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

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The use of a ventilation strategy with high positive end-expiratory pressure (PEEP) that is intended to recruit collapsed alveoli and to prevent recurrent collapse can reduce alveolar protein influx in experimental acute lung injury (ALI). This could affect the pulmonary response to treatment with surfactant, since plasma proteins inhibit surfactant function. We studied the effect of exogenous surfactant on lung mechanics after 4 h of mechanical ventilation with high or low PEEP. Twenty-two adult male Sprague–Dawley rats were anaesthetized, tracheotomized and submitted to pressure-controlled mechanical ventilation with 100% oxygen. One group served as healthy controls (n=6). In the remaining animals acute lung injury was induced by repeated lung lavages to obtain a Pa_{O_2}<13 kPa during ventilation with a peak inspiratory pressure (PIP) of 26 cm H_2O and a PEEP of 6 cm H_2O. These animals were allocated randomly to ventilation with high PEEP (n=8; 100 breaths min^{-1}, I:E=1:1, PIP 35 cm H_2O, PEEP 18 cm H_2O) or to conventional mechanical ventilation (PIP 28 cm H_2O, PEEP 8 cm H_2O; n=8; ventilated control group). After 4 h of ventilation, all animals were given surfactant (120 mg kg^{-1}) via the trachea and ventilation was continued for 15 min. At the end of the study, pressure–volume curves were constructed to measure total lung capacity at 35 cm H_2O (TLC_{35}) and maximal compliance (C_{max}), and bronchoalveolar lavage was then used to measure alveolar protein influx. After lavage, Pa_{O_2} remained around 13 kPa in the ventilated control group and was >66 kPa in the high-PEEP group. After surfactant treatment, Pa_{O_2} increased to >53 kPa in both groups. In the ventilated control group alveolar protein influx was greater and TLC_{35} and C_{max} were lower than in the high-PEEP group. We conclude that the pulmonary response to exogenous surfactant after mechanical ventilation in experimental ALI is improved when a ventilation strategy with high PEEP is used.

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Methods of mechanical ventilation that prevent repeated alveolar collapse are thought to prevent worsening of lung injury during ventilation of surfactant-deficient lungs.[1 2] High positive end-expiratory pressure (PEEP) decreases the accumulation of lung water and protein leakage and prevents intra-alveolar oedema in experimental acute lung injury (ALI).[3–5] A decrease in protein transfer into the alveoli has important consequences for pulmonary function, since proteins inhibit surfactant function in a dose-dependent way.[6] In previous studies in an animal model of ALI, we showed that ventilation with high PEEP attenuated the decrease in lung function, and attributed this effect to a decrease in protein transfer into the alveoli.[3 7]

Exogenous surfactant improves lung function in the respiratory distress syndrome (RDS) of the premature newborn, giving better gas exchange, lung mechanics and outcome.[8] Since hypoxia and poor lung mechanics in ALI are also caused by a lack of active surfactant, the efficacy of surfactant replacement in ALI is being studied experimentally and clinically.[9 10 11] Surfactant therapy in adults is expensive and the large amount of surfactant that is needed is difficult to obtain. If protein influx could be reduced in ALI patients, the surfactant dose could be reduced so that surfactant replacement would be practicable.

We considered the hypothesis that the improvement in lung function after surfactant replacement would be better if

ventilation with a high PEEP were used to decrease alveolar protein influx. We therefore ventilated surfactant-depleted rats with a high or a low PEEP, and then assessed gas exchange and lung mechanics in response to surfactant treatment.

Methods

The study was approved by the University's animal experiments committee, and the care and handling of the animals conformed with European Community guidelines (86/609/EC). We studied 22 adult male Sprague–Dawley rats (body weight 270–330 g). After induction of anaesthesia with 2% enflurane and 65% nitrous oxide in oxygen, a polyethylene catheter was inserted into a carotid artery to draw arterial blood samples. Before tracheostomy, the animals received 60 mg kg^{-1} pentobarbital sodium i.p. (Nembutal®; Algin, Maassluis, The Netherlands). After tracheostomy, muscle relaxation was induced by pancuronium bromide 1 mg kg^{-1} i.m. (Pavulon®; Organon Teknika, Boxtel, The Netherlands), and the rats were then immediately connected to a ventilator. The animals were mechanically ventilated with a Servo Ventilator 300 (Siemens-Elema, Solna, Sweden) in constant-pressure, time-cycled mode at an inspired oxygen concentration ($F_{I_{O_2}}$) of 1.0, a frequency of 30 breaths min^{-1} (b.p.m.), a peak inspiratory pressure (PIP) of 12 cm H_2O, a PEEP of 2 cm H_2O, and an inspiratory/expiratory (I:E) ratio of 1:2. Anaesthesia was maintained with pentobarbital sodium 40 mg kg^{-1} h^{-1} i.p.; muscle relaxation was maintained with pancuronium bromide 1 mg kg^{-1} h^{-1} i.m. Body temperature was kept within the normal range with a heating pad. Six animals were killed immediately after induction of anaesthesia and served as healthy controls.

