Surfactant impairment after mechanical ventilation with large alveolar surface area changes and effects of positive end-expiratory pressure

S. J. C. VERBRUGGE, S. H. BÖHM, D. GOMMERS, L. J. I. ZIMMERMAN AND B. LACHMANN

Summary

We have assessed the effects of overinflation on surfactant function and composition in rats undergoing ventilation for 20 min with 100% oxygen at a peak inspiratory pressure of 45 cm H_2O, with or without PEEP 10 cm H_2O (groups 45/10 and 45/0, respectively). Mean tidal volumes were 48.4 (SEM 0.3) ml kg^{-1} in group 45/0 and 18.3 (0.1) ml kg^{-1} in group 45/10. Arterial oxygenation in group 45/0 was reduced after 20 min compared with group 45/10 (305 (71) *vs* 564 (10) mm Hg); maximal compliance of the P–V curve was decreased (2.09 (0.13) *vs* 4.16 (0.35) ml cm H_2O^{-1} kg^{-1}); total lung volume at a transpulmonary pressure of 5 cm H_2O was reduced (6.5 (1.0) *vs* 18.8 (1.4) ml kg^{-1}) and the Gruenwald index was less (0.22 (0.02) *vs* 0.40 (0.05)). Bronchoalveolar lavage fluid from the group of animals who underwent ventilation without PEEP had a greater protein concentration (2.18 (0.11) *vs* 0.76 (0.22) mg ml^{-1}) and a greater minimal surface tension (37.2 (6.3) *vs* 24.5 (2.8) mN m^{-1}) than in those who underwent ventilation with PEEP. Group 45/0 had an increase in non-active to active total phosphorus compared with non-ventilated controls (0.90 (0.16) *vs* 0.30 (0.07)). We conclude that ventilation in healthy rats with peak inspiratory pressures of 45 cm H_2O without PEEP for 20 min caused severe impairment of pulmonary surfactant composition and function which can be prevented by the use of PEEP 10 cm H_2O. (*Br. J. Anaesth.* 1998; **80**: 360–364)

Keywords: ventilation, mechanical; ventilation, positive end-expiratory pressure; lung, damage; lung, surfactant; rat

Pioneering work by Mead and Collier in 1959 showed that mechanical ventilation in dogs resulted in a progressive decrease in pulmonary compliance which was reversed by periodic forced inflations.[1] Greenfield, Ebert and Benson related these changes in compliance to pulmonary surfactant by demonstrating increased surface tension in lung extracts from dogs whose lungs were ventilated with peak inspiratory pressures of 28–32 cm H_2O for 1–2 h.[2]

Intra-alveolar oedema in healthy rats subjected to intermittent positive pressure ventilation (IPPV) at high inflation pressures (HIPPV), without positive end-expiratory pressure (PEEP), was first demonstrated by Webb and Tierney[3] and later confirmed by Dreyfuss and colleagues.[4] Dreyfuss and colleagues showed that damage to lung tissue resulting in oedema formation began at the capillary endothe-lium and progressed rapidly to the alveolar epithelium within 20 min.[4] Using PEEP 10 cm H_2O and the same peak inspiratory pressure, the lung was partially preserved from this high permeability oedema.[4 5] A subsequent study showed that the main determinant of lung oedema formation was end-inspiratory lung volume and attributed the effect of PEEP to a decrease in lung capillary hydrostatic pressure.[6]

We postulate a different explanation for the effect of PEEP which may prevent impairment of the pulmonary surfactant system by reducing the large changes in alveolar surface area which occur during mechanical ventilation with large tidal volumes. The balance of hydrostatic forces is altered when surfactant is impaired: pressure within the alveolar fluid lining is reduced, applying more "suction" to the interstitial space. If PEEP reduces surfactant impairment, this prevents suction-induced pulmonary oedema. To assess this hypothesis, we conducted a study to measure changes in surfactant function and composition after mechanical ventilation with high lung volumes or with PEEP when changes in lung volume were less.

Materials and methods

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

Twenty-four adult male Sprague–Dawley rats, weighing 290–350 g, were anaesthetized with 2% halothane and 65% nitrous oxide in oxygen, tracheotomized and a catheter inserted into a carotid artery. During the experiment anaesthesia was maintained with pentobarbital 60 mg kg^{-1} i.p. (Nembutal; Algin BV, Maassluis, the Netherlands); neuromuscular block was produced with pancuronium 2.0 mg kg^{-1} i.m. (Pavulon; Organon Technika, Boxtel, the Netherlands).

