Effects of Surfactant on Lung Injury Induced by Hyperoxia and Mechanical Ventilation in Rabbits

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We evaluated the effects of exogenous surfactant on lung injury caused by 100% oxygen and mechanical ventilation in rabbits. Surfactant-treated rabbits (n=9) were ventilated with 100% oxygen for 36 hours and bovine surfactant was given via the trachea 12 hours after the start of mechanical ventilation. Saline-treated (n=9) rabbits were treated identically, except that they received saline without surfactant. There were no significant changes in hemodynamics, lung mechanics, or arterial oxygen tension during artificial ventilation.

Albumin concentration in the bronchoalveolar lavage fluid (BALF) of saline-treated rabbits was slightly higher than those in surfactant-treated rabbits and significantly higher than in non-treated rabbits. C3a concentration in BALF was significantly higher in saline-treated rabbits than in surfactant-treated and non-treated rabbits. In addition, the wet-to-dry lung weight ratio was significantly lower in surfactant-treated rabbits than in saline-treated rabbits (5.06 ± 0.10 vs. 5.67 ± 0.14, $P < 0.05$).

Light microscopy revealed hyaline membrane formation in saline-treated rabbits, but fewer changes were observed in surfactant-treated rabbits. Electron microscopy revealed extensive endothelial cell destruction in saline-treated rabbits, while such changes except endothelial cell swelling were not observed in surfactant-treated rabbits.

We conclude that exogenous surfactant attenuated lung injury caused by oxygen exposure and ventilation. (Key words: hyperoxia, ventilation, pulmonary surfactant, bronchoalveolar lavage fluid, endothelial ultrastructure)

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Mechanical ventilation with high concentrations of oxygen is often used to care for critically ill patients. However, it is well known that prolonged use of high concentrations of oxygen can injure the respiratory system[1–6].

Recent studies have demonstrated that administration of exogenous surfactant to animals exposed to hyperoxia mitigates the arterial hypoxemia and abnormal lung mechanics, and improves survival in spontaneously breathing rabbits under hyperoxia[7,8]. However, few data are available on surfactant prophylaxis against lung injury

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induced by hyperoxia with artificial ventilation.

In animals spontaneously breathing under hyperoxia, the earliest morphological changes in the lung are observed in the capillary endothelial cell ultrastructure[2,6,9], and are followed by an accumulation of inflammatory blood cell elements in the lung, and release of mediators[10–12].

In this study, we evaluated the effects of surfactant on the analysis of BALF as well as lung ultrastructure, in rabbits exposed to 100% oxygen during mechanical ventilation.

Materials and Methods

Twenty-five male rabbits weighing 2.0–2.4 kg were randomly divided into 3 groups as follows: 1) 9 were ventilated with inspired oxygen (O_2) for 36h and were given surfactant; 2) 9 were ventilated with 100% O_2 for 36h and were given saline instead of surfactant; and 3) 7 were not treated and were given food and water ad libitum.

The ventilated rabbits were anesthetized with 20 mg of ketamine hydrochloride. A tracheostomy was performed aseptically and a 3.5 mm uncuffed endotracheal tube was inserted and tied in place. Anesthesia and paralysis were maintained with intermittent intravenous ketamine and pancuronium bromide. The rabbits were then connected to an infant ventilator Sechrist (IV100B, Anaheim, California). The inspired O_2 concentration was set to 100% and the tidal volume was set 10 $ml \cdot kg^{-1}$. Respiratory rate was adjusted to produce an initial Pa_{CO_2} of 35–40 mmHg. Via femoral cutdown a catheter was placed in the distal aorta to monitor arterial pressure and to take samples for blood gas analysis. Central venous pressure was monitored via a catheter in an external jugular vein. The animals were placed on a heating pad under a radient heat lamp, and body temperature was kept between 36° and 38°C. Ringer's lactate solution was continuously infused at 4 $ml \cdot kg^{-1}$ per hour. Prophylactic antibiotics (10,000 $U \cdot kg^{-1}$ of penicillin G given intravenously every 12 hours; gentamicin, 8 $mg \cdot kg^{-1}$/day, given topically every 12 hours) were administered to prevent bacterial colonization and superimposed infection.

