Surfactant abnormalities after single lung transplantation in dogs: Impact of bronchoscopic surfactant administration

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Objective: Disturbances of the alveolar surfactant system have been implicated in the pathogenesis of reperfusion injury. The aim of this study was to evaluate the influence of exogenous surfactant administration on surfactant properties in a model of single lung transplantation.

Methods: We performed heterologous, left lung transplantation (+4°C ischemia; 24 hours, Euro-Collins solution) in 6 foxhounds (untreated) and in 6 animals that received calf lung surfactant extract (Alveofact) prior to explantation (only donor lung; 50 mg/kg body weight) and immediately after onset of reperfusion (both lungs, 200 mg/kg body weight). Separate but synchronized ventilation of each lung was performed, in a volume-controlled, pressure-limited mode, with animals in prone position. Bronchoalveolar lavage fluids were collected in pretransplantation lungs (control), after 24 hours of ischemia prior to transplantation (0 hours) and 6 and 12 hours after reperfusion in both the grafts and the recipient native lungs.

Results: Ischemic storage per se did not provoke any changes of the surfactant system; however, severe alterations occurred within 6 hours of reperfusion, resulting in a severe loss of surface activity, including a decrease in the percentage of the large surfactant aggregate fraction, reduction of the surfactant apoproteins SP-B and SP-C, the dipalmitoyl molecular species of phosphatidylcholine and phosphatidylglycerol within the large surfactant aggregate fraction. These abnormalities were restricted to the graft, with virtually normal surfactant function and composition being found in the recipient native lung. Surfactant administration fully normalized the biochemical and largely improved the biophysical surfactant properties, alongside maintenance of lung gas exchange properties.

Conclusions: Severe surfactant abnormalities occur exclusively in the graft when performing separate, synchronized ventilation of each lung to attenuate ventilator-induced lung injury. Bronchoscopic surfactant administration provides protection against these abnormalities and may be a therapeutic strategy in lung transplantation.

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Pulmonary surfactant is a highly surface-active lipoprotein complex covering the alveoli of the lung. The major constituents of this material are phospholipids (approximately 80% phosphatidylcholine (PC) and 10% phosphatidylglycerol) and the surfactant apoproteins (SP)-A, SP-B, SP-C, and SP-D.¹ Both surfactant lipids and apoproteins act in concert to facilitate rapid surfactant adsorption to the air-liquid interface and to reduce the alveolar surface tension to near zero values at end-expiration.² Dipalmitoyl-phosphatidylcholine (DPPC), phosphatidylglycerol,

SP-B, and SP-C were found to be particularly relevant in this context. By this far-reaching reduction of surface tension, stabilization of alveoli is enabled, thereby keeping shunt flow at low numbers. An intact surfactant system is thus a prerequisite for breathing at normal transpulmonary pressures and ascertains highly efficient gas exchange. Accordingly, surfactant abnormalities have been encountered in different states of acute respiratory distress including classic acute respiratory distress syndrome (ARDS) triggered by systemic inflammatory events³ and severe pneumonia necessitating mechanical ventilation,⁴ and transbronchial surfactant administration was recently shown to acutely improve gas exchange and to reduce pulmonary shunt flow in patients with ARDS.⁵⁻⁷

Reperfusion injury is a frequent complication of lung transplantation, representing a major threat to these patients. Although the mechanisms underlying this clinical condition are not precisely known, surfactant alterations might be well operative as many of the inflammatory events observed under conditions of reperfusion injury resemble those in ARDS.⁸ Accordingly, the potential use of an exogenous surfactant therapy in lung transplantation was suggested 10 years ago.⁹ Ultrastructural analysis of surfactant after experimental ischemia-reperfusion injury revealed a significant reduction in the biophysically active tubular myelin and a corresponding increase in the less active unilamellar surfactant components.¹⁰ In addition, in experimental studies of lung transplantation in dogs, including varying ischemia times (6-38 hours),¹¹⁻¹⁴ surfactant abnormalities were noted and included altered phospholipid profiles, reduced content of SP-A, decreased relative amounts of large surfactant aggregates, and loss of surface activity. However, no detailed analysis of the fatty acid composition and the molecular species of PC was hitherto performed, and data on the hydrophobic surfactant apoprotein SP-C and on the influence of plasma protein leakage in posttransplantation lungs are currently not available. We therefore analyzed biochemical and biophysical surfactant changes in canine lungs undergoing 24 hours of cold ischemia and 12 hours of reperfusion to explore the impact of bronchoscopic surfactant administration.