After neuromuscular block, the animals were connected to a ventilator (Servo Ventilator 300, Siemens-Elema, Solna, Sweden) in a pressure-controlled mode, at an ($F_{I_{O_2}}$) of 1.0, frequency 30 bpm and an I/E ratio of 1:2. In order to re-open the

SERGE J. C. VERBRUGGE, MSC, STEPHAN H. BÖHM, MD, DIEDERIK GOMMERS, MD, BURKHARD LACHMANN, MD, PHD (Department of ANAESTHESIOLOGY); LUC J. I. ZIMMERMAN, MD, PHD (Department of Paediatrics); Erasmus University Rotterdam, Postbox 1738, 3000 DR Rotterdam, The Netherlands. Accepted for publication: November 11, 1997.

Table 1 Tidal volume (V_T) (ml kg^{-1}), mean arterial pressure (MAP) (mm Hg) and blood-gas tensions (torr) in the three groups who underwent ventilation during the study (mean (SEM)). Inter- and intra-group comparisons: ANOVA with Bonferroni post-test if ANOVA $P<0.05$. Significance difference compared with: #$t=0$ min: ‡$t=1$ min; §$t=1$, 10 min; ★group 7/0; and †group 45/0

	Time (min)	Group		
		7/0	45/10	45/0
V_T	0	12.2 (1.2)	18.2 (2.2)★†	46.4 (2.7)★
	1	12.2 (1.2)	17.8 (2.1)★†	49.5 (2.7)★
	5	11.5 (1.0)	17.9 (2.7)★†	51.0 (2.1)★
	10	10.4 (1.3)#	18.5 (2.7)★†	49.6 (2.2)★
	15	10.2 (1.1)#	18.9 (2.7)★†	46.3 (2.7)★
	20	10.2 (1.2)#	18.9 (2.7)★†	44.9 (2.6)★
Pa_{O_2}	1	522 (18)	511 (18)	492 (12)
	10	497 (16)	525 (13)	524 (17)
	20	508 (24)†	564 (10)†‡	305 (71)§
Pa_{CO_2}	1	35.3 (3.3)†	31.5 (1.9)	23.4 (1.9)
	10	37.6 (3.8)†	42.0 (3.1)†	21.1 (1.8)
	20	39.6 (3.4)†	43.1 (3.8)†‡	24.8 (1.7)
MAP	0	113.1 (5.2)	97.0 (9.4)	103.5 (10.9)
	1	134.8 (5.7)	65.2 (7.9)★	77.3 (9.3)★
	10	136.5 (7.7)	85.4 (8.6)★	92.3 (5.8)★
	20	137.6 (5.8)	97.6 (15.3)	64.7 (8.1)★

Table 2 Recovery of lavage fluid and post-mortem data for maximal compliance (Cmax), Gruenwald index, V_5, total lung weight, total phosphorus, protein concentration of BAL and surface tension in all four groups after the ventilation period (mean (SEM)). Inter-group comparisons: ANOVA with Bonferroni post-test if ANOVA $P<0.05$. ★Group 45/0 significantly different compared with all other groups

	Group			
	Control	7/0	45/10	45/0
Recovery BAL fluid (%)	74.4 (1.9)	75.6 (2.2)	75.0 (3.2)	73.3 (1.2)
Cmax (ml cm H$_2$O^{-1} kg^{-1})	4.11 (0.23)	4.49 (0.48)	4.16 (0.35)	2.09 (0.13)★
Gruenwald index	0.41 (0.04)	0.46 (0.08)	0.40 (0.05)	0.22 (0.02)★
V_5 (ml kg^{-1})	17.0 (1.5)	16.0 (0.8)	18.8 (1.4)	6.5 (1.0)★
Total lung weight (g)	1.9 (0.1)	2.0 (0.1)	2.5 (0.1)	3.9 (0.1)★
Total phosphorus (µmol)	3.8 (0.9)	3.6 (0.3)	4.5 (0.2)	3.1 (0.3)
Protein concn BAL (mg ml^{-1})	0.44 (0.18)	0.30 (0.06)	0.76 (0.22)	2.18 (0.11)★
Minimal surface tension (mN m^{-1})	24.3 (1.3)	26.6 (1.8)	24.5 (2.8)	37.2 (6.3)★