Lung mechanics were measured at 1, 12, 24 and 36 hours after the start of ventilation. Each measurement was performed after an arterial blood specimen was obtained for analysis of Pa_{O_2}, Pa_{CO_2} and pH (ABL2, Copenhagen, Denmark). Lung mechanics were measured by the passive expiratory flow-volume technique as described by LeSouef[13]. The airflow was measured with a Fleisch 00 pneumotachograph and a differential pressure transducer (model MP-45, Validyne Engineering Corp., Northridge, Calif.). Airway pressure was measured at the proximal end of the pneumotachometer with a semiconductor pressure trans ducer (model P-300 501G Copal Electronics Corp., Tokyo, Japan). Volume was determined for each breath by digital integration of airflow using a respiration monitor (Aivision, Tokyo, Japan) and a personal computer (PC 9801 VM11, NEC, Tokyo, Japan). The lungs were inflated and the airflow was interrupted at 20 cm H_2O. The occlusion was rapidly released after a plateau in airway pressure was achieved. Compliance and resistance were then analyzed by the personal computer.

Twelve hours after the start of mechanical ventilation, Surfactant-TA (Tokyo Tanabe, Tokyo, Japan) was instilled in 8 rabbits. This surfactant is an organic solvent extract of minced cow lung that had been supplemented with dipalmitoylphosphatidylcholine (DPPC), palmitic acid and tripalmitin. One hundred milligrams of the powdered surfactant was dispersed

Table 1. Blood gas analysis and lung mechanics

	1h	12h	24h	36h
Surfactant				
pH	7.39 ± 0.01	7.37 ± 0.02	7.40 ± 0.01	7.37 ± 0.02
Pa_{O_2} (mmHg)	520 ± 10	520 ± 5	496 ± 6	499 ± 15
compliance ($ml \cdot cmH_2O^{-1}$)	3.12 ± 0.17	2.84 ± 0.41	2.95 ± 0.39	3.00 ± 0.42
resistance ($cmH_2O \cdot l^{-1} \cdot sec^{-1}$)	122 ± 8	98 ± 15	124 ± 13	95 ± 13
PIP (cmH_2O)	11.6 ± 0.8	13.8 ± 0.9	12.8 ± 0.4	13.1 ± 0.4
Saline				
pH	7.39 ± 0.01	7.38 ± 0.02	7.37 ± 0.02	7.38 ± 0.01
Pa_{O_2} (mmHg)	529 ± 9	515 ± 5	496 ± 18	516 ± 20
compliance ($ml \cdot cmH_2O^{-1}$)	2.72 ± 0.24	2.69 ± 0.31	2.43 ± 0.33	2.78 ± 0.34
resistance ($cmH_2O \cdot l^{-1} \cdot sec^{-1}$)	106 ± 9	117 ± 20	109 ± 11	110 ± 18
PIP (cmH_2O)	12.8 ± 0.7	14.1 ± 0.6	14.3 ± 0.7	13.4 ± 0.7

PIP: peak inspiratory pressure
no significant difference between and within the groups

in 2.5 ml of normal saline. Surfactant-treated rabbits received 100 $mg \cdot kg^{-1}$ of Surfactant-TA in 2.5 $ml \cdot kg^{-1}$ of saline. This was instilled into the lung via a catheter. During this time, to ensure that the instillate was evenly distributed throughout the lung, the animals were rotated properly and it was instilled in 5 aliquots. The animals were manually ventilated after each instillation. This procedure was done over 15 minutes to avoid hypoxemia.

