

Exogenous surfactant improves survival and surfactant function in ischaemia–reperfusion injury in minipigs

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ABSTRACT: Reperfusion injury is the major cause of early morbidity and mortality after lung transplantation. This complication has been experimentally linked to dysfunction of pulmonary surfactant. Therefore, the hypothesis that reperfusion injury might be preventable by exogenous surfactant treatment was tested.

Left lungs of minipigs were exposed to 120 min of ischaemia, and the lungs were then reperfused for up to 7 h. Animals were divided into a control group and a surfactant group (n=5 each). The surfactant group received 50 mg·kg⁻¹ Alveofact® intrabronchially via a bronchoscope at the beginning of the ischaemic period. Bronchoalveolar lavage was performed at baseline before ischaemia and 90 min after reperfusion.

Surfactant treatment significantly improved short-term survival. Pulmonary vascular resistance increased markedly in control animals leading to right heart failure and death within 3 h after reperfusion whereas the surfactant-treated animals survived the 7 h observation period. After reperfusion, alveolar accumulation of neutrophils and exuded proteins was present in both groups to the same extent. Surfactant activity after reperfusion deteriorated markedly in the control group but was preserved in the surfactant group.

In conclusion, early surfactant treatment alleviates the deterioration of surfactant function and improves survival in this minipig model of ischaemia–reperfusion injury. *Eur Respir J 1999; 13: 1037–1043.*

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Ischaemia–reperfusion injury has a major impact on morbidity and mortality in the early postoperative course after lung transplantation [1], and it is a feared complication following pulmonary thromboendarterectomy [2]. Several mechanisms are involved in ischaemia–reperfusion injury including activation of leukocytes, platelets and complement, as well as release of oxygen free radicals and other inflammatory mediators [3]. Recent research has highlighted the possibility that surfactant alterations contribute to ischaemia–reperfusion injury [4–7]. Surfactant is composed of a complex mixture of mainly phospholipids and surfactant-associated proteins [8]. Surfactant reduces the surface tension at the air–liquid interface of the lungs, thereby preventing alveolar collapse and airway closure at end expiration. Moreover, its high surface pressure prevents alveolar oedema. Surfactant dysfunction occurs through a wide variety of mechanisms which include inhibition by exuded plasma proteins [9–11], dilution from alveolar oedema [12, 13], enzymatic cleavage of surfactant components [14–16] and type II cell injury. In reperfusion injury, it has been suggested that surfactant dysfunction occurred due to inhibition by exuded plasma proteins and that dysfunctioning surfactant promotes alveolar oedema, thus leading to a vicious circle.

The degree of surfactant dysfunction in reperfusion injury is similar to the impairment of surfactant found in acute respiratory distress syndrome (ARDS) [17, 18]. The first clinical trials have evaluated the use of exogenous surfactant in the management of patients with ARDS with encouraging results [19, 20]. The possible role of surfactant replacement therapy in reperfusion injury was also addressed in studies of experimental lung transplantation [21–24] and for the management of lung transplant recipients with severe reperfusion injury [25].

To further study the efficacy of exogenously administered surfactant in reperfusion injury, a minipig model of ischaemia–reperfusion injury was designed and the effect of surfactant application on the severity of reperfusion damage, surfactant function, and survival studied.

Materials and methods

Animals

Ten female minipigs (Ellegaard, Dalmose, Denmark) weighing 30–35 kg were used in this study. Animals received humane care in strict compliance with the "Principles of Laboratory Animal Care" formulated by the

National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" [26]. The study was approved by the State Ethics Committee of Lower Saxony, Germany.

Experimental procedure

Respiratory mechanics. The animals were orotracheally intubated (7.5 mm tube) after initial bolus injection of 0.2 mg fentanyl and 0.25 g thiopental. General anaesthesia was maintained by infusion of fentanyl ($0.1 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) and thiopental ($80 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), together with pancuronium ($2 \text{ mg}\cdot\text{h}^{-1}$) for relaxation. The minipigs were mechanically ventilated in a pressure-limited mode (tidal volume (V_T) $12 \text{ mL}\cdot\text{kg}^{-1}$, breathing frequency $15 \text{ breaths}\cdot\text{min}^{-1}$, maximum inspiratory pressure 30 hPa , positive end-expiratory pressure (PEEP) 5 hPa , inspiratory expiratory ratio (I:E) 1:1) with an EVITA-2-respirator (Dräger Werke, Lübeck, Germany) at a constant inspiratory oxygen fraction (F_{I,O_2}) of 0.5 throughout the experiment. Parameters of mechanical ventilation (minute volume (V'_E), expiratory tidal volume ($V_{T,e}$), plateau pressure (P_{plat}) and respirator compliance (C)) were documented at baseline (see below) and every 30 min during reperfusion.