atelectatic lung areas induced by the surgical procedure, mechanical ventilation with a peak airway pressure of 26 cm H$_2$O and a PEEP of 6 cm H$_2$O was applied for 30 s. The animals were allocated randomly to one of four groups ($n=6$ in each group). Animals in group 7/0 underwent ventilation with a peak pressure of 7 cm H$_2$O without PEEP; group 45/0 underwent ventilation with a peak pressure of 45 cm H$_2$O without PEEP; and group 45/10 underwent ventilation with a peak pressure of 45 cm H$_2$O and a PEEP of 10 cm H$_2$O. Ventilatory frequency was set at 60 bpm in group 7/0 and 25 bpm in group 45/10 to maintain (Pa_{CO_2}) within the normal range. Ventilatory frequency in group 45/0 was the same as in group 45/10. The control group was killed immediately after the surgical procedure without undergoing mechanical ventilation. After ventilation for 20 min, the other animals were killed by an overdose of pentobarbital via the penile vein, followed by KCl.

Blood samples obtained from the carotid artery were measured (ABL 505, Radiometer, Copenhagen, Denmark) 1, 10 and 20 min after 30 s of ventilation with pressures of 26/6. Mean arterial pressure was measured using a Statham P23XL transducer (Spectramed, Oxnard, CA, USA) and recorded (Siemens Sirecust 404–1, Danvers, MA, USA).

During mechanical ventilation, animals were placed in a volume-constant body plethysmograph to allow continuous recording of tidal volume. A pressure transducer (Validyne model DP 45–32, Validyne Engineering Co., Northridge, CA, USA) recorded pressure changes which were sampled at 10 Hz using a 12-bit analogue-to-digital converter (DAS 1800, Keithley MetraByte, Taunton, MA, USA).

After the animals were killed, a static pressure–volume plot was recorded using conventional techniques.[7] Maximal compliance (Cmax) was defined as the steepest part of the pressure–volume deflation curve,[8] and was determined separately for each animal. The Gruenwald index, defined as ($2V_5 + V_{10})/2V$max, where V_5, V_{10} and Vmax = lung volumes above functional residual capacity (FRC) at transpulmonary pressures of 5, 10 and 35 cm H$_2$O, respectively, was calculated.[9] FRC was taken to be total lung volume at a transpulmonary pressure of 5 cm H$_2$O (V_5)[10]; total lung weight was recorded. The lungs were lavaged with saline–CaCl$_2$ 1.5 mmol litre^{-1}.[11] The active component of surfactant in the bronchoalveolar lavage fluid 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. The protein concentration of BAL was determined using the Bradford method (Bio-Rad protein assay, Munich, Germany).[13] Some of the re-suspension (20 µl) of the active surfactant part was used for biophysical analysis of minimal surface tension after 50 cycles on a pulsating bubble surfactometer (PBS; Electronics Corporation, Tonowanda, NY, USA), as described by Enhorning.[14]

STATISTICAL ANALYSIS

Statistical analysis was performed using the Instat 2.0 biostatistics package (GraphPad software, San Diego, CA, USA). Intra-group comparisons were analysed using ANOVA. Inter-group comparisons were analysed with repeated measures ANOVA. If ANOVA resulted in $P<0.05$, a Bonferroni post-test was performed. All data are reported as mean (SEM).

Results

Data followed a normal distribution. Tidal volume, (Pa_{O_2}), (Pa_{CO_2}) and mean arterial pressure over time in the three groups are shown in table 1. During the study, tidal volumes differed markedly between groups (table 1). At $t=1$ min, (Pa_{O_2}) was comparable in the three study groups and remained stable in groups 7/0 and 45/10; (Pa_{O_2}) in group 45/0 decreased significantly after 10 min.