Saline-treated rabbits were treated exactly as the surfactant-treated rabbits, except that saline without surfactant was instilled into the trachea.

At the end of the experiment, the rabbits were killed by an intravenous injection of thiamylal (100 $mg \cdot kg^{-1}$). The heart and lungs were removed en bloc. Through the right mainstem bronchus 40 ml of saline with EDTA at 4°C was slowly infused and then withdrawn. This procedure was repeated 5 times. The BALF was analyzed for cell count and cell differentiation. A cytocentrifuged preparation (Cytospin 2, Shandon Southern Products Ltd., England) of the BALF was stained with Wright-Giemsa for cell differentiation. The cells in the fluid were counted by a Coulter Counter and the Burker-Turk method. The fluid was centrifuged at 250g at 4°C for 10 min to remove the cells. The cell-free supernatant was divided into tubes and stored at −70°C until assayed. The following variables were then measured.

1) Activated components of complement C3a and C5a were quantified by a radioimmunoassay (Amersham, Bucks, UK).

2) Albumin concentration was determined by nephelometry with IgG fraction coat anti-rabbit albumin (Cappel, Pennsylvania).

3) Phospholipid concentration was determined with phospholipase D and choline oxidase[14].

The left upper lobe was weighed and then dried to a constant weight at 60°C in an oven. The ratio of wet weight to dry weight was then obtained.

To prepare tissues, a catheter was inserted into the left lower lobe and fixed by instillation of 4% glutaraldehyde and 4% paraformaldehyde buffered to pH 7.4 with phosphate buffer, at 40 cm H_2O. The specimens fixed for light microscopic study were postfixed in a 10% formaldehyde so-

Table 2. Analysis of bronchoalveolar lavage fluid

	Surfactant	Saline	no treatment
n	9	9	7
total white blood cells (cells·μl^{-1})	308 ± 85*	345 ± 36†	211 ± 74
%PMN	18.7 ± 4.5*	22.2 ± 6.0†	< 1
Albumin (mg·dl^{-1})	4.62 ± 1.30	9.40 ± 3.21†	1.14 ± 0.40
phospholipid (mg·dl^{-1})	12.0 ± 2.54*,††	4.00 ± 0.9	4.14 ± 0.51
C3a (ng·dl^{-1})	38.0 ± 7.6	64.5 ± 5.0†,††	37.8 ± 8.3
C5a (ng·dl^{-1})	7.0 ± 1.0	7.3 ± 2.8	7.4 ± 1.5
Wet-to-dry weight ratio	5.06 ± 0.10	5.67 ± 0.14†,††	4.90 ± 0.17

All the values indicating concentrations are corrected for 200 ml of BALF.
*Significant difference: surfactant vs no treatment ($P < 0.05$)
†Significant difference: saline vs no treatment ($P < 0.05$)
††Significant difference: surfactant vs saine ($P < 0.05$)

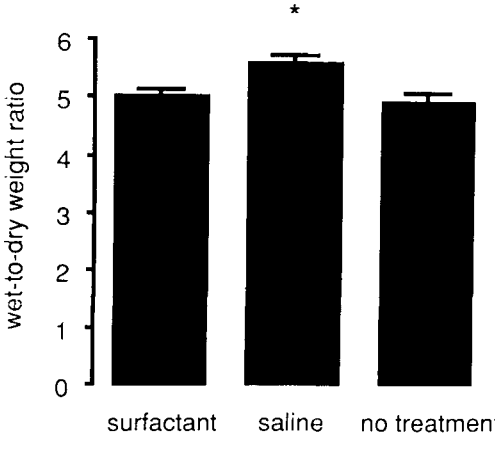

Fig. 1. Wet to dry ratio obtained from left upper lobe. Values are given mean ± SEM. $P < 0.05$.

lution, embedded in paraffin wax, and stained with hematoxylin and eosin. Forty blocks from randomly chosen specimens of surfactant-treated rabbits or saline-treated rabbits were used for electron microscopic study and they were postfixed in 1% osmium tetroxide, dehydrated in ethanol, and embedded in epon. All the specimen were blinded to the examiner.

