Anesthesiology
1998; 89:467–74


# *Exogenous Surfactant Preserves Lung Function and Reduces Alveolar Evans Blue Dye Influx in a Rat Model of Ventilation-induced Lung Injury*

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

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

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

***Conclusions:*** **Exogenous surfactant preceding high peak inspiratory lung volumes prevents impairment of oxygenation, lung mechanics, and minimal surface tension of bronchoalveolar lavage fluid and reduces alveolar influx of Evans blue dye. These data indicate that surfactant has a beneficial effect on ventilation-induced lung injury. (Key words: Lung mechanics; mechanical ventilation; PEEP; pulmonary surfactants.)**

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

Peak inspiratory overstretching by overinflation of the lungs alone, however, cannot explain ventilation-induced lung injury, because 10 cm $H_2O$ at the same degree of overdistension (*i.e.*, the same peak inspiratory pressure) in this animal model has been shown to reduce permeability edema and to prevent lung parenchymal injury almost completely.[2,3] One study attributed this reduction in permeability edema by PEEP to a decrease in the pulmonary capillary hydrostatic pressure,[4] which reduces formation of edema when the pressure balance between (1) plasma colloid oncotic pressure, (2) capillary hydrostatic pressure, (3) interstitial oncotic pressure, and (4) alveolar surface tension at the alveolocapillary barrier is shifted away from the alveolar direction.[5] A recent study by our group in the same rat model, however, showed a reduction in the amount of surface tension-reducing surfactant components after

Received from the Department of Anesthesiology, Erasmus University Rotterdam, Rotterdam, The Netherlands. Submitted for publication December 29, 1997. Accepted for publication April 24, 1998. Supported by the International Foundation for Clinically Oriented Research (IFCOR).

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20 min of overinflation of the lungs without PEEP. Impairment of the surfactant system could be prevented with 10 cm $H_2O$ PEEP,[6] which prevented the conversion of surface active tubular myelin-like forms of surfactant (large aggregates) into nonactive components that represent small vesicular structures (small aggregates). Gross and Narine were the first to show that conversion of active into nonactive surfactant subfractions is dependent on cyclic changes in surface area *in vitro*.[7] Studies by Veldhuizen *et al. in vivo* have confirmed that conversion is dependent on the change in alveolar surface area associated with mechanical ventilation.[8] These studies suggest that the reduction in alveolar flooding by PEEP is partially caused by its preservation of the surfactant system, suggesting that ventilation-induced surfactant changes play a role in the development of alveolar flooding.

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

## Materials and Methods

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

### *Studies on the Effects of Exogenous Surfactant*

In the first set of experiments, 36 rats were divided randomly into six groups, anesthetized with 65% nitrous oxide/33% oxygen/2% enflurane (Ethrane®; Abbott, Amstelveen, The Netherlands), and tracheotomized. A metal cannula was inserted into the trachea. The operation area was infiltrated with 30 mg/kg lidocaine (Xylocaine®; Astra Pharmaceutica BV, Rijswijk, The Netherlands).

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

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

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

After 20 min of mechanical ventilation, all animals were killed with an overdose of pentobarbital sodium through the penile vene. A sixth group of animals was killed immediately after tracheotomy in an identical way. Static pressure–volume diagrams were then recorded using conventional techniques.[10] For these measurements, the thorax and diaphragm were opened. The animals were placed into a volume- and temperature-constant body box, and the lungs were reexpanded with pure nitrogen up to a pressure of 35 cm $H_2O$ and subsequently deflated again. This procedure was performed to reopen lung areas that became atelectatic after this surgical procedure. The lungs were then reinflated immediately, starting from a pressure of 0 cm $H_2O$ and proceeding in steps of 1 cm $H_2O$ up to an intraalveolar pressure of 35 cm $H_2O$, and subsequently deflated in steps of 1 cm $H_2O$. This was done by changing the PEEP level on the ventilator while in continuous positive airway pressure mode (Servo Ventilator 300).