Methods

Materials

A calf lung surfactant extract (Alveofact), consisting of phospholipids, neutral lipids, and the 2 hydrophobic surfactant proteins SP-B and SP-C,¹⁵ was kindly given to us by Dr H. Weller (Thomae, Biberach, Germany). A monoclonal antibody against SP-B (8B5E) and a polyclonal antibody against SP-C were kindly provided by Dr Y. Suzuki (Chest Disease Research Institute, Kyoto, Japan) and Dr W. Steinhilber (Altana Pharm, Konstanz, Germany), respectively.

Transplantation Model

Details of the transplantation procedure and of the ventilator settings are given in the companion article.^{15a} In summary, the dogs (12 donors, 12 recipients, both sexes, 27-33 kg, weight-matched, all receiving humane care in accordance with the Guide for the Care and Use of Laboratory Animals [National Academies Press, Washington, DC, 1996]) were randomly assigned to either the control group (6 donors, 6 recipients) or the surfactant treatment group (6 donors, 6 recipients). Donors underwent left lateral thoracotomy. In the surfactant group, 50 mg/kg body weight (BW) Alveofact was instilled bronchoscopically in 10 divided doses stepwise into each segment of the left lung. No transbronchial fluid application was performed in the control group. Lungs were then perfused with 60 mL/kg of 4°C cold, modified Euro-Collins solution (including 100 µg/L prostaglandin E₂ [PGE₂]). The lungs were inflated to a pressure of 20 cm H₂O, the trachea was stapled, and the heart-lung block was stored at 4°C for 24 hours. Recipients also underwent left lateral thoracotomy. The left lung was clamped, excised and lavaged ex situ ("baseline"). The donor left lung was implanted subsequently. The preserved and stored but nontransplanted right donor lung was lavaged ex situ to assess storage-induced changes ("postischemia"). The transplanted left lung and the native right lung of the recipient were then ventilated separately but synchronized in a master and slave modus in a volume-controlled, pressure-limited fashion. Upon ventilation of the donor lung, reperfusion was started and time was set to zero. In the surfactant group, a second dose (200 mg/kg BW) of Alveofact was administered bronchoscopically into both lungs at divided doses, directly upon onset of reperfusion and positioning of the animals in the prone position. No application of fluids was performed in the control group.

Bronchoalveolar Lavage Technique

All lavages (baseline, postischemia, and native lung and graft postreperfusion) were performed ex vivo except for the bronchoscopically performed lavage 6 hours postreperfusion. In case of ex vivo lavage, the main bronchus of the lower lobe was cannulated and fixed by a transbronchial suture, and 500 mL of sterile saline solution was carefully instilled and reaspirated 3 times by a passive pressure gradient of 40 cm H₂O. The overall fluid recovery was 79.2% ± 4.9% (mean ± standard error of the mean [SEM]). The 6-hour postreperfusion lavages were obtained by placing the bronchoscope in wedge position into a segment bronchus of the left and right upper lobe and by lavaging this segment with 10 × 20 mL of sterile saline solution, with a fluid recovery of 69.2% ± 2.3% (mean ± SEM). The fractions were pooled, filtered through sterile gauze, and centrifuged at 200g (10 minutes, 4°C).

Surfactant Analysis

Lipid extraction, quantification of total phospholipid and protein concentrations, and characterization of phospholipid profiles (high-performance thin-layer chromatography) were performed as recently published.⁴ PC fatty acids and plasmalogens (alkenyl-acyl-PC) were determined using gas chromatography as previously described.^{16,17} The ratio of the detected dimethylacetals to fatty acid methyl esters indicated the relative amount of plasmalogens within the PC fraction. The profile of molecular species of PC was analyzed following phospholipolytic cleavage of the polar head

TABLE 1. Phospholipid content of BALF, relative content of LSA and phospholipid/protein ratio