Data are expressed as means ± SEMs. Statistical analysis was performed using analysis of variance, followed by Scheffé's test.

Results

No rabbit died as a result of hyperoxia. Table 1 shows the hemodynamics, lung mechanics and the results of arterial blood gas analysis.

None of the rabbits had significant changes in arterial oxygen tension or hemodynamics throughout the experiment. Compliance and resistance did not change significantly in either of the two ventilated groups.

There was a significant difference in the total number of BAL cells between saline-treated rabbits and non-treated rabbits (table 2). The BALF of the non-treated rabbits contained mainly macrophages. PMNs accounted for less than 1% of the white blood cells in fluid from non-treated rabbits, but they accounted for 16.0 ± 6.6% of the cells in fluid in surfactant-treated rabbits, and 21.5 ± 6.5% in fluid from saline-treated rabbits.

Biochemical data obtained from the

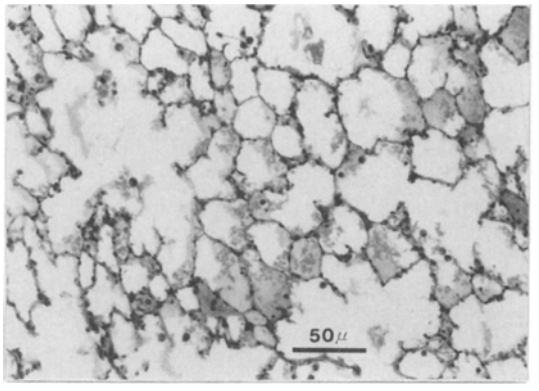

Fig. 2. Light microscopy (H&E, original magnification ×100)

Saline-treated rabbits. Hyaline membrane formation is seen at the alveolar level. Macrophages and neutrophils are seen in the alveolar space.

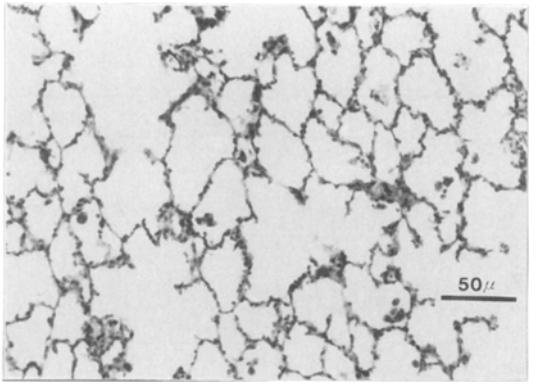

Fig. 3. Light microscopy (H&E, original magnification ×100)

Surfactant-treated rabbits. Hyaline membrane formation is not clear.

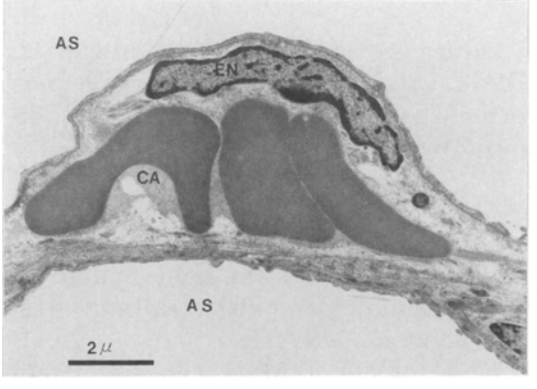

Fig. 4. Electron microscopy (original magnification ×3500) of saline-treated rabbits. Capillary endothelial cells were extensively destructed.