Pressure changes in the body box were recorded (Validyne model DP 45-32; Validyne Engineering Co., Northridge, CA) at a sampling rate of 10 Hz using a (12-bit) analog-to-digital converter (DAS 1800; Keithley MetraByte, Taunton, MA) and stored in a computer. With the rat still in the body box, the pressure signals from the body box were calibrated for known volume changes immediately after pressure-volume recordings, by injection of known volumes of air into the body box, using a precise syringe. The maximal compliance ($C_{max}$) was defined as the steepest part of the pressure-volume deflation curve and was determined separately for each animal.[9] The Gruenwald index, defined as $(2 \cdot V_5 + V_{10})/2 \cdot V_{max}$, where $V_5$, $V_{10}$, and $V_{max}$ are the lung volumes higher than functional residual capacity at transpulmonary pressures of 5, 10, and 35 cm $H_2O$, was calculated.[11] Functional residual capacity was estimated by measuring total lung volume at a transpulmonary pressure of 5 cm $H_2O$ ($V_5$) as previously described.[12] For this measurement, the lungs and the heart were removed from the thorax. After dissection from the heart, the lungs were reexpanded with nitrogen up to a pressure of 35 cm $H_2O$ to reopen lung areas that became atelectatic during excision. The lungs were then left to deflate against a positive pressure of 5 cm $H_2O$, which was chosen to compensate for the loss of negative intrathoracic pressure. The total weight of lungs (W) was registered, and the lungs were then immersed in saline at a present depth to measure the upward force (F). According to the principle of Archimedes, this force is caused by fluid displacement equal to the volume of the lungs. $V_5$ was then calculated as $0.99 \cdot F - 0.94 \cdot W$.[12]

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

*Permeability Studies*

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

Vascular permeability was quantified by the extravasation of Evans blue dye over 19 min (Sigma, Steinheim, Germany), which correlates well with the extravasation of radiolabeled albumin at high rates of plasma leakage.[17] The dye (30 mg/ml) was filtered with a 0.22-$\mu$m Millipore filter (MILLEX-GV; Millipore Products Division, Bedford, MA) before use.[18] One minute after starting mechanical ventilation and after tracheotomy in the control group, Evans blue dye (30 mg/kg) was injected through the penile vene. Nineteen minutes after injection of Evans blue dye, the lungs were lavaged once with warm saline (30 ml/kg). The lavage was centrifuged at 400 × *g* to remove cells and cellular debris. The high amount of surfactant dissolved in the bronchoalveolar lavage (BAL) was shown to disturb photospectrometric measurements of concentration of Evans blue dye. Pilot experiments (not reported) measuring the extinction of the chloroform layer at 620 nm at various concentrations of Evans blue dye in saline after Bligh Dyer extraction demonstrated that Evans blue dye does not dissolve in chloroform but completely dis-

solves in a water-methanol phase. Therefore, 1 ml BAL was used for extraction of phospholipids according to Bligh and Dyer[14] to separate phospholipids in a chloroform layer from Evans blue dye in the water-methanol phase.

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

The absorbance of water-methanol extracts of Evans blue dye from BAL and of the formamide tissue extracts of Evans blue dye were determined against a water-methanol and formamide blank at a 620-nm wavelength and by interpolation from a standard curve of Evans blue dye in the range 0.5-10.0 $\mu$g/ml in water-methanol and formamide, respectively.[18] It was demonstrated (data not reported) that, after Bligh Dyer extraction, there are no substances in the BAL of animals with lung edema not given Evans blue dye that affect the absorbance for water-methanol at 620 nm. The total amount of Evans blue dye (in milligrams) recovered from the BAL and in the tissue was calculated.

*Statistical Analysis*

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

## Results

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

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

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

## Discussion

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

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

Important evidence regarding the role of capillary hydrostatic pressure in inducing edema in the HIPPV rat model comes from the effect of 10 cm $H_2O$ PEEP, which was shown to reduce edema infiltration.[1,3] This effect was attributed to hemodynamic alterations resulting from PEEP, which reduce filtration pressure over the alveolocapillary membrane.[4] Infusion of dopamine to correct the decrease in systemic arterial pressure that occurs with PEEP ventilation was shown by Dreyfuss

