitreerd') in lage concentraties, totdat de bloedgassen verbeteren. In *Hoofdstuk 6* wordt broncho-alveolaire lavage uitgevoerd voorafgaand aan surfactant therapie teneinde het intrapulmonaire eiwitgehalte te verlagen en daardoor de surfactant-inhibitor ratio te verhogen. Voor deze studie werd het rat-lavage model zodanig veranderd dat long-gelaveerde dieren eerst 3 uur werden beademd vóór surfactant therapie om een ernstigere longbeschadiging te induceren. Verschillende surfactant regimes werden geëvalueerd: een surfactant bolus met of zonder voorafgaande long-lavage, met fysiologische zoutoplossing of verdunde surfactant suspensie. Aangetoond werd dat long-lavage met verdunde surfactant suspensie samen met toediening van exogeen

surfactant resulteerde in een aanhoudende verbetering van de longfunktie, terwijl de longfunktie in de volgende 3 uren verslechterde bij de andere regimes. We suggereren dan ook dat, wanneer vergeleken met long-lavage met fysiologische zoutoplossing, long-lavage met verdunde surfactant suspensie minder schadelijk is voor de long, meer van de krachtige surfactant inhibitoren verwijdert en bijdraagt aan een meer gelijkmatige surfactant distributie over de longen. Bronchoscopische toediening van een grote hoeveelheid exogeen surfactant is met succes toegepast in patiënten met ARDS. Met een flexibele fiberoptische bronchoscoop kan een longkwab eenvoudig worden gespoeld en we suggereren daarom de longen te laveren met een verdunde surfactant suspensie alvorens surfactant toe te dienen, teneinde te besparen op exogeen surfactant in de behandeling van ernstige ARDS.

In de *Hoofdstukken 7 en 8* wordt het effect van verschillende beademingsvormen geëvalueerd. In *Hoofdstuk 7* worden de effecten van 'high-frequency-oscillatory-ventilation' (HFOV) op longfunktie en histopathologie vergeleken met die van conventionele mechanische beademing (CMV) na toediening van surfactant aan long-gelaveerde konijnen. Om de door de beademing geïnduceerde longschade te minimaliseren, werd HFOV gebruikt in combinatie met de zgn. 'high-lung-volume-strategy'. Aangetoond werd dat surfactant gecombineerd met CMV resulteerde in dezelfde aanhoudende verbetering van gasuitwisseling als gezien wordt bij HFOV en surfactant. Daarbij liet histopathologisch onderzoek geen verschil in longschade zien tussen HFOV en CMV na surfactant therapie. Uit deze resultaten werd de conclusie getrokken dat surfactant therapie met CMV net zo effektief is in preventie van longschade t.g.v. beademing als HFOV in combinatie met surfactant, zolang alveoli open zijn en open gehouden worden om 'shear stress' te vermijden. In een andere studie onderzochten we de invloed van verschillende ventilator instellingen bij CMV op het effect van exogene surfactant therapie betreffende gasuitwisseling, eiwit influx en omzetting van aktief in niet-aktief surfactant in long-gelaveerde ratten (zie *Hoofdstuk 8*). Getoond werd dat PEEP een belangrijke impact heeft op het effekt van exogene surfactant therapie op arteriële oxygenatie en dat instellingen waarbij kleine druk-amplitudes gecombineerd worden met een hoge PEEP het beste de grote aggregaten van exogeen surfactant (de oppervlakte-aktieve component) preserveren en resulteren in een lagere eiwit concentratie van de BAL vloeistof. Verder werd aangetoond dat CMV met een beademingsvorm waarbij auto-PEEP optreedt effektiever is in het verwijderen van kooldioxide (CO_2), vergeleken met een vorm waarbij dezelfde PEEP-waarde alleen

door statische PEEP tot stand komt.

In *Hoofdstuk 9* bestudeerden we het gebruik van stikstof monoxide (NO) in combinatie met exogeen surfactant. Bij long-gelaveerde konijnen werden de bloed-gassen eerst verhoogd, óf door toediening van een lage dosis exogeen surfactant (25 mg/kg) óf door de PEEP te verhogen van 6 naar 10 cm H_2O, voordat de inhalatie van stikstof monoxide werd gestart. Na stikstof monoxide inhalatie werd alleen in de dieren die een lage dosis surfactant ontvingen een extra stijging van de oxygenatie waargenomen. De controle groep, die alleen stikstof monoxide inhaleerde, liet geen significante verandering in oxygenatie zien. Uitgaande van deze resultaten, postuleerden we de hypothese dat voortgaande atelectase de effectieve afzet van dit geïnhaleerde agens in de alveoli vermindert en dat surfactant het geventileerde gebied in de long vergroot door heropening van eerder gecollabeerde longblaasjes; geïnhaleerd stikstof monoxide verbetert selectief de perfusie van deze geventileerde gebieden, wat tot het additionele effect van exogeen surfactant op het effekt van stikstof monoxide inhalatie leidt. In het huidige onderzoek stellen we dat heropening van alveoli die tot stand gebracht wordt door exogeen surfactant gunstiger is met betrekking tot de verbetering van de arteriële oxygenatie door geïnhaleerd stikstof monoxide dan die door PEEP, omdat er sprake is van lagere drukken in de long.

