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EXPERIMENTAL

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Treatment of ventilation-induced lung injury with exogenous surfactant

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Abstract *Objective*: It has been demonstrated that pulmonary surfactant plays a role in the pathophysiology of ventilation-induced lung injury (VILI). Therefore, we investigated whether exogenous surfactant might restore lung function and lung mechanics in an established model of VILI.
Design: Prospective, randomized, animal study.
Setting: Experimental laboratory of a university.
Subjects: Twenty-four adult male Sprague-Dawley rats.
Interventions: First, a group of six animals were killed immediately after induction of anesthesia and used as healthy controls. Then, in 18 rats, VILI was induced by increasing peak inspiratory pressure (PIP) to 45 cmH_2O without positive end-expiratory pressure (PEEP) for 20 min. Thereafter, animals were randomly divided into three groups of six animals each: one group was killed immediately after VILI and served as VILI-control. In the other two groups, ventilator settings were changed to a PIP of 30 cmH_2O and a PEEP of 10 cmH_2O, and a respiratory rate of 40 bpm. One group received a bolus of surfactant and the other group received no treatment.
Measurements and results: Blood gas tension and arterial blood pressures were recorded every 30 min for 2 h. After the study period, a pressure-volume curve was recorded. Then, a broncho-alveolar lavage (BAL) was performed to determine protein content, minimal surface tension, and surfactant composition in the BAL fluid. Oxygenation, lung mechanics, surfactant function and composition were significantly improved in the surfactant-treated group compared to the ventilated and non-ventilated control groups.
Conclusion: We conclude that exogenous surfactant can be used to treat VILI.

Key words Ventilation-induced lung injury · Mechanical ventilation · Pulmonary surfactant · Animal · Rat

Introduction

It is known that modes of mechanical ventilation which allow alveolar end-expiratory collapse and/or end-inspiratory alveolar overstretching lead to decreases in lung compliance [1, 2, 3, 4] and gas exchange [5], and result in atelectasis, pulmonary edema, pneumonitis, and fibrosis [6, 7]. Development of intra-alveolar edema in healthy rats subjected to intermittent positive pressure ventilation at high inflation pressures, without positive end-expiratory pressure (PEEP), was first demonstrated by Webb and Tierney, and was later confirmed by Drey-

fuss and colleagues who suggested that high inspiratory lung volumes induce endothelial and epithelial overstretching leading to microvascular injury [8, 9]. However, it is increasingly realized that impairment of the surfactant system plays a key role in the mechanism of ventilation-induced lung injury (VILI) in the above-mentioned model [5, 10, 11, 12]; further, it has been shown that surfactant function is impaired by pulmonary edema constituents [13, 14, 15]. Loss of surfactant function will increase the surface tension at the air-liquid interphase of the alveolar walls [1, 2, 3], which will lead, amongst other things, to alveolar collapse and to an increased suction force on the pulmonary interstitium resulting also in alveolar edema [5, 8, 9, 10, 11, 12]. Continuous re-expansion and collapse during the ventilatory cycles causes epithelial and endothelial damage mainly due to shear forces [5, 10]. In addition, we have shown that exogenous surfactant administration preceding mechanical ventilation with high peak inspiratory lung volumes without PEEP, could partially prevent VILI which is characterized, for example, by impaired gas exchange and lung mechanics [11]. In this study, we wanted to investigate whether exogenous surfactant is able to restore gas exchange and lung mechanics in VILI.