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

| Time (min) | 45/0 | Saline | S50 | S100 | S200 |
|---|---|---|---|---|---|
| $Pa_{O_2}$ | | | | | |
| 1 | 495.8 ± 28.2* | 481.9 ± 102.8*†‡ | 510.4 ± 42.0†‡ | 578.7 ± 25.4*§ | 587.5 ± 25.4§ |
| 10 | 518.2 ± 40.7* | 412.5 ± 123.4†‡ | 499.3 ± 142.3 | 566.8 ± 52.1* | 632.4 ± 39.2 |
| 20 | 307.5 ± 186.8† | 322.0 ± 150.7† | 443.1 ± 191.0 | 457.7 ± 114.8 | 608.4 ± 37.7 |
| $Pa_{CO_2}$ | | | | | |
| 1 | 22.6 ± 4.1 | 24.7 ± 3.0 | 24.4 ± 5.4 | 23.7 ± 4.0 | 23.6 ± 2.7*¶ |
| 10 | 20.6 ± 3.9 | 22.7 ± 4.0 | 21.1 ± 3.3 | 18.9 ± 2.7 | 18.5 ± 1.6 |
| 20 | 23.7 ± 4.4 | 21.8 ± 7.0 | 21.5 ± 3.9 | 19.1 ± 3.7 | 18.2 ± 2.6 |

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

* Statistical significance *versus* t = 20 min.

† Statistical significance *versus* group S200.

‡ Statistical significance *versus* group S100.

§ Statistical significance *versus* group 45/0.

¶ Statistical significance *versus* t = 10 min.

and Saumon to abolish partially the reduction in pulmonary edema induced by PEEP[4]; however, this effect was only partial, and because pulmonary artery pressure was not recorded in their study, it cannot be excluded that the transpulmonary filtration pressure after infusion of dopamine was higher than in the animals ventilated without PEEP.[4] Therefore, the possibility that other factors contribute to the development of intraalveolar edema cannot be excluded.

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

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

| | Control | 45/0 | Saline | S50 | S100 | S200 |
|---|---|---|---|---|---|---|
| Recovery BAL fluid (%) | 74.6 ± 4.7 | 76.6 ± 4.2 | 73.3 ± 2.9* | 71.6 ± 1.3* | 75.6 ± 4.7 | 81.3 ± 6.6 |
| $C_{max}$ (ml/cm$H_2O$/kg) | 3.9 ± 0.7 | 2.3 ± 0.5 | 2.5 ± 0.7† | 2.9 ± 0.6* | 3.1 ± 0.6 | 4.2 ± 0.9§ |
| Gruenwald index | 0.47 ± 0.13 | 0.25 ± 0.08*† | 0.28 ± 0.10*† | 0.23 ± 0.09*† | 0.37 ± 0.07 | 0.52 ± 0.21 |
| $V_5$ (ml) | 18.2 ± 4.1 | 6.0 ± 2.5† | 4.1 ± 2.4*†‡ | 7.4 ± 3.2 | 9.2 ± 2.9§ | 15.4 ± 3.5§ |
| Total phosphorus (SA) | 0.8 ± 0.4 | 1.3 ± 0.3 | 1.2 ± 0.2*‡ | 3.0 ± 0.9*†‡ | 4.9 ± 1.3†§ | 5.8 ± 2.7†§ |
| Total phosphorus (LA) | 3.0 ± 1.6 | 2.0 ± 0.7* | 2.6 ± 1.0* | 6.3 ± 1.6§ | 7.7 ± 2.5* | 17.0 ± 6.5†§ |
| Min surf tension crude (mN/m) | 28.5 ± 6.5 | 40.0 ± 1.5*† | 46.1 ± 0.7*†‡ | 39.5 ± 6.8*† | 37.7 ± 7.7*† | 29.7 ± 2.6 |
| Min surf tension (mN/m) | 24.8 ± 2.9 | 38.4 ± 5.9† | 45.0 ± 3.2*†‡¶ | 14.8 ± 10.5*†‡§ | 6.2 ± 7.9†§ | 1.8 ± 1.5†§ |

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

* Statistical significance *versus* group S200.

† Statistical significance *versus* group control.

‡ Statistical significance *versus* group S100.

§ Statistical significance *versus* group 45/0.

¶ Statistical significance *versus* group S50.