Haemodynamics. Following thoracotomy, a 7 F flow-directed, balloon-tipped Swan-Ganz-catheter (No. 131F7; Baxter, Irvine, CA, USA) was placed into the main stem of the pulmonary artery under manual guidance. In addition, left atrial and arterial catheters were placed (18 G LeatherCath; Vygon, Aachen, Germany). After stabilization for 10 min, baseline haemodynamics, blood gases, and ventilator variables were obtained. The cardiac output was measured by the thermodilution technique and calculated using an analogue computer system (Cardiac output computer COM-2; Baxter). Pulmonary vascular resistance (PVR) was computed using the standard equation. The alveolar-arterial oxygen pressure difference (P_{A-a,O_2}) was computed according to the following standard simplified equation: $P_{A-a,O_2} = \text{alveolar oxygen tension } (P_{A,O_2}) - \text{arterial oxygen tension } (P_{a,O_2})$, where $P_{A,O_2} = F_{I,O_2} (\text{barometric pressure} - 47) - \text{arterial carbon dioxide tension } (P_{a,CO_2}) / \text{respiratory quotient } (R)$. In the latter equation, 47 is the airway water vapour pressure at 37°C in torr, and R is assumed to be 0.8. Haemodynamic variables were determined at baseline and every 30 min during reperfusion.

Lung preservation and reperfusion. After baseline haemodynamic monitoring, the left lung was prepared for flush preservation. Heparin ($200 \text{ immunizing units } (\text{IE})\cdot\text{kg}^{-1}$) was given 30 min before lung perfusion with preservation solution. The left pulmonary artery and left pulmonary veins were clamped, while ventilation of both lungs was continued. A catheter was placed into the left pulmonary artery. The pulmonary veins were incised and the left lung was selectively perfused for 15 min with 1 L of Euro-Collins solution at 4°C via the pulmonary artery catheter [27]. At the end of flush preservation, the veins were sutured. After 2 h of ischaemia, the lung was reperfused by first opening the venous clamp followed by the arterial clamp (time after

reperfusion=0 min). The circulation was allowed to stabilize for 15 min before the contralateral right pulmonary artery and bronchus were clamped. The thorax remained open throughout the experiment. To prevent early right heart failure, adrenalin was started after reperfusion at $0.1 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ to maintain cardiac output and systemic arterial pressure. Whenever cardiac output fell below $2.5 \text{ L}\cdot\text{min}^{-1}$ or mean systemic arterial pressure was below 8.0 kPa (60 mmHg), the adrenalin dose was increased by $0.2 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ up to a maximum dose of $3 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. The experiment was terminated 7 h after reperfusion.

Surfactant treatment

Animals were alternately assigned to either an untreated control group ($n=5$) or treated intrabronchially with exogenous surfactant ($50 \text{ mg}\cdot\text{kg}^{-1}$ Alveofact®; provided by K. Thomae, Biberach, Germany) ($n=5$). In this study, Alveofact® was chosen for surfactant supplementation because it has been shown that this bovine lipid extract surfactant has a good efficacy to resist protein inhibition [11]. Surfactant was segmentally instilled into the left lung by bronchoscopy at the beginning of the 120-min ischaemic period. Starting in the lower lobe, the bronchoscope was placed into a segment in a wedge position and an aliquot of surfactant ($\sim 4 \text{ mL}$) was gently instilled, followed by 5 mL of air. After ~ 1 min the bronchoscope was slowly drawn back and the procedure was continued in a neighbouring segment. The instillation procedure took ~ 15 min. Sham bronchoscopies with saline instillation were not carried out in the control group because of presumed negative effects on alveolar surfactant balance.