In the remaining animals, acute lung injury was induced by repeated bronchoalveolar lavage (BAL) (32 ml kg^{-1}) with warm saline (37°C).[12] Lavage was repeated as often as necessary to produce a Pa_{O_2} <13 kPa at a PIP of 26 cm H_2O and a PEEP of 6 cm H_2O. Within 10 min after the last lavage, the animals were randomized to two study groups (eight animals in each group). In the first group, mechanical ventilation according to the open lung concept was applied. In this strategy, collapsed alveoli are recruited by applying a high PIP and kept open with a high PEEP, and Pa_{O_2} can be kept >65 kPa.[3] In the present study, we used identical ventilator settings. First, the lungs were opened by increasing PIP to 40 cm H_2O and PEEP to 20 cm H_2O, and the I:E ratio was set at 1:1. After 2–3 min, PIP was decreased to 35 cm H_2O and PEEP to 18 cm H_2O, and arterial blood gas samples were taken. Ventilator settings remained unchanged for the remainder of the study period. In the second group, which served as ventilated controls, ventilator pressure was increased by 2 cm H_2O (PIP/PEEP 28/8 cm H_2O). These ventilator settings were based on results from a previous study using this model, which found that at these settings Pa_{O_2} remained stable at a low value of about 13 kPa for 4 h at an $F_{I_{O_2}}$ of 1.0, indicating that large parts of the lungs remain collapsed and protein transfer into the alveoli is increased.[3] In the present study, both ventilated groups were treated with surfactant at a dose of 120 mg kg^{-1} after 4 h of ventilation. The surfactant used was isolated from minced pig lungs, processed as described previously.[13] The freeze-dried material was suspended in warm saline to a concentration of 40 mg ml^{-1} and given into the trachea after the animals had been disconnected from the ventilator. The surfactant suspension was given as a bolus followed by a bolus of air (12 ml kg^{-1}), directly into the endotracheal tube via a syringe, and this was followed immediately by reconnection to the ventilator. After administration of surfactant, ventilation was continued for 15 min with the previous ventilator settings. This short interval was chosen because maximal improvement of lung function by exogenous surfactant occurs 2–5 min after administration, and because the inactivation of surfactants by proteins occurs instantaneously.

Arterial blood gas samples were taken before lavage, after lavage, hourly for 4 h after lavage, and immediately and 15 min after surfactant administration. The samples were analysed for Pa_{O_2} and Pa_{CO_2} with an electrochemical blood gas analyser (ABL 505; Radiometer, Copenhagen, Denmark).

At the end of the experiment, the animals were killed with an overdose of pentobarbital. Static pressure–volume curves were recorded using conventional techniques. Total lung capacity was defined as lung volume at inflation with a distending pressure of 35 cm H_2O (TLC_{35}). Maximal compliance (C_{max}) was defined as the steepest part of the deflation limb of the pressure–volume curve. After the pressure–volume recordings, BAL was performed five times with saline–$CaCl_2$ 1.5 mmol $litre^{-1}$. The active surfactant component in the BAL fluid was separated from the non-active surfactant component by differential centrifugation followed by subsequent phosphorus analysis, and the ratio of non-active to active components (small aggregate:large aggregate ratio=SA:LA ratio) was calculated as described by Veldhuizen and colleagues.[14] The protein concentration of the BAL fluid was determined using the Bradford method[15] (Bio-Rad, Munich, Germany).

Statistical analysis was performed with the Instat 2.0 biostatistics package (GraphPad, San Diego, CA, USA) under Windows 95. For blood gases, intergroup comparisons were analysed using the alternative (Welch) *t*-test, while intragroup comparisons were analysed using repeated measures analysis of variance (ANOVA). All other data were analysed with ANOVA. If ANOVA resulted in $P<0.05$, a Tukey–Kramer post-test was performed. All data are reported as mean (SD) and $P<0.05$ was considered statistically significant.