	Baseline	0 hours post ischemia	6 hours post reperfusion	12 hours post reperfusion
Phospholipid ($\mu\text{g/mL}$)				
Standard treatment				
Donor lung		264.0 $\pm$ 33.6 R	71.9 $\pm$ 23.5 L	146.7 $\pm$ 12.8 L
Native lung	158.2 $\pm$ 20.3		52.2 $\pm$ 10.9	137.9 $\pm$ 42.2
Surfactant treatment				
Donor lung		182.2 $\pm$ 50.2 R	499.0 $\pm$ 75.5 L*	857.5 $\pm$ 202.6 L†
Native lung	158.2 $\pm$ 35.3		418.9 $\pm$ 169.0	539.6 $\pm$ 69.1*
Phospholipid/protein ratio				
Standard treatment				
Donor lung		0.58 $\pm$ 0.05 R	0.11 $\pm$ 0.02 L	0.22 $\pm$ 0.15 L
Native lung	0.70 $\pm$ 0.07		0.39 $\pm$ 0.08	0.28 $\pm$ 0.07
Surfactant treatment				
Donor lung		0.53 $\pm$ 0.10 R	1.11 $\pm$ 0.41 L†	0.56 $\pm$ 0.11 L
Native lung	0.59 $\pm$ 0.16		2.20 $\pm$ 0.63†	1.50 $\pm$ 0.26†
LSA (in % of total PL)				
Standard treatment				
Donor lung		85.0 $\pm$ 4.2 R	29.7 $\pm$ 9.0 L	65.1 $\pm$ 12.7 L
Native lung	94.4 $\pm$ 3.2		70.1 $\pm$ 4.4	76.2 $\pm$ 13.2
Surfactant treatment				
Donor lung		71.9 $\pm$ 10.3 R	81.2 $\pm$ 8.6 L†	82.5 $\pm$ 7.9 L
Native lung	82.5 $\pm$ 5.3		87.3 $\pm$ 6.5†	85.7 $\pm$ 3.9

BALF, Bronchoalveolar lavage fluid; LSA, large surfactant aggregates; PL, phospholipids; R, right donor lung after ischemia; L, transplanted graft (left donor lung).

Given are the phospholipid concentration of BALF, the phospholipid/protein ration, and the relative amount of large surfactant aggregates (in % of total PL) for the donor lung and the native lung in the standard treatment and the surfactant-treated group. Baseline = explanted left recipient lung. Mean $\pm$ SEM of 6 independent experiments is given.

* $P < .001$, † $P < .05$, ‡ $P < .01$, comparison between untreated and treated organs.

group with phospholipase C and conversion of the resulting diacylglycerols with 1-naphthylisocyanate by means of high-performance liquid chromatography following a variation of the method described by Ruestow and colleagues.¹⁸ SP-B and SP-C were quantified using recently described enzyme-linked immunosorbent assay techniques,^{19,20} with isolated canine SP-B and SP-C²¹ serving as standard. For characterization of relative content of large surfactant aggregates (LSA) and surface activity, bronchoalveolar lavage fluid (BALF) was centrifuged at 48,000g (1 hour, 4°C), the pellet was resuspended in a small volume of 0.9% NaCl containing 3 mmol/L CaCl_2 and assessed for the phospholipid (PL) content. Recovery of PL in the pellet (=LSA) was used to calculate relative LSA content. Surface activity of the LSA fraction, adjusted to 2 mg/mL PL always, was determined using a pulsating bubble surfactometer (Electronetics, New York, NY) as previously described.^{4,22} The surface tension after 5 minutes of film oscillation and at minimum bubble radius (γ_{min}) is given. If possible, the LSA preparations were recombined with purified BALF proteins and reanalyzed for surface activity as outlined previously.⁴

Statistics

All results are given as mean $\pm$ SEM and were analyzed by a biostatistician (Moredata GmbH, Giessen, Germany). Statistical analysis of differences between surfactant-treated lungs and controls was performed by testing principle significant diversity first (Kruskal-Wallis H-test), followed by comparison with a nonparametric test (Mann-Whitney U test). A Wilcoxon matched-pair test was used to compare baseline values with each time point.

Results

Ischemic storage of the donor lungs did not evoke a significant influx of plasma proteins or cells into the alveolar compartment. After reperfusion, however, a marked inflammatory response was encountered in both the graft and the native organ, with markedly increased permeability, pronounced neutrophil influx, and increased alveolar protein levels. As a result, lung compliance and gas exchange of the graft, and in the further course also of the native lung, progressively deteriorated (see companion paper^{15a}).

Concerning the surfactant properties, ischemic storage of the lungs per se did not evoke any significant change in the surfactant composition. In detail, unchanged concentrations of total phospholipids, phospholipid/protein ratio, and relative amount of large surfactant aggregates (LSA; all Table 1) were observed. Moreover, phospholipid-profiles (Table 2) and the pattern of molecular species of PC (Figure 1 and Table 4) in the lungs undergoing ischemic storage largely corresponded to the findings under baseline conditions. Similarly, relative content of palmitic acid and plasmalogens in PC were unaltered after 24 hours of cold ischemic storage (Table 2). The relative amount of the 2 hydrophobic surfactant apoproteins SP-B and SP-C within the LSA fraction was found to range from $\sim 1.1\%$ (SP-B; wt/wt of total PL) to $\sim 0.5\%$ (SP-C) and did not change upon ischemic