Table 2 shows recovery of BAL fluid and post-mortem data for Cmax, Gruenwald index, V_5, total lung weight, total phosphorus, protein concen-
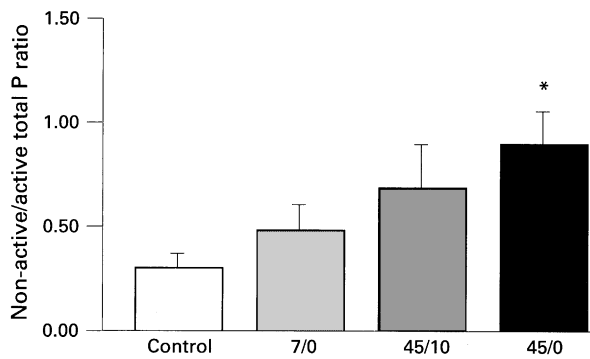
Figure 1 Ratio of non-active to active total phosphorus (P). Group 45/0 had a significant conversion of active to non-active total P during the ventilation period compared with controls. Values are mean (SEM). *P<0.05 compared with the control group.

tration of BAL and minimal surface tension for the three study groups and the control group. There was no significant difference between group 7/0 and the control group. However, there were significant differences between group 7/0 and group 45/0 for all variables except total phosphorus. Figure 1 shows that the ratio of non-active to active surfactant was greater in group 45/0 compared with controls. Group 45/10 showed no impairment of any variable compared with the other groups or with controls. In addition, all variables were significantly different between groups 45/10 and 45/0.

Discussion

In this study we used an established rat model of ventilation-induced lung injury first developed by Webb and Tierney[3] and later investigated by Dreyfuss and colleagues.[4 5] The role of changes in pulmonary surfactant, however, has never been investigated in this animal model. The study was designed to better understand the relation between changes in lung morphology and permeability, and changes in the pulmonary surfactant system. We used established techniques to characterize the pulmonary surfactant system. Significant changes in surfactant function and composition occurred after lung overinflation without PEEP for a period as short as 20 min. Surfactant composition, characterized by significant conversion of active to non-active surfactant, was changed after ventilation with HIPPV, without PEEP, compared with controls who did not undergo ventilation. Impairment of surfactant function after lung overinflation was associated with impairment of lung mechanics and an increase in minimal surface tension of lung lavage fluid extracts. Impairment of surfactant composition and function caused by lung overinflation was prevented by the use of PEEP 10 cm H_2O. These findings support the hypothesis that the beneficial effect of PEEP in this model of ventilation-induced lung injury is mediated by prevention of impairment of surfactant composition and function.

Protein concentration after mechanical ventilation with high lung volumes was increased in BAL fluid in group 45/0; PEEP 10 cm H_2O prevented protein accumulation. These data are consistent with previous studies in this model which showed less accumulation of lung water, lung protein permeability and

absence of intra-alveolar oedema with PEEP 10 cm H_2O during ventilation with high peak inspiratory lung volumes.[3 5]

The exact mechanism of ventilation-induced lung injury, and contributory factors, are still disputed. Experiments in rats with high peak inspiratory pressure ventilation of 45 cm H_2O, where peak inspiratory volume was limited by thorax restriction, have shown clearly that high peak inspiratory pressures alone do not induce lung injury.[5] However, a high peak inspiratory lung volume with peak inspiratory ovestretching alone can also not explain ventilation-induced lung injury, as the use of PEEP 10 cm H_2O at identical peak inspiratory lung volumes almost completely prevented histologically assessed lung injury.[5] Although a role for surfactant in lung injury caused by lung overinflation was suggested,[5] our data are the first to show the association of surfactant changes in lung injury with mechanical ventilation using high lung volumes.

Several mechanisms are involved in the changes in surfactant function during mechanical ventilation. First, mechanical ventilation combined with over-inflation enhances the release of surfactant from pneumocytes type II into the alveolus.[15-18] This material may be lost from the alveoli into the airways by compression of the surfactant film if the alveolar surface area becomes smaller than the surface area occupied by the surfactant molecules, so that surface-active material moves into the airways.[19 20] This mechanism of surfactant depletion after mechanical ventilation was first shown by Faridy in the isolated rat lung.[20] Mechanical ventilation increased the surface activity of lavage fluid of the pulmonary airways; this change in activity was dependent on the duration of ventilation and size of the tidal volume.[20] Studies by Wyszogrodski and colleagues have shown that PEEP prevents a decrease in lung compliance and surface activity of lung extracts, indicating prevention of loss of alveolar surfactant function during lung overinflation.[19] It was suggested that PEEP prevents alveolar collapse and thus maintains the end-expiratory volume of alveoli at a higher level, thereby preventing excessive loss of surfactant in the small airways during expiration.[21]