Bronchoalveolar lavage

Two bronchoalveolar lavage (BAL) procedures were performed in all animals. The initial BAL was performed in the right middle lobe at baseline. A second BAL was obtained from the lingula of the left lung 90 min after reperfusion. Briefly, a fiberoptic bronchoscope (Olympus P10, Tokyo, Japan) was wedged into the appropriate bronchus and the lavage carried out with warm saline solution in five aliquots of 20 mL. Following instillation, each aliquot was aspirated with gentle suction. The BAL fluid (BALF) was pooled and the recovered volume recorded ("recovery"). The fluid was filtered through sterile gauze and then centrifuged at $250 \times g$ for 10 min to obtain a cell free supernatant, which was stored at -28°C until further analysis. From the cell pellet the total number of cells in the BALF was counted and a differential cell count was performed using standard techniques.

Protein and phospholipid analysis

Aliquots of the cell free supernatant were used for determination of protein concentrations according to LOWRY *et al.* [28] and of phospholipid concentrations according to the method of BARTLETT [29]. The latter assay is based on a phosphorus determination carried out on the lipids extracted with chloroform/methanol according to BLIGH and DYER [30]. All assays were performed in duplicate and the mean value was reported.

Surfactant isolation and aggregate separation

The cell-free supernatant was centrifuged at $48,000 \times g$ for 60 min at 4°C to pellet large surfactant aggregates (LA). The supernatant, containing small surfactant aggregates (SA), was removed and the LA pellet was resuspended in Ringer's solution. The phospholipid contents of the LA pellets and the SA supernatants were determined as described above. By adding Ringer's solution, the phospholipid concentration of the LA suspension was adjusted to $1 \text{ mg}\cdot\text{mL}^{-1}$ for studying surface properties with the pulsating bubble surfactometer (Electronetics, Buffalo, NY, USA).

Surface activity evaluated with the pulsating bubble surfactometer

Surface activity of BALF was measured with a pulsating bubble surfactometer [31]. Briefly, $40 \mu\text{L}$ of the LA suspension, which had been adjusted to a phospholipid concentration of $1 \text{ mg}\cdot\text{mL}^{-1}$, was used for filling the sample chamber with a micropipette. The surface tension used for statistical analysis of this study was the value at minimal bubble size (γ_{min}), registered after 5 min of bubble pulsation at a rate of $20 \text{ cycles}\cdot\text{min}^{-1}$ and at a temperature of 37°C . Before starting bubble pulsation, the initial surface tension (γ_0) after bubble formation was measured and the adsorption rate (γ_{ads}) was evaluated by determining surface tension 10 s after formation of a bubble. All analogue data were digitalized and recorded by computer.

Statistics

Results are expressed as mean \pm SEM. Unless otherwise stated, the parameters after reperfusion were analysed at 120 min and at the individual end-points which were shortly before death or sacrifice. Within treatment groups the differences before and after reperfusion were tested by Wilcoxon's rank-sum test. Differences between the study groups (control *versus* surfactant) were tested by the Mann-Whitney U-test [32]. A p-value of <0.05 was considered significant.

Results

Survival, haemodynamics, respiratory mechanics, and oxygenation

Following reperfusion, all control animals developed a rapidly progressive increase of PVR that uniformly resulted in fatal right heart failure after 186 ± 26 min. In contrast, surfactant-treated animals exhibited a less severe increase in PVR and all except one survived until sacrifice (table 1).

In control animals, cardiac output decreased from $2.68\pm 0.17 \text{ L}\cdot\text{min}^{-1}$ at baseline to $1.60\pm 0.36 \text{ L}\cdot\text{min}^{-1}$ after 120 min of reperfusion and went down further to $0.74\pm 0.28 \text{ L}\cdot\text{min}^{-1}$ shortly before death. In contrast, cardiac output decreased significantly less in the surfactant group ($2.92\pm 0.12 \text{ L}\cdot\text{min}^{-1}$ at baseline, $2.46\pm 0.22 \text{ L}\cdot\text{min}^{-1}$ after 120 min of reperfusion and $1.78\pm 0.18 \text{ L}\cdot\text{min}^{-1}$ shortly before death/sacrifice; table 1). In addition, control animals required