TABLE 2. Phospholipid profile and relative content of palmitic acid and plasmalogens in phosphatidylcholine

	Baseline	0 hours post ischemia	6 hours post reperfusion	12 hours post reperfusion
Phosphatidylcholine				
Standard treatment				
Donor lung		85.9 ± 1.8 R	85.1 ± 1.9 L	86.0 ± 1.4 L
Native lung	84.6 ± 2.5		84.7 ± 3.0	83.8 ± 1.3
Surfactant treatment				
Donor lung		82.6 ± 2.2 R	85.5 ± 1.6 L	86.9 ± 1.6 L
Native lung	82.3 ± 1.8		85.9 ± 1.8	85.5 ± 1.7
Phosphatidylglycerol				
Standard treatment				
Donor lung		9.9 ± 1.5 R	7.7 ± 1.1 L	6.4 ± 1.1 L
Native lung	10.4 ± 1.9		9.1 ± 1.7	10.8 ± 1.6
Surfactant treatment				
Donor lung		12.7 ± 1.6 R	9.5 ± 1.1 L	9.8 ± 1.5 L*
Native lung	11.8 ± 0.9		10.0 ± 1.1	9.1 ± 1.6
Phosphatidylinositol				
Standard treatment				
Donor lung		1.3 ± 0.2 R	2.0 ± 1.0 L	2.2 ± 1.0 L
Native lung	1.7 ± 0.2		1.9 ± 0.4	1.2 ± 0.2
Surfactant treatment				
Donor lung		1.3 ± 0.3 R	1.3 ± 0.5 L	0.5 ± 0.1 L*
Native lung	1.6 ± 0.6		1.0 ± 0.1	0.6 ± 0.1*
Sphingomyelin				
Standard treatment				
Donor lung		2.1 ± 0.6 R	3.9 ± 0.6 L	2.7 ± 0.6 L
Native lung	2.1 ± 0.7		1.6 ± 0.4	1.7 ± 0.4
Surfactant treatment				
Donor lung		1.8 ± 0.3 R	1.6 ± 0.4 L	1.4 ± 0.2 L*
Native lung	1.6 ± 0.6		1.4 ± 0.4	2.1 ± 0.7
16:0 (% of PC)				
Standard treatment				
Donor lung		64.9 ± 0.5 R	46.8 ± 4.2 L	49.2 ± 3.6 L
Native lung	63.6 ± 1.1		59.9 ± 3.1	60.7 ± 2.7
Surfactant treatment				
Donor lung		65.3 ± 0.9 R	61.0 ± 2.9 L*	63.8 ± 1.0 L†
Native lung	65.0 ± 0.6		64.3 ± 0.5	64.0 ± 0.8
Plasmalogens (% of PC)				
Standard treatment				
Donor lung		4.1 ± 0.2 R	5.2 ± 1.2 L	5.0 ± 0.9 L
Native lung	3.9 ± 0.3		5.1 ± 0.7	4.6 ± 0.7
Surfactant treatment				
Donor lung		3.6 ± 0.2 R	5.8 ± 0.3 L	5.9 ± 0.1 L
Native lung	3.6 ± 0.3		5.3 ± 0.5	5.6 ± 0.2

PC, Phosphatidylcholine; PL, phospholipids; R, right donor lung after ischemia; L, transplanted graft (left donor lung).

Given are the relative concentrations of PC, phosphatidylglycerol, phosphatidylinositol, and sphingomyelin (in % of total PL), the relative content of palmitic acid (16:0) in percent (w/w) of all phosphatidylcholine fatty acids, and the relative content of plasmalogens in PC for the donor lung and the native lung in the standard treatment and the surfactant-treated group. Phospholipids with a relative distribution lower than 1.0% under baseline conditions (lysophosphatidylcholine, cardiolipin, phosphatidylethanolamine, phosphatidylserine) are not given. The mean ± SEM of 6 independent experiments is given.

* $P < .05$, † $P < .01$, comparison between untreated and treated organs.

storage (Table 3). In accordance, the minimum surface tension ($\gamma_{\min}$) was consistently found to range below 5 mN/m in these samples (Figure 2).