Results

Blood gases before and immediately after lavage were similar for the ventilated groups (Fig. 1 and Table 1). None of the animals died during the 4 h and 15 min observation periods. In the group ventilated with high PEEP, Pa_{O_2} increased to the prelavage value and remained stable during the 4 h ventilation period, but decreased to 61.8 (5) kPa after surfactant administration. In the control group, Pa_{O_2} did not improve until surfactant was administered, after which Pa_{O_2} increased to 54.0 (17) kPa.

Pa_{CO_2} data are given in Table 1. In both ventilated groups, Pa_{CO_2} increased after lavage and remained unchanged during the study period (Table 1).

Tidal volume after lavage was similar in the two ventilated groups: 10.2 (0.6) and 10.9 (0.7) ml kg^{-1} for the high-PEEP and control groups respectively.

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

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

Figure 3 shows the pressure–volume curves. On deflation to 15 cm H_2O, lung volume was less only in the ventilated control group. Below 15 cm H_2O, lung volume in both ventilated groups was less than in the healthy controls. Maximal compliance (C_{max}) was lower in the ventilated control group than in the high-PEEP group and the healthy controls (Table 2).

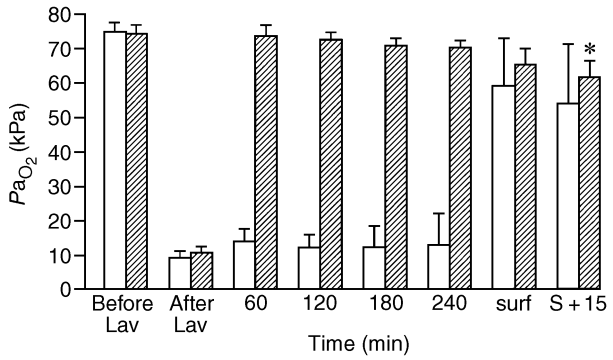

Fig 1 Pa_{O_2} values (mean (SD), kPa) of the ventilated groups. Hatched bars, high-PEEP group; open bars, ventilated control group with low PEEP; Before Lav, before lavage; After Lav, after lavage; surf, after surfactant administration; S+15, 15 min after surfactant administration. *P<0.05 versus 240 min.

Table 1 Pa_{CO_2} values (mean (SD), kPa) for the ventilated groups, before lavage (Healthy), immediately after lavage, 60, 120, 180 and 240 min after lavage, immediately after surfactant administration (Surfactant), and 15 min after surfactant administration (Surfactant+15 min). *P<0.05 versus Healthy

	High PEEP	Control
Healthy	4.9 (1.0)	5.0 (0.8)
Lavage	6.7 (0.9)*	7.2 (1.3)*
60 min	7.6 (1.8)*	6.7 (1.6)*
120 min	7.1 (1.5)*	7.0 (1.8)*
180 min	7.5 (1.6)*	7.3 (1.9)*
240 min	7.6 (2.0)*	7.6 (1.6)*
Surfactant	7.1 (2.4)*	7.7 (1.8)*
Surfactant+15 min	7.8 (2.6)*	7.8 (2.1)*

Discussion

We have shown that surfactant therapy after 4 h of mechanical ventilation can improve total lung capacity and compliance more effectively if ventilation with a high PEEP is used. Protein concentration in the BAL fluid of the high-PEEP group was lower than in the ventilated controls, in which a low PEEP was used.

In the ventilated control group, TLC_{35} and C_{max} were lower than in the healthy controls. In the high-PEEP group this difference was not found (Table 2 and Fig. 3). Surfactant has the unique ability to reduce surface tension at the air–liquid interface in the alveoli and to reduce alveolar radius, thus improving lung distensibility and alveolar stability. Plasma proteins decrease the ability of surfactant to decrease surface tension.[6] We suggest that the poor response of lung mechanics in the low-PEEP group after administration of surfactant was caused by the greater degree of protein transfer into the alveoli in this group. These results are consistent with previous results obtained in this model, in which mechanical ventilation with high PEEP decreased protein transfer into the alveoli and improved lung mechanics.[3 7]

An important factor in decreasing protein influx is probably the prevention of damage to the alveolar epithelium by reducing the shear forces from repeated alveolar collapse.[4] PEEP can also shift fluid from the alveoli to the interstitium by decreasing the pressure gradient across the alveolar–capillary membrane.[16] Furthermore, there is evidence that PEEP prevents the loss of surfactant from the

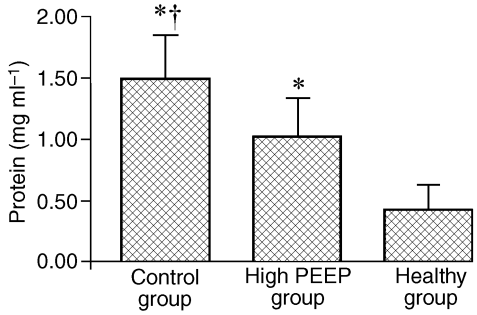

Fig 2 Protein concentration (mean (SD), ml mg^{-1}) of the BAL fluid. *P<0.05 versus healthy controls (Healthy Group); †P<0.05 versus the high-PEEP group. Control Group, ventilated controls.