Table 1. – Survival, haemodynamic and oxygenation data

	Control	Surfactant	p-value
Survival min	186 \pm 26	408 \pm 12	0.009
CO $\text{L}\cdot\text{min}^{-1}$			
Baseline	2.68 \pm 0.17	2.92 \pm 0.12	0.30
After 120 min	1.60 \pm 0.36	2.46 \pm 0.22	0.10
At death/sacrifice	0.74 \pm 0.28	1.78 \pm 0.18	0.02
PVR $\text{dynes}\cdot\text{cm}\cdot\text{s}^{-5}$			
Baseline	321 \pm 94	429 \pm 86	0.35
After 120 min	1889 \pm 813	1014 \pm 223	0.47
At death/sacrifice	3307 \pm 1041	1482 \pm 178	0.18
PAP mmHg			
Baseline	16 \pm 3	18 \pm 3	0.75
After 120 min	33 \pm 6	38 \pm 7	0.47
At death/sacrifice	28 \pm 4	37 \pm 7	0.25
SAP mmHg			
Baseline	66 \pm 2	75 \pm 5	0.18
After 120 min	44 \pm 10	71 \pm 7	0.08
At death/sacrifice	21 \pm 4	56 \pm 7	0.009
LAP mmHg			
Baseline	6 \pm 1	7 \pm 1	0.60
After 120 min	6 \pm 2	6 \pm 2	0.92
At death/sacrifice	6 \pm 2	4 \pm 1	0.53
P_{a,O_2} mmHg			
Baseline	231 \pm 24	271 \pm 32	0.08
After 120 min	127 \pm 53	157 \pm 28	0.25
At death/sacrifice	50 \pm 12	56 \pm 4	0.46
P_{a,CO_2} mmHg			
Baseline	44 \pm 2	37 \pm 4	0.10
After 120 min	53 \pm 7	45 \pm 5	0.75
At death/sacrifice	62 \pm 13	59 \pm 11	0.81
$P_{\text{A-a},\text{O}_2}$ mmHg			
Baseline	91 \pm 23	56 \pm 31	0.08
After 120 min	187 \pm 48	164 \pm 29	0.35
At death/sacrifice	257 \pm 10	253 \pm 8	0.81
HR $\text{beats}\cdot\text{min}^{-1}$			
Baseline	74 \pm 12	85 \pm 8	0.35
After 120 min	110 \pm 17	97 \pm 5	0.99
At death/sacrifice	110 \pm 25	108 \pm 5	0.60
Adrenalin $\mu\text{g}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$			
Baseline	0	0	
After 120 min	1.50 \pm 0.52	0.26 \pm 0.01	0.04
At death/sacrifice	1.56 \pm 0.51	0.31 \pm 0.02	0.04

CO: cardiac output; PVR: pulmonary vascular resistance; PAP: mean pulmonary artery pressure; SAP: mean systemic arterial pressure; LAP: mean left arterial pressure; P_{a,O_2} : arterial oxygen tension; P_{a,CO_2} : arterial carbon dioxide tension; $P_{\text{A-a},\text{O}_2}$: alveolar-arterial oxygen pressure difference; HR: heart rate. Data are reported before left lungs were made ischaemic (baseline), after 120 min of reperfusion and shortly before death or sacrifice (at death/sacrifice). Values are given as mean \pm SEM. (1 mmHg=0.133 kPa.)

much higher doses of adrenalin to maintain cardiac output and systemic arterial blood pressure compared to surfactant-treated animals (table 1). Left atrial pressures remained normal in both groups throughout the entire experiment (table 1).

At baseline, P_{a,O_2} and $P_{\text{A-a},\text{O}_2}$ values were similar in the control and in the surfactant group. Moreover, oxygenation variables were not different in the two groups during the initial 2 h of reperfusion or shortly before death or sacrifice (table 1). In contrast, $V'E$ markedly decreased and compliance deteriorated during reperfusion in untreated control animals, whereas surfactant treatment alleviated the loss of compliance and prevented deterioration of $V'E$ (fig. 1).