In contrast, transplantation of the graft and reperfusion over 12 hours resulted in far-reaching alterations of the

surfactant system. The total concentration of phospholipids was reduced in the 6-hour BALF obtained by bronchoscopy, as was the relative amount of LSA (Table 1). Due to the markedly increased alveolar protein values, the phospholipid/protein ratio was decreased by nearly 1 order of

Figure 1. Relative amount (in percent of all phosphatidylcholine molecular species, *left panel*) and absolute concentration (*right panel*) of dipalmitoylated phosphatidylcholine (DPPC) molecular species from BALF of treated and untreated grafts and native lungs during reperfusion over 12 hours. Depicted are the mean $\pm$ SEM of 6 independent experiments. * $P < .05$, ** $P < .01$, comparison between untreated and treated organs. The 0-hour values of the native lung represent the baseline values (excised lung from the recipient) and the 0-hour values of the graft represent the postischemia values (obtained by bronchoalveolar lavage [BAL] of the nontransplanted lung at the end of the ischemia period).

magnitude (Table 1). The relative amount of SP-B and SP-C within the LSA fraction was found to be reduced by $\sim 50\%$ within 6 hours after reperfusion (Table 3). At 12 hours after onset of reperfusion, some of these changes had partially resolved (PL, LSA, SP-C, SP-B).

Concerning the phospholipid profile, only minor changes were encountered upon reperfusion of the ischemic lungs, including a significant reduction in phosphatidylglycerol and a small increase in phosphatidylinositol and sphingomyelin (Table 2). Although the relative concentration of PC did not change over the entire reperfusion period, profound alterations of the fatty acid and molecular species profile of PC were observed, with a marked decline in the degree of palmitoylation of this phospholipid (see Figure 1 and Tables 2 and 4). In detail, the percentage of palmitic acid within the PC class was reduced from 65% under baseline conditions to $\sim 50\%$ after 12 hours of reperfusion ($P < .001$, Table 2). Concomitantly, unsaturated PC species, mostly oleic acid (18:2) and arachidonic acid (20:4), were increased (data not shown). These changes were even more prominent for the dipalmitoylated PC (DPPC, Figure 1 and Table 4): the relative amount of DPPC (given as % of total PC) was reduced from $\sim 44\%$ under baseline conditions to 30% after

6 hours ($P < .001$) and maintained at this low level over the rest of the reperfusion period. Instead, 16:0/18:1, 16:0/18:2, 16:0/20:4, and 16:0/trans18:1 species increased and entirely compensated for this loss of DPPC. As a result, the absolute DPPC quantities in the lavage fluid, reflecting changes in the molecular species profile as well as total changes of PL and PC concentrations, were reduced by $>50\%$ (Figure 1). The relative concentrations of PC plasmalogens slightly increased during the reperfusion period (see Table 2).

The surface activity of LSA from the graft progressively deteriorated during reperfusion. The minimum surface tension ($\gamma_{\min}$) increased from ~ 2 mN/m at baseline in ischemic but nonperfused lungs to ~ 15 mN/m after 6 hours and to ~ 16 mN/m after 12 hours of reperfusion (Figure 2, *left panel*). In addition, a further increase in the $\gamma_{\min}$ values was encountered upon reconstitution of the LSA fraction with the isolated and concentrated supernatant proteins originating from the same lavage (reconstitution performed at the same ratio as observed in original BALF): $\gamma_{\min}$ values then increased from 3 mN/m (baseline) to ~ 21 mN/m after 6 hours and ~ 26 mN/m after 12 hours of reperfusion (Figure 2, *right panel*). Reductions in PC palmitic acid content were significantly correlated with the increase in

TABLE 3. Concentration of SP-B and SP-C in large surfactant aggregates

	Baseline	0 hours post ischemia	6 hours post reperfusion	12 hours post reperfusion
SP-B (% of PL)				
Standard treatment				
Donor lung		1.22 ± 0.30 R	0.58 ± 0.12 L	1.06 ± 0.34 L
Native lung	1.02 ± 0.17		0.97 ± 0.21	0.90 ± 0.23
Surfactant treatment				
Donor lung		1.24 ± 0.27 R	1.30 ± 0.25 L*	1.84 ± 0.55 L
Native lung	1.17 ± 0.30		1.53 ± 0.21	1.65 ± 0.07*
SP-C (% of PL)				
Standard treatment				
Donor lung		0.72 ± 0.21 R	0.15 ± 0.19 L	0.29 ± 0.15 L
Native lung	0.52 ± 0.09		0.33 ± 0.15	0.82 ± 0.31
Surfactant treatment				
Donor lung		0.69 ± 0.09 R	1.29 ± 0.17 L*	1.88 ± 0.25 L†
Native lung	0.42 ± 0.05		0.82 ± 0.10*	1.25 ± 0.46

SP-B, Surfactant apoprotein B; SP-C, surfactant apoprotein C; PL, phospholipids; R, right donor lung after ischemia; L, transplanted graft (left donor lung). Concentrations of the hydrophobic surfactant proteins SP-B and SP-C in large surfactant aggregates (in % of phospholipids) for the donor lung and the native lung in