Het werk dat gepresenteerd wordt in dit proefschrift laat zien dat de respons op exogeen surfactant verbeterd kan worden door verschillende factoren, zoals het gebruik van een natuurlijk surfactant met een hoge aktiviteit, vroegtijdige toediening ervan in het ziekte proces, verwijdering van inhibitoren door longlavage met een verdunde surfactant suspensie, combinatie met mechanische beademing met een voldoende hoge PEEP en kleine drukamplitudes, en combinatie met geïnhaleerd stikstof monoxide. Het is te verwachten dat door de verbeterde respons kleinere hoeveelheden exogeen surfactant nodig zullen zijn met waarschijnlijk betere resultaten. De exorbitant hoge prijs en het niet beschikbaar zijn van grote hoeveelheden exogeen surfactant, maken dat surfactant therapie in volwassenen nog niet haalbaar is. Het is daarom belangrijk verder onderzoek te doen naar manieren om de de hoeveelheid toe te dienen surfactant zo laag mogelijk te houden voor de behandeling van ARDS en ARDS-achtige aandoeningen.

Abbreviations

ANOVA	analysis of variances
ARDS	acute respiratory distress syndrome
BAL	broncho-alveolar lavage
BE	base excess
bpm	breathS per minute
BW	body weight
°C	degree Celsius
C_{dyn}	dynamic lung compliance
cm	centimeter
CMV	conventional mechanical ventilation
CPBP	cardiopulmonary by-pass
C_{st}	static lung compliance
DPPC	dipalmitoylphosphatidylcholine or lecithin
DTPA	diethylene triamine pentaacetic acid
FEV_1	forced expiratory volume in one second
FiO_2	fraction oxygen in inspired gas
FRC	functional residual capacity
FVC	forced vital capacity
g	gram
h	hour
HCl	hydrochloric acid
HFOV	high-frequency oscillatory ventilation
ICU	intensive care unit
I/E ratio	inspiratory/expiratory ratio
im	intramuscular
ip	intraperitoneal
iv	intravenous
l	liter
kPa	kilopascal (pressure)
MAP	mean arterial pressure
MawP	mean airway pressure
mg	milligram
min	minute

ml	milliliter
mmHg	millimeters of mercury (pressure)
NaCl	sodium chloride
NO	nitric oxide
p	probability
PaO$_2$	arterial oxygen partial pressure or arterial oxygenation
PaCO$_2$	arterial carbon dioxide partial pressure
PEEP	positive end-expiratory pressure
PG	phosphatidylglycerol
Ppeak	peak inspiratory pressure
PIP	peak inspiratory pressure
P/L ratio	protein/lipid ratio
ppm	parts per million
PV curve	pressure volume curve
RDS	respiratory distress syndrome
SA/LA ratio	small (non-active) to large (active) surfactant aggregate ratio
SD	standard deviation
SF$_6$	sulfur hexafluoride
S/I ratio	surfactant/inhibitor ratio
SP	surfactant protein (A, B, C, or D)
TLC	total lung capacity
T$_V$	tidal volume

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Curriculum vitae

De schrijver van dit proefschrift werd geboren op 23 mei 1964 te Gorinchem. In 1982 behaalde hij het VWO-diploma aan het Maurik College te Vught. In datzelfde jaar begon hij de studie Geneeskunde aan de Rijksuniversiteit te Gent in België en behaalde in 1985 het kandidaats examen. Aansluitend werd de studie Geneeskunde voortgezet aan de Erasmus Universiteit te Rotterdam, alwaar hij in 1989 het doctoraal examen behaalde. Gedurende zijn studie vervulde hij student-assistentschappen bij Prof. dr B. Lachmann op de afdeling anesthesiologie, bij dr W. Trouerbach op de afdeling experimentele radiologie en bij dr J. Grashuis op de afdeling medische informatica.

Vanaf 1990 is hij coördinator van een project rond dr toepassing van long surfactant in volwassen patienten met akuut longfalen. Naast deze werkzaamheden werd wetenschappelijk onderzoek verricht naar het gebruik van long surfactant onder leiding van Prof. dr B. Lachmann. Gedurende dit onderzoek werd samengewerkt met verschillende vakgroepen: biochemie van de 'Friedrich-Alexander-Universität' te Erlangen in Duitsland, anesthesiologie van de 'Lunds Universitet' te Lund in Zweden, pathologie van de 'Ruhr-Universität' te Bochum in Duitsland en neonatologie van het Sophia Kinderziekenhuis te Rotterdam.

In 1994 begon hij zijn co-assistentschappen, welke in maart 1996 werden afgesloten met het artsexamen. Daarna werkte hij aan de afronding van dit proefschrift. Vanaf 1 oktober 1998 is hij arts-assistent op de afdeling anesthesiologie van het Dijkzigt Ziekenhuis te Rotterdam onder leiding van Prof. dr W. Erdmann.