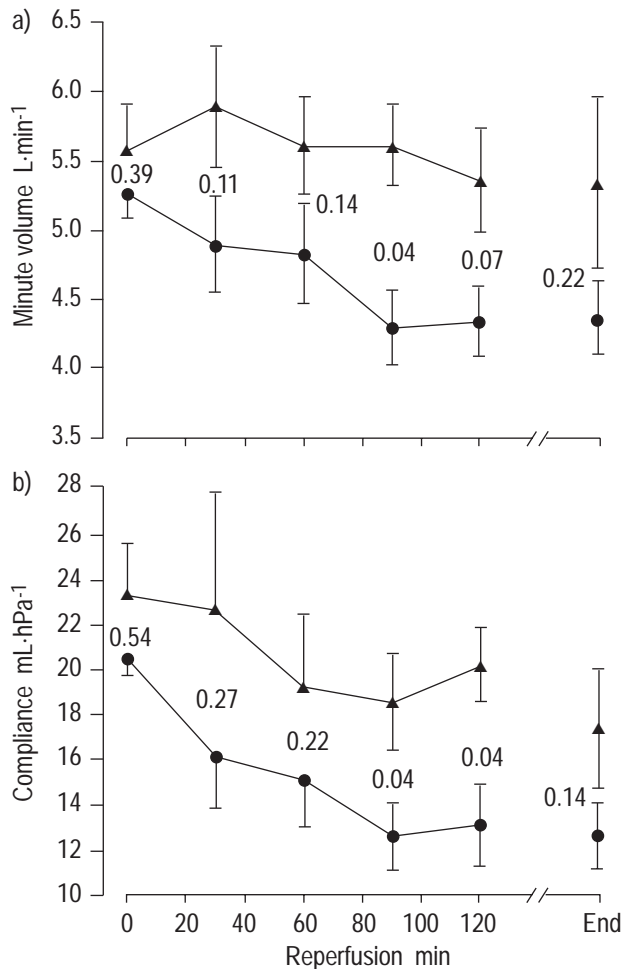


Fig. 1. – Minute volume (a) and respirator compliance (b) during the 120 min of reperfusion and shortly before death or sacrifice (End) in untreated control animals (●) and in surfactant-treated animals (▲). Values are given as mean±SEM and exact p-values are shown.

Cells in bronchoalveolar lavage fluid

The total cell numbers recovered from BALF at baseline did not differ between the control and the surfactant-treated group (table 2). After reperfusion, there was a slight but nonsignificant increase of total cells in both groups which was more pronounced in the surfactant group.

Analysis of differential cell counts showed a similar distribution in the control and in the surfactant group at baseline with ~90% macrophages as the predominant cells. After reperfusion, there was a marked increase in neutro-

phils (>50%), accompanied by a decrease in macrophages, and a slight increase in lymphocytes in both groups without a difference between control and surfactant-treated animals (table 2).

Phospholipids and proteins in bronchoalveolar lavage fluid

Phospholipid concentrations in BALF from both groups were not different at baseline. In the control group, the phospholipid concentration remained unchanged after reperfusion compared to baseline. Exogenous surfactant treatment increased the phospholipid concentration compared to pretreatment values ($p=0.04$, fig. 2a). The difference between the control and surfactant group was close to statistical significance ($p=0.08$).

Proteins in the BALF at baseline were low in both groups but increased 15–20-fold after reperfusion without a difference between the two groups (fig. 2b). High-speed centrifugation co-pelleted proteins leading to protein concentrations in the LA pellet of $790\pm 292 \mu\text{g}\cdot\text{mL}^{-1}$ and $757\pm 178 \mu\text{g}\cdot\text{mL}^{-1}$ in control and surfactant-treated animals, respectively. At baseline, the ratio of protein to phospholipid as a marker of the inhibitory potential on surfactant function was not different between the control and the surfactant group. Reperfusion resulted in a marked increase in the protein to phospholipid ratio in control animals whereas the treatment with exogenous surfactant alleviated this increase (fig. 3a).

The ratio of SA:LA was determined as an indicator for metabolic conversion of the surfactant material. As seen in figure 3b, the SA:LA was low under baseline conditions in both groups and increased dramatically after reperfusion in the control group ($p=0.04$), but was unchanged in lavage from surfactant-treated animals ($p=0.50$).

Surface activity evaluated with the pulsated bubble surfactometer

The adsorption rate γ_{ads} , defined as the surface tension of the uncycled bubble 10 s after bubble formation, was $\sim 28 \text{ mN}\cdot\text{m}^{-1}$ in both study groups at baseline (fig. 4a). In the control group, the γ_{ads} showed higher values after reperfusion ($p=0.14$), whereas in the surfactant group there was a slight decrease of the adsorption rate values ($p=0.69$). After reperfusion the difference in γ_{ads} was significant between the control and the surfactant group ($p=0.02$).

Surface tension at minimal bubble size after five minutes of pulsation (γ_{min}) was $\sim 2 \text{ mN}\cdot\text{m}^{-1}$ in both groups at

Table 2. – Cellular analysis of bronchoalveolar lavage

	Control			Surfactant			p-value ⁺
	Baseline	Reperfusion	p-value*	Baseline	Reperfusion	p-value*	
Total cells· μL^{-1}	136±19	173±87	0.99	156±45	293±150	0.35	0.33
Macrophages %	95±2	48±22	0.11	91±2	37±13	0.07	0.72
Lymphocytes %	4±2.1	16±11	0.29	5±1.4	10±4.5	0.29	0.48
Neutrophils %	1±0.3	53±19	0.11	1±0.8	53±18	0.07	0.72

Values are given as mean±SEM. Bronchoalveolar lavage was performed before ischaemia (baseline) and after 90 min of reperfusion. *: p-values for within treatment group comparisons (baseline versus reperfusion); ⁺: p-values for between treatment group comparisons (control versus surfactant).