Material and methods

Animal preparation

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

The study was performed in 24 adult male Sprague-Dawley rats (body weight 280–350 g). Anesthesia was induced with 2 % enflurane and 65 % nitrous oxide in oxygen, and a polyethylene catheter was inserted into a carotid artery for drawing arterial blood samples and continuous monitoring of arterial blood pressure. Immediately after induction of anesthesia, six animals were killed, the thorax was opened, and static pressure-volume curves (P-V curves) were recorded and a bronchoalveolar lavage (BAL) was performed. These animals served as a healthy non-ventilated control (healthy). In the remaining animals, before tracheostomy, the animals received 30 mg/kg pentobarbital sodium, i.p. (Nembutal, Algin, Maassluis, The Netherlands). After tracheostomy, muscle relaxation was induced by pancuronium bromide 0.6 mg/kg, i.m. (Pavulon, Organon Teknika, Boxtel, The Netherlands) immediately followed by connection to a ventilator and a pressure transducer for continuous monitoring of arterial blood pressure. The animals were mechanically ventilated with a Servo Ventilator 300 (Siemens-Elema, Solna, Sweden) in a pressure constant time-cycled mode, at an inspired oxygen concentration (FiO_2) of 1.0, frequency of 30 breaths per minute (bpm), peak inspiratory pressure (PIP) of 12 cmH_2O, positive end-expiratory pressure (PEEP) of 2 cmH_2O, and inspiratory/expiratory (I/E) ratio of 1:2. Anesthesia was maintained with pentobarbital sodium 30 mg/kg per hour, i.p.; muscle relaxation was maintained with pancuronium bromide 0.6 mg/kg per hour, i.m. Body temperature was kept within normal range by means of a heating pad.

Experimental design

In order to produce VILI, PIP was increased to 45 cmH_2O and PEEP was decreased to zero for 20 min; other settings were not changed. Thereafter, PIP was decreased to 26 cmH_2O and PEEP was increased to 6 cmH_2O for 5 min, in order to increase arterial CO_2 tension. These ventilator settings were chosen based on a pilot study (unpublished data) in which we observed that when animals were ventilated at 45/0 cmH_2O (PIP/PEEP, respectively) for 20 min and then ventilated at 30/10 cmH_2O, the animals died from severe hypocapnia. Then, the animals were disconnected from the ventilator to allow some edema fluid (1 ± 0.5 ml) to flow from the lungs; after this procedure the animals were randomized.

Experimental groups

The animals were randomized to one of three groups (n = 6). The first group (surfactant), received a bolus of exogenous surfactant (100 mg/kg) intratracheally. The surfactant used was isolated from minced pig lungs, that were processed as previously described [16]. The surfactant suspension, at a concentration of 40 mg/ml, was administered as a bolus followed by a bolus of air 28 ml/kg, directly into the endotracheal tube via a syringe, and was immediately followed by re-connection to the ventilator. Mechanical ventilation was continued at a PIP of 30 cmH_2O, PEEP of 10 cmH_2O, I/E ratio of 1:2, FiO_2 1.0, and respiratory rate of 40 bpm for 2 h. These ventilator settings, were chosen based on results of a preliminary study which showed that applied ventilation pressures of 26/6 cmH_2O (PIP, PEEP, respectively) and 28/8 cmH_2O were too low to keep animals alive for an observation period of 2 h. The second group (ventilated) did not receive exogenous surfactant, but received a sham bolus of air 28 ml/kg intratracheally and was mechanically ventilated at the same settings as the surfactant group. The third group of animals (VILI-control) was killed after the 5-min ventilation period of 26/6 with an overdose of pentobarbital and was used as a non-treated, non-ventilated control group.

Gas exchange and hemodynamics

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

Pressure-volume curves

At 120 min after exogenous surfactant therapy all animals were killed with an overdose of pentobarbital sodium injected through the penile vein. Then static P-V curves were recorded. After the thorax and diaphragm were opened, the tracheostomy catheter was connected to a pressure transducer (Validyne model DP 45–32, Validyne Engineering, Northridge, Calif., USA) with a syringe attached to it, and pressures were recorded on a polygraph (Grass model 7B, Grass Instrument, Quincy, Mass., USA). Using a syringe filled with nitrogen (N_2) the lungs were first inflated (within 10 s) to an airway pressure of 35 cmH_2O, which was main-

tained for 5 s, followed by deflation to an airway pressure of 0 cmH_2O. Then the lungs were re-inflated in steps of 0.5 ml until an airway pressure of 35 cmH_2O was reached. Each inflation step took 1–2 s followed by a 5-s pause to allow pressure equilibration. After this, in the same way, the lungs were then deflated until an airway pressure of 0 cmH_2O was reached. The volume of N_2 left in the syringe was recorded. Maximal compliance (C_{max}) was calculated from the steepest part of the deflation limb [16]. Total lung capacity (TLC_{35}) was defined as lung volume at inflation with a distending pressure of 35 cmH_2O [17].