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

| Time (min) | Control | 45/0 | S100 | S200 | S400 |
|---|---|---|---|---|---|
| $Pa_{O_2}$ | | | | | |
| 1 | | 535.9 ± 24.3 | 514.4 ± 51.4 | 538.2 ± 47.3 | 542.9 ± 22.6 |
| 10 | | 507.2 ± 79.4 | 561.8 ± 38.3 | 560.0 ± 39.3 | 555.9 ± 36.6 |
| 20 | | 280.1 ± 114.1* | 408.4 ± 154.5 | 555.9 ± 31.1‡§ | 585.0 ± 38.1‡§ |
| $Pa_{CO_2}$ | | | | | |
| 1 | | 26.9 ± 2.7 | 24.0 ± 1.4 | 27.5 ± 4.1 | 27.0 ± 5.1 |
| 10 | | 23.4 ± 3.3* | 19.6 ± 1.6* | 21.4 ± 3.9* | 20.0 ± 1.4* |
| 20 | | 21.5 ± 3.4* | 20.0 ± 2.7* | 22.3 ± 5.1* | 19.4 ± 2.1* |
| Evans Blue tissue (mg) | 0.11 ± 0.05 | 0.64 ± 0.08† | 0.61 ± 0.25† | 0.58 ± 0.12† | 0.55 ± 0.14† |
| Evans Blue BAL (mg) | 0.06 ± 0.01 | 0.94 ± 0.36† | 0.53 ± 0.26 | 0.43 ± 0.40‡ | 0.28 ± 0.15‡ |
| Evans Blue total (mg) | 0.17 ± 0.04 | 1.58 ± 0.43† | 1.14 ± 0.44† | 1.01 ± 0.45†‡ | 0.83 ± 0.21‡ |

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

* Statistical significance *versus* t = 1 min.

† Statistical significance *versus* group control.

‡ Statistical significance *versus* group 45/0.

§ Statistical significance *versus* group S100.

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

Once protein infiltration has started, plasma-derived proteins dose-dependently inhibit surfactant,[28,29] resulting in a vicious circle of more influx of fluid and protein as a result of increased surface tension with further surfactant inactivation by plasma-derived proteins and more destabilization of the small airways. In the current study, exogenous surfactant at a dose of 200 mg/kg preceding HIPPV prevented a decrease in arterial oxygenation after 20 min of HIPPV and preserved the Gruenwald index, $C_{max}$, and $V_5$ at control values. These findings indicate that exogenous surfactant preceding HIPPV is able to preserve normal end-expiratory lung stability even after 20 min of HIPPV. This end-expiratory alveolar stabilization attributable to exogenous surfactant is likely caused by a more advantageous protein-phospholipid ratio, which is a critical factor for normal surfactant function.[29] There are two reasons for this more beneficial ratio. First, there was a higher amount of surfactant present in the BAL-accessible space, as evidenced by the higher amount of total phosphorus of surface-active large and nonsurface-active small aggregates in the animals given exogenous surfactant (table 2). Second, the reduction in surface tension over the alveolocapillary barrier toward normal levels by exogenous surfactant reduced influx of protein. The large aggregate resuspension of the group given 200 mg/kg exogenous surfactant showed more potential to reduce surface tension than in nonventilated controls (table 2). When the influence of surfac-

tant-inhibiting proteins in the BAL-accessible space was included, however, the net surface tension-reducing potential was normalized to the level of controls, as evidenced by the normalization of the minimal surface tension of the crude lavage on the pulsating bubble surfactometer in group S200 (table 2).

Such disturbance of surfactant function may be the reason for repeated collapse and reexpansion of the lung and, thus, for ventilation-induced lung parenchymal damage.[26] It may be suggested, therefore, that surfactant changes are (partially) responsible for the lung parenchymal damage previously demonstrated in this animal model.[2] Such a relationship has been shown previously by Nilsson *et al.* in prematurely delivered rabbits. It was shown that exogenous surfactant preceding mechanical ventilation with both constant tidal volumes (10 ml/kg) and constant peak inspiratory pressures increases lung-thorax compliance and reduces epithelial lesions.[30] Further studies need to be conducted to test such a hypothesis in this HIPPV-induced lung injury model.

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

Our data show that administration of exogenous surfactant preceding 20 min of overinflation of the lungs without PEEP reduces accumulation of Evans blue dye in the BAL-accessible space and preserves end-expiratory lung stability. These data indicate that exogenous surfactant changes have a beneficial effect on ventilation-induced lung injury.

The authors thank Paul Huygen and Wim Holland for assistance with body box measurements; the Department of Pediatrics, Erasmus University, Rotterdam, for assistance with phospholipid measurements; and Laraine Visser-Isles for English language editing.

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