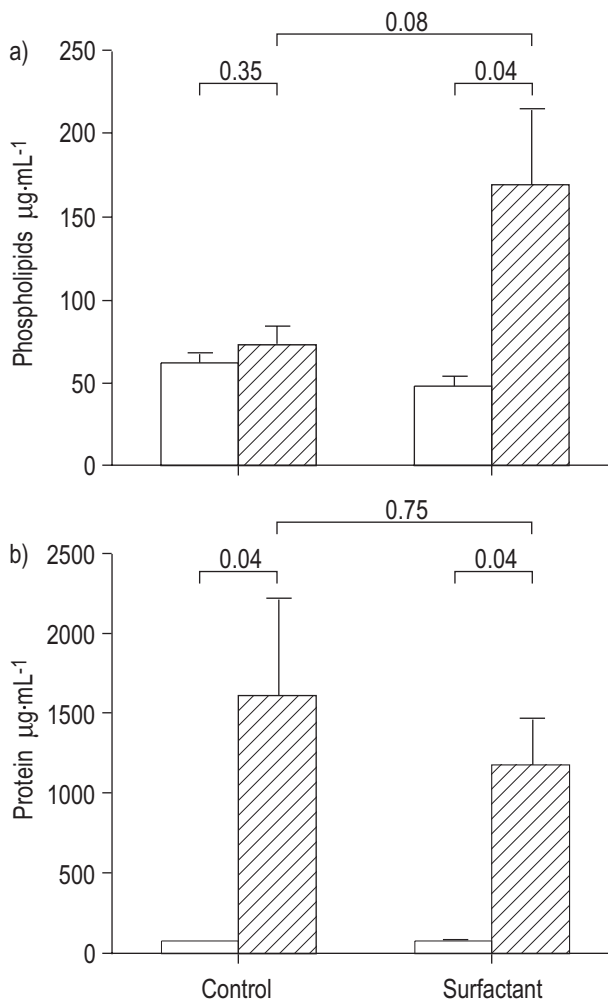


Fig. 2. – Phospholipids (a) and proteins (b) in bronchoalveolar lavage fluids obtained at baseline before ischaemia (□) and after 90 min of reperfusion (▨) in untreated control animals and in surfactant-treated animals. Values are given as mean±SEM and exact p-values are shown.

baseline (fig. 4b). After reperfusion, minimum surface tension increased in the control group ($p=0.23$) but was unchanged in BALF from surfactant-treated animals ($p=0.69$) resulting in a significant difference of γ_{\min} between the two study groups ($p=0.01$).

Maximum survival in control animals was 270 min, whereas surfactant-treated animals survived at least 360 min. Animals with better survival had better surfactant function analysed after reperfusion. Adsorption rate, γ_{ads} , was $<25 \text{ mN}\cdot\text{m}^{-1}$ in all five surfactant-treated animals, while only one animal in the control group had a γ_{ads} of $<25 \text{ mN}\cdot\text{m}^{-1}$. In addition, γ_{\min} was $<2 \text{ mN}\cdot\text{m}^{-1}$ in all surfactant-treated animals whereas only two out of five control animals had such low γ_{\min} values.

Discussion

Reperfusion injury is characterized by a disturbance of the capillary–endothelial barrier with leakage of plasma proteins into the alveoli. In this study in pig lungs, ischaemia–reperfusion injury resulted in severe exudation of proteins into the alveoli with increased numbers of neutrophils. Surface activity of surfactant recovered from BALF after