Gruenwald index

The Gruenwald index which characterizes the surfactant system in situ [18], was calculated from the P-V curve, defined as $(2V_5+V_{10})/2V_{max}$, where V_5, V_{10} and V_{max} are the lung volumes at transpulmonary pressures of 5, 10 and 35 cmH_2O from the deflation limb, respectively.

Functional residual capacity (FRC)

After P-V recordings, the lungs were removed en bloc and weighed, and lung volume at an airway pressure of 5 cmH_2O (V_5) was determined by fluid displacement. A positive pressure of 5 cmH_2O was chosen to compensate for the loss of transpulmonary pressure in the open chest [19]. The total lung volume at this distending pressure was considered close to FRC.

Bronchoalveolar lavage

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

Minimal surface tension

Minimal surface tension of the crude lavage was determined by means of a modified Wilhelmy balance (E. Biegler, 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 (1 × 1 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/m (mN/m). Surface tension characteristics of a BAL sample are measured after application on the surface of the saline-filled trough. In this study, 300 μl of BAL fluid was applied to the surface of the trough; minimal surface tension was measured after three cycles [22].

Statistical data analysis

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

Results

Figure 1 shows the PaO_2 levels during the whole study period. After the ventilator settings were set at 26/6 cmH_2O for 5 min the PaO_2 decreased below 100 torr in all animals. The surfactant group showed a significant increase in PaO_2 values to pre-VILI levels ($P < 0.001$), and were maintained during the 2-h study period. In the ventilated control group, mean PaO_2 values remained below 200 torr during the 2-h study period: the difference between the values in the ventilated group and the surfactant group was significant throughout the study ($P < 0.001$).

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

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

Figure 2 shows the deflation limbs from the P-V curves. The surfactant group had TLC_{35}, and C_{max} values comparable with the healthy group, and significantly

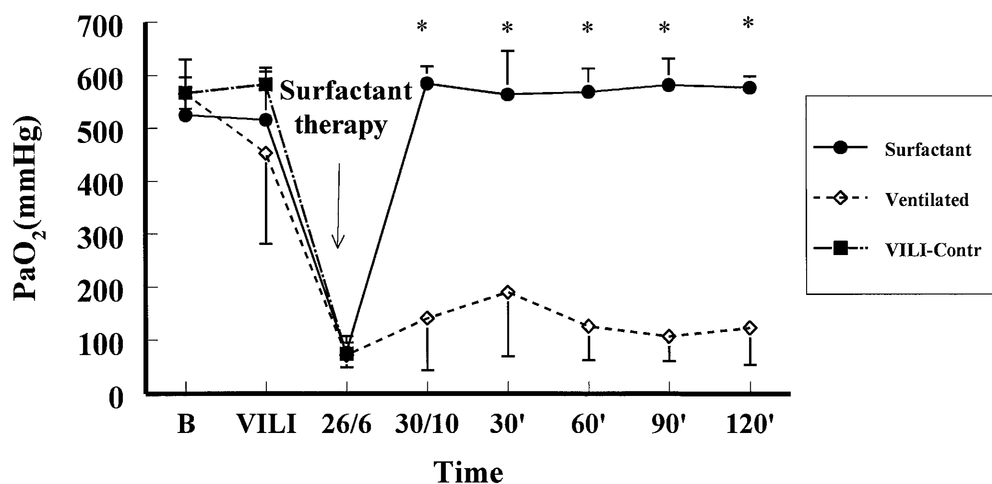

Fig. 1 Arterial oxygen tension (mean ± standard deviation) during the whole study period. *B* = basal, *VILI* = ventilation with peak inspiratory pressure (PIP) of 45 cmH_2O without PEEP after 20 min; 26/6 = after 5 min at PIP 26 cmH_2O, 6 cmH_2O PEEP; 30/10 = 5 min PIP 30 cmH_2O, 10 cmH_2O PEEP. * indicates significant difference between surfactant group and the ventilated control group