AS: alveolar space, EN: Endothelial cell, CA: capillary lumen

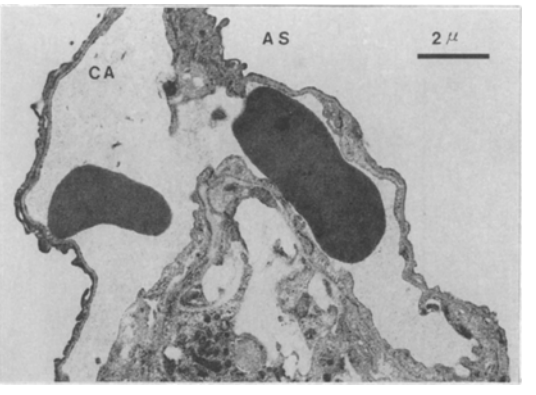

Fig. 5. Electron microscopy (original magnification ×3500) of surfactant-treated rabbits. Relatively normal appearance of capillary endothelium.

AS: alveolar space, CA: capillary lumen

supernatant of BALF after 36h of exposure to 100% O_2 are shown in table 2. The phospholipid level in surfactant-treated rabbits was significantly higher than in other groups. The albumin concentration in saline-treated rabbits was significantly higher than in non-treated rabbits and was slightly higher (P=0.2) than in surfactant-treated rabbits. The concentration of C3a in BALF was higher in the saline-treated rabbits than the surfactant-treated and non-treated rabbits. There was no significant difference in the concentration of C5a among groups.

In contrast, the wet-to-dry lung weight ratio in surfactant-treated rabbits was almost the same as that in non-treated rabbits (5.06 ± 0.10, 4.90 ± 0.17), but significantly lower than

that in the saline-treated rabbits (5.67 ± 0.14, $P < 0.05$) (fig. 1).

Light microscopic findings in saline-treated rabbits included hyaline membrane formation and existence of inflammatory cells in alveolar spaces (fig. 2). In contrast, there were inflammatory cells, but there was relatively little hyaline membrane formation in surfactant-treated rabbits (fig. 3). Electron microscopy revealed extensive endothelial cell destruction (fig. 4), endothelial cell swelling, and interstitial edema in saline-treated rabbits. There was no extensive destruction of the endothelium in surfactant-treated rabbits, although endothelial cell swelling was observed (fig. 5).

Discussion

In the present study we found that endothelial destruction and interstitial edema occurred without impairment of lung mechanics during exposure to 100% oxygen under mechanical ventilation and that early administration of exogenous surfactant attenuated this lung injury.

In our model, exposing rabbits to 100% O_2 with continuous mechanical ventilation for 36 hours induced an influx of neutrophils into the lungs, and an increase in the albumin concentration in BALF, with no changes in hemodynamics or arterial oxygen tension. This is similar to the finding that in baboons after 4 days of mechanical ventilation neutrophil recruitment and protein leakage preceded abnormalities in gas exchange[15]. Increased albumin in BALF with no changes in lung function was observed in humans spontaneously breathing 100% O_2[16]. Increased permeability is a common initial response to hyperoxia with or without artificial ventilation.

Matalon et al. found that endothelial permeability to water increased after 48 hours of hyperoxia in rabbits, but there were no changes in arterial oxygen tension or permeability to macromolecules[17]. At this stage, morphological change was not observed by electron microscopy[2]. The present study showed that alveolar protein leakage and pulmonary endothelial cell destruction could occur before the arterial blood gas values became abnormal. These changes occurred somewhat earlier than those observed in the previous reports when artificial ventilation was not used[7,8,17]. High tidal volume ventilation or high pressure ventilation is reported to cause lung damage[18,19]. In this study such management was not done (table 1). However, it is conceivable that the effect of a combination of positive pressure ventilation and hyperoxia should differ from that of spontaneous breathing under hyperoxia. Further study is needed to clarify the effect of ventilation on lung injury induced by hyperoxia.

in vitro and *in vivo* studies have shown that surfactant is depleted by hyperoxia[20,21]. Gross and Smith have shown that BALF in mice and rabbits exposed to normobaric hyperoxia has low levels of phospholipid and abnormal surface tension[20]. In addition, when albumin leaks into the alveolar spaces, the capacity to lower surface tension is diminished[22–26]. In the saline-treated rabbits in the present study, phospholipid was not decreased although the albumin concentration in BALF was significantly higher than in not-treated rabbits. However, we found no changes in compliance. This may be because the concentration of albumin was not high enough to increase surface tension.