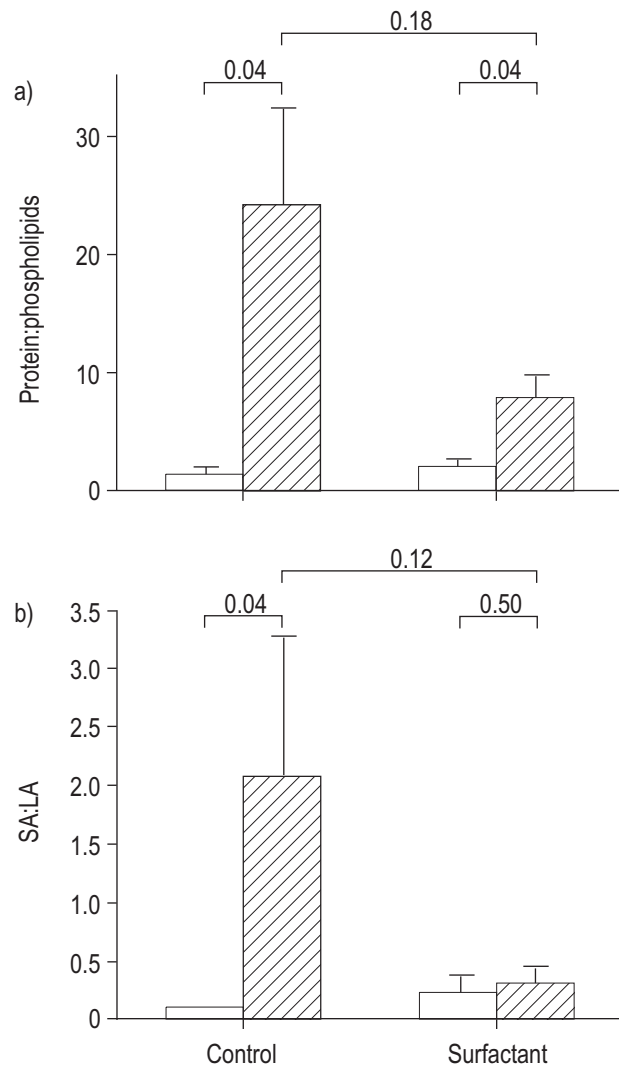


Fig. 3. – The protein to phospholipids ratio (a) and small surfactant aggregates (SA) to large surfactant aggregates (LA) ratio (b) in bronchoalveolar lavage fluids obtained at baseline before ischaemia (□) and after 90 min of reperfusion (▨) in untreated control animals and in surfactant-treated animals. Values are given as mean±SEM and exact p-values are shown.

reperfusion was markedly impaired in untreated animals, probably as a result of surfactant inhibition by exuded proteins. Plasma proteins like albumin, fibrinogen, and haemoglobin are well known inhibitors of surfactant function [9–11]. In contrast to control animals, surface activity in surfactant-treated animals was preserved, although proteins in BALF were present to the same extent. In particular, the total amount of proteins that co-pelleted during the isolation procedure of surfactant did not differ between the control and surfactant-treated animals. Therefore, other mechanisms may account for the impaired surfactant function after reperfusion. It seems reasonable to assume that altered surfactant composition contributed to the observed differences of surfactant activity. On the one hand, it has been shown that exogenous surfactant increased the percentage of well-functioning heavy subtype surfactant while on the other hand, surfactant supplementation with elevation of phospholipids and surfactant-specific proteins might improve the surfactant