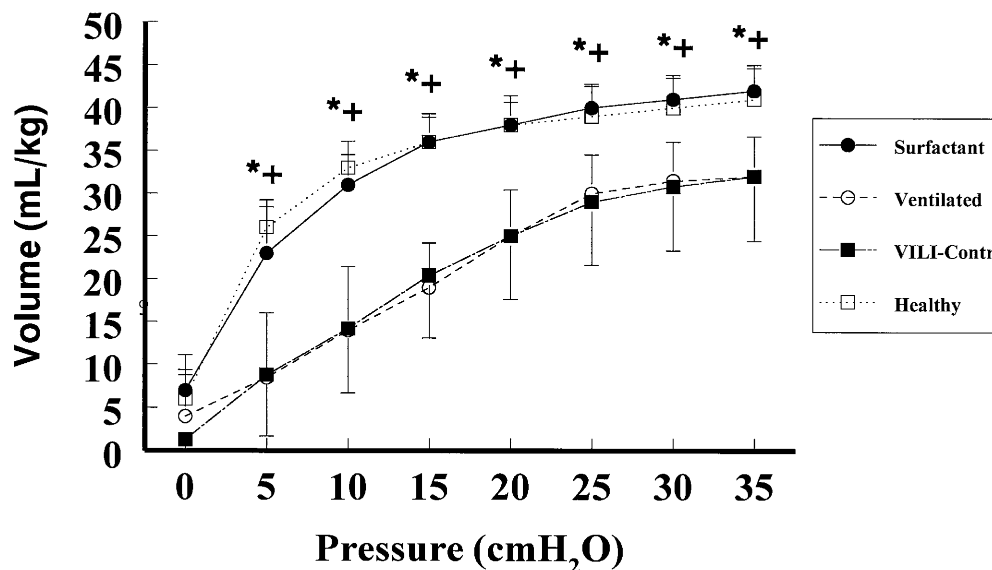

Fig. 2 Deflation limbs from the pressure-volume curves, (mean ± standard deviation). * indicates significant difference between the surfactant group and the two control groups, + indicates significant difference between the healthy group and the two control groups

higher than both the VILI-control and the ventilated control groups.

Discussion

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

In the present study, 20 min of ventilation with high peak-inspiratory pressures without PEEP resulted in pulmonary edema and hypoxemia, and in impairment of the surfactant system. The latter is characterized by a decrease in pulmonary compliance, V_5, and Gruenwald index. The exact mechanism by which the lung damage is produced by artificial ventilation is not yet entirely clear, but the role of surfactant changes is increasingly realized [1, 2, 3, 4, 5, 11, 12, 23, 24]. Two primary mechanisms of surfactant inactivation by mechanical ventilation have been described. In the first mechanism, mechanical ventilation enhances surfactant release from the pneumocytes type II into the alveolus [1, 2, 3, 4]. This material is subsequently lost into the small airways as a result of compression of the surfactant film when the surface of the alveolus becomes smaller than the surface occupied by the surfactant molecules, so that surface active material moves into the airways [1, 4, 24]. The second mechanism describing the surfactant changes associated with mechanical ventilation, is based on the observation that the alveolar surface area changes associated with mechanical ventilation result in the conversion of large surface-active surfactant aggregates into small nonsurface-active surfactant aggregates [5,

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

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

* vs baseline $P < 0.05$
** vs surfactant $P < 0.05$

Table 2 Amount of recovered broncho-alveolar lavage *(BAL)* fluid, amount of protein recovered from *BAL*, total phosphorus of small aggregates *(SA)* and total phosphorus of large aggregates *(LA)*, non-active/active total phosphorus ratio *(SA/LA ratio)*, minimal surface tension *(Min surf)* of crude *BAL* fluid, *Gruenwald Index*, total lung volume at a transpulmonary pressure of 5 cmH_2O (V_5), lung volume above FRC at pressure 35 cmH_2O (TLC_{35}) and maximum compliance (C_{max}). Values are mean ± standard deviation