Table 2 Data on maximal compliance (C_{max}, ml cm H_2O^{-1} kg^{-1}), total phosphorus (μmol ml^{-1}) recovered from the BAL fluid, and SA:LA ratio (mean (SD)). *P<0.05 versus healthy controls (Healthy), †P<0.05 versus high-PEEP group

Group	C_{max}(ml cm H_2O^{-1} kg^{-1})	Total phosphorus(μmol ml^{-1})	SA:LA ratio
Healthy	13.4 (1.1)	0.14 (0.06)	0.31 (0.12)
High PEEP	10.0 (2.6)	0.32 (0.08)*	0.36 (0.17)
Control	6.8 (1.2)*†	0.29 (0.06)*	0.47 (0.19)

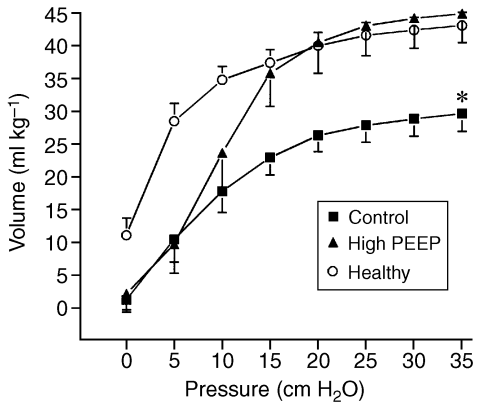

Fig 3 Deflation limbs of the pressure–volume curves (mean (SD)). Volume is lung volume above FRC. On deflation to 15 cm H_2O, lung volume was significantly decreased only in the ventilated control group (Control). Below 15 cm H_2O, lung volume in both ventilated groups was lower than in the healthy controls (Healthy). *P<0.05 versus healthy controls.

alveoli. This will prevent an increase in surface tension, which could increase protein influx from capillary to alveolus.[5]

Before administration of surfactant, Pa_{O_2} was >66 kPa in the high-PEEP group, indicating that the ventilator settings were sufficient to open up the lungs and keep them open; this confirms previous experience (Fig. 1).[3 7] The decrease in oxygenation after surfactant administration is attributable to the bolus of liquid in which the surfactant was suspended, which partially filled the alveoli, causing a decrease in diffusion.[9]

No differences were found between the ventilated groups in non-active and active surfactant aggregates (SA:LA ratio). This supports the report of Veldhuizen *et al*., who showed that the conversion of LA into SA depends on alveolar area cycling and time.[17] Alveolar cycling is determined by tidal volume, which was similar in the two ventilated groups.

An important factor in successful treatment with surfactant in RDS of the premature infant is giving the surfactant shortly after birth, to reduce the time in which proteins can transfer into the surfactant-deficient alveoli. In the adult ALI patient, where the surfactant deficiency is secondary to lung injury, protein concentration in the alveoli is increased.[18] Mixing exogenous surfactant with plasma proteins decreases the ability of surfactant to improve gas exchange, and larger amounts of surfactant are necessary to overcome the inhibitory effects.[6] This study shows that surfactant therapy can be improved by attenuating the increase in protein transfer into the alveoli. It has been suggested that a decrease in alveolar protein concentration, for example by lobe-wise lung lavage, could improve the effect of exogenous surfactant on pulmonary function.[19] Our study suggests that if the lungs are kept open with a high PEEP during the ventilation period before surfactant treatment, the efficacy of surfactant treatment can be improved. Because the inhibition of surfactant by proteins is dose-dependent, a reduction in protein influx during mechanical ventilation before surfactant administration is important, as it could reduce the surfactant dose, reduce costs and improve outcome in ALI.

In conclusion, the improvement in lung function after surfactant treatment in experimental ALI is better if a high PEEP is used to decrease alveolar protein influx. Clinical studies are necessary to assess the effects of high PEEP in ALI patients who might benefit from surfactant therapy, in increasing the efficacy of surfactant replacement and improving the outcome.

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