Second, the pulmonary surfactant system can be divided into active and non-active subfractions by differential centrifugation.[11] The active subfractions, which represent tubular, myelin-like forms of surfactant, are the metabolic precursors of the non-active components, which represent small vesicular structures.[22] Gross and Narine were the first to show that conversion of active to non-active surfactant subfractions is dependent on cyclic changes in surface area *in vitro*.[23] To maintain an adequate pool of functional surfactant subfractions in the airspaces *in vivo*, it is necessary to maintain a balance between secretion, uptake and clearance of the active and non-active surfactant subfractions.[22] Recent *in vivo* studies by Veldhuizen and colleagues in rabbits attributed surfactant conversion to a change in alveolar surface area associated with mechanical ventilation.[24 25] They found that changing ventilatory frequency[24] or the level of PEEP[25] did not affect the rate of conversion but that conversion of surfactant subfractions is dependent on tidal volume and time.[24] We found a significant conversion of active to

non-active surfactant in group 45/0 compared with non-ventilated controls, but no significant conversion in the two other groups who underwent ventilation compared with controls. We suggest that the large tidal volume in group 45/0 was able to induce significant surfactant conversion within 20 min. However, this 20-min period was too short to cause a significant difference in surfactant conversion in the groups undergoing ventilation with lower tidal volumes compared with non-ventilated controls. The exact mechanisms underlying surfactant conversion as a result of changes in surface area are unknown.

An important function of pulmonary surfactant is to aid fluid balance in the lung and prevent pulmonary oedema from increased suction forces at the alveolo-capillary barrier.[26] Loss of surfactant function with an increase in surface tension at the air–liquid interphase on the alveolar walls decreases the pressure in the alveolar fluid, altering the pressure gradient across the alveolo-capillary membrane in the alveolar direction.[26–28] *In vitro* and *in vivo* studies have shown that pulmonary oedema, and in particular plasma-derived proteins in this oedema, are capable of inactivating surfactant in a dose-dependent manner.[29–32] This further decreases the pressure in the alveolar fluid and thus causes further surfactant inactivation. When this vicious circle of surfactant inactivation has started, the resulting protein-rich pulmonary oedema accounts for much of the surfactant alterations seen in group 45/0. The importance of subtle primary changes in the pulmonary surfactant system in increasing the pressure gradient across the alveolo-capillary membrane, initiating a subsequent cascade of protein inactivation, was recently shown in a model of surfactant perturbation by dioctyl sodium sulphosuccinate, which does not cause any other damage to the alveolo-capillary barrier.[28] The study also showed that changes in surfactant make the lung vulnerable to damage by mechanical ventilation.[28]

Findings in animals with induced acute respiratory failure and in patients with acute respiratory distress syndrome (ARDS) suggest that changes in the pulmonary surfactant system play a central role in this disease process.[33] Irrespective of the cause, decreased surfactant function increases the forces acting at the air–liquid interface of the alveolus and can lead to consequences such as decreased pulmonary compliance, decreased FRC with end-expiratory alveolar collapse, right-to-left shunt and hypoxaemia with anaerobic metabolism.[33] Such changes necessitate the use of mechanical ventilation to maintain adequate oxygen delivery to the tissues. Mechanical ventilation may, however, perpetuate the alterations in the pulmonary surfactant system, as found in this study, indicating that mechanical ventilation with high peak inspiratory pressures in patients with ARDS may impair the function of those alveoli that are still intact. The data also suggest that it is important to use PEEP in these patients to preserve normal surfactant function of alveoli that are not yet affected by the disease process.

We conclude that ventilation of the lungs of healthy rats with high peak inspiratory volumes at peak inspiratory pressure of 45 cm H_2O without PEEP caused severe impairment of pulmonary surfactant composition and function. PEEP prevented this impairment of the surfactant system, probably by reducing the amount of change in alveolar surface area, which prevents: surfactant displacement from the alveolar air–liquid interface into the small airways; increased conversion of active to non-active surfactant subfractions; and increased hydrostatic forces over the alveolo-capillary barrier which could lead to a self-propagating mechanism of surfactant inactivation.

Acknowledgements

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