	Healthy	VILI-control	Ventilated	Surfactant
Recovery BAL fluid (%)	90 ± 1	90 ± 1	90 ± 1	90 ± 1
Protein recovered from BAL (mg)	12.8 ± 4.3	38.3 ± 1.3*,**	30.1 ± 8.4**	25.2 ± 4.2**
SA (mmol)	0.53 ± 0.1*	1.2 ± 0.1*	1.2 ± 0.2*	2.6 ± 0.6
LA (mmol)	1.8 ± 0.2	1.0 ± 0.2*	1.3 ± 0.5*	11 ± 2
SA/LA ratio	0.39 ± 0.05	1.3 ± 0.22*,**	1.0 ± 0.33*,**	0.22 ± 0.08
Min surf (mN/m)	22.8 ± 2.5*	32.2 ± 2.6*,**	29.5 ± 1.1*,**	17.3 ± 2.2
Gruenwald Index	1 ± 0.01	0.20 ± 0.08*,**	0.37 ± 0.2*,**	0.96 ± 0.06
V_5 (ml/kg)	24.3 ± 5.6	3.5 ± 0.5*,**	5.5 ± 0.5*,**	13.0 ± 1.0**
TLC_{35} (ml/kg)	41 ± 3.6	32 ± 8*,**	32 ± 5*,**	42 ± 3
C_{max} (ml/kg)	4 ± 0.5	1.8 ± 0.7*,**	1.6 ± 0.3*,**	3.2 ± 0.7

* vs surfactant $P < 0.05$
** vs healthy $P < 0.05$

25, 26]. These two mechanisms will lead to alveolar collapse and protein infiltration [5, 8, 9, 10, 11, 12] in which the latter leads to further inactivation of surfactant [13, 14, 15]. These mechanisms produce self-perpetuating changes which require higher ventilator pressures which may finally be responsible for more parenchymal damage [5, 8, 9, 10, 11, 12].

In the current study, we used an exogenous surfactant to replace the surfactant lost and/or inactivated during VILI, trying to re-establish the physiological surface tension at the air-liquid interface. The exogenous surfactant used contains 1–2% of the surfactant proteins B and C which are a pre-requisite for a rapid adsorption at the air-liquid interface. In the surfactant group a significant increase in arterial oxygen tension levels, comparable with basal values, was seen within 5 min and was sustained during the 2-h study period. At the end of the study period TLC_{35}, C_{max}, and the Gruenwald index, were significantly higher in the surfactant group compared with the ventilated and VILI-control groups, and not significantly different from the healthy control group. Additionally, in the surfactant group a low minimal surface tension of the BAL fluid was observed. It is known that one of the most important functions of the pulmonary surfactant system is the mechanical stabilization of the lung alveoli during end-expiration. This is achieved by decreasing the surface tension in parallel with the decrease in alveolar radius [27]. Conversely, a high surface tension will promote alveolar collapse during deflation of the lung [1, 2, 3, 4, 8, 9, 10]. Based on our results, we assume that the alveolar surface tension was restored by exogenous surfactant in the surfactant group, providing, together with PEEP, open alveoli resulting in almost normal arterial oxygen tension. In contrast, the ventilated control group showed an impaired gas exchange and decreased alveo-

lar stability, probably caused by the demonstrated high surface tension in the BAL fluid of this group.

Another important function of pulmonary surfactant is stabilization of the fluid balance in the lung and prevention of pulmonary edema [27, 28, 29]. Therefore, loss of surfactant function will lead to alveolar edema which dilutes and inactivates the pulmonary surfactant. The protein level from the crude BAL fluid was significantly higher in all groups exposed to VILI compared with the healthy group. Therefore, the application of exogenous surfactant had no additional effect on the resolution of this edema during the 2-h study period. More studies are needed to determine if there are any changes in lung water, microvascular permeability, and histological parameters of edema when exogenous surfactant is used after VILI.

Although the model of VILI used in the present study does not directly represent the clinical situation, in some situations (e.g., when high inspiratory lung volumes are applied) some comparisons may be drawn. It is known, however, that mechanical ventilation may damage the lung in the presence or absence of pre-existing lung disease and produces a similar pattern of injury as that observed during ARDS [23]; mechanical ventilation can induce lung parenchymal damage especially in the surfactant-deficient parts of the ARDS lungs and may further induce surfactant changes in those parts of the ARDS lung which still have an adequately functioning surfactant system [12]. Therefore, we hypothesize that the clinical relevance of our study may be that exogenous surfactant can be used not only to prevent VILI, but also as a treatment after VILI, restoring the surfactant function in those alveolar units already damaged and preventing damage of the intact alveolar units.

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

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