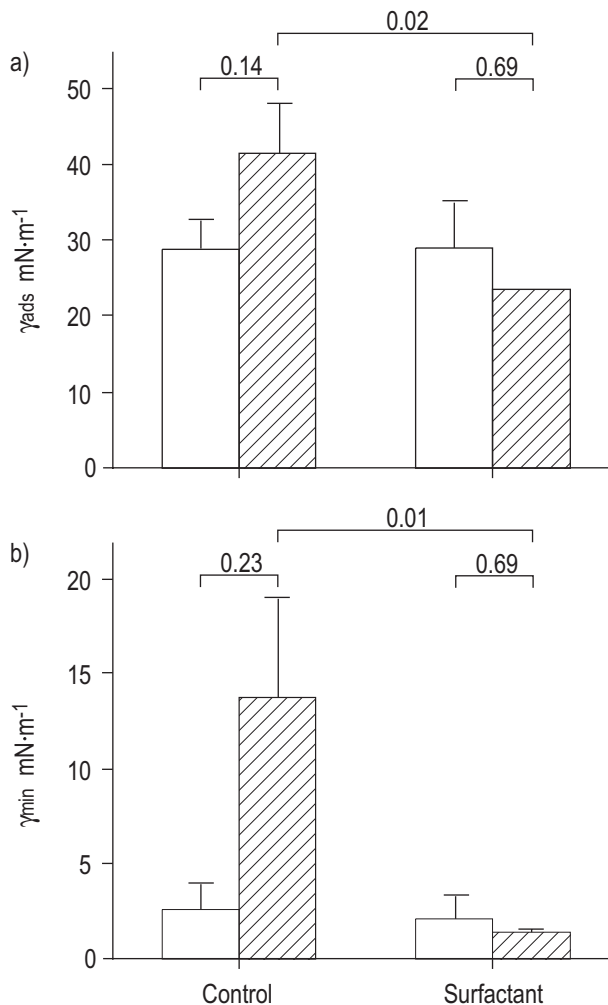


Fig. 4. – Biophysical properties of surfactant recovered from bronchoalveolar lavage fluids obtained at baseline before ischaemia (□) and after 90 min of reperfusion (▨) in untreated control animals and in surfactant-treated animals. Biophysical properties are determined as surface tensions after a 10 s adsorption period (γ_{ads} ; a) and at minimum bubble size after 5 min of film oscillation (γ_{min} ; b). Values are given as mean \pm SEM and exact p-values are shown.

profile, leading to enhanced surfactant activity. In addition, changes of surfactant composition after administration of exogenous surfactant might also have led to a higher resistance against surfactant inhibition by proteins. These findings that ischaemia–reperfusion injury caused surfactant dysfunction which can be preserved by exogenous surfactant treatment are in good accordance with data from the literature [4–6, 21–24, 33]. In addition, it is suggested that preservation of surfactant function might improve the outcome of ischaemia–reperfusion injury.

The experimental model of ischaemia in minipigs led to a reliable and severe reperfusion injury which invariably caused right heart failure and death within 3–4 h. Remarkably, right heart failure did not occur in surfactant-treated animals and these animals required less adrenalin to maintain cardiac output. Surprisingly, oxygenation was similar in untreated control and surfactant-treated animals. The most likely explanation for this paradoxical finding is a reduction of hypoxic pulmonary vasoconstriction in the surfactant group, since $\dot{V}'E$ during reperfusion was higher

in these animals compared to the control group (fig. 1). If so, the fact that the oxygenation index of the control group was not lower than the surfactant group might reflect a highly effective matching of perfusion to the reduced ventilated lung areas in the control group at the expense of increased pulmonary vasoconstriction. Another, but more speculative, explanation would be that surfactant might have some indirect effects on the regulation of pulmonary vascular tone. For instance, it has been shown that surfactant compounds can stimulate nitric oxide production of rat alveolar macrophages [34, 35], which in turn could lead to vasodilation of precapillary pulmonary arteries.

Previous studies on the effects of surfactant treatment on oxygenation in reperfusion injury have yielded conflicting results. In accordance with the present findings, NOVICK *et al.* [23] have demonstrated that treatment of transplanted dogs with instilled surfactant before reperfusion did not improve oxygenation. In contrast, ERASMUS *et al.* [22] have shown that surfactant treatment immediately before reperfusion improved oxygenation in experimental lung transplantation in rats. Interestingly, when surfactant was administered before lung procurement, oxygenation was significantly improved during reperfusion, suggesting that surfactant might have superior effects when given before the ischaemic damage [23]. The different effects of surfactant treatment on oxygenation in the various studies might be due to differences in species, dosing, timing, surfactant preparations and models used.

The present study has several limitations. Firstly, the number of animals per group was rather small. However, despite these low numbers, significant effects of surfactant were demonstrable. A further limitation of this study could be the fact that the control group did not undergo sham bronchoscopy with instillation of saline as a placebo dosing procedure. A sham bronchoscopy was chosen not to be carried out because saline instillation in ischaemia–reperfusion injury might itself have negative effects on alveolar surfactant balance. Finally, this study provides no information on long-term outcome since it was designed to investigate the acute postoperative phase. However, it has been shown that successful early treatment of ischaemia–reperfusion injury has some long lasting positive effects on pulmonary function after lung transplantation [36].

In conclusion, ischaemia–reperfusion injury in minipigs leads to deterioration of surfactant function. Exogenous surfactant treatment improves surfactant function and short-term survival. Thus, supplementation of surfactant may be an effective treatment for reperfusion injury. Further studies are needed to unravel the optimal strategies of surfactant supplementation for the prevention and treatment of reperfusion injury. Moreover, future work should concentrate on treatment modalities that protect endogenous surfactant function in reperfusion injury.

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