Rabbits receiving surfactant had a significantly lower wet-to-dry weight ratio of lung tissue than those given saline. Surfactant administration has been shown to reduce the increased permeability caused by hyperoxia, and to lower the wet-to-dry weight ratio[7,8], but the mechanism by which this oc-

curs is not known.

Considering that the saline-treated rabbits had normal phospholipid levels, an excess of surfactant, rather than compensation for a surfactant deficit, may have protected them against the increase in lung water caused by hyperoxia. Hills proposed that surfactant has water repellent properties through which fluid is repelled from the alveoli[27,28]. This leads us to the hypothesis that excess water is repelled by an excess of surfactant.

One of the important findings of this study is that lung endothelial cells in surfactant-treated rabbits were less damaged than those in saline treated-rabbits. It is thought that reactive oxygen species play a role in the pathogenesis of hyperoxia-induced lung injury[29,30]. Several reports have shown that surfactant can work beneficial effects against toxic oxygen species through various mechanism[31–33]. They have shown that hyperoxic injury in rats can be limited by he intravenous and intraperitoneal administration of liposomes which are made from DPP C and contain superoxide dismutase and catalase. DPPC alone also limits lipid peroxidation. Matalon reported that increased levels of lung surfactant in the alveolar space can reduce extracellular H_2O_2 levels[34]. Furthermore, Hayakawa et al. showed that superoxide production from macrophages is suppressed by surfactant[35].

The PMN is thought to play an important role in the development of lung injury caused by hyperoxia[1]. Although it does not participate in the initial events, PMNs seem to mediate subsequent injury, by releasing superoxides and other enzymes such as collagenase and elastase. This results in a phenomenon similar to the adult respiratory distress syndrome (ARDS). Complement is known to be a chemotactic factor, and is thought to lead to leucocyte aggregation. C3a and C5a in BALF are reportedly elevated in ARDS[36]. Fox et al.[10] reported that chemotactic factor is increased in association with PMNs in hyperoxia. They also reported that the number of PMNs in BALF correlates with the degree of lung injury[10]. In the present study, C3a levels were significantly higher in the saline-treated group than in the surfactant group. PMNs in BALF did not significantly differ between the groups, but the degree of injury did differ. Merrit et al. observed that 24 hours after administration, patients given surfactant had more neutrophils than did controls[37]. Damiano also observed an influx of neutrophils into dog lung alveoli after instillation of sterile saline[38]. It seems that an increased number of PMNs does not always correlate with lung injury when surfactant is used.

In addition, the fact that the complement was not activated in the surfactant-treated rabbits implies that the surfactant did not evoke inflammation via the complement system, and that surfactant suppresses the activation of chemotactic factors in hyperoxic lung injury.

Clinical data have shown that giving surfactant to preterm infants with respiratory distress syndrome attenuates lung injury by improving gas exchange, reducing airway pressure and improving lung compliance[39,40]. Maeta studied the effect of exogenous surfactant in artificially ventilated premature baboons during hyperoxia[41]. They explained that functional abnormalities were alleviated and that there were fewer histologic features of bronchopulmonary dysplasia, resulting in rapid weaning and reduced barotrauma. In the present study, surfactant was given when lung mechanics were within the normal range, and this attenuated lung edema and endothelial injury.

We conclude that excess surfactant can protect against lung injury induced

by hyperoxia and mechanical ventilation, through a mechanism other than a reduction in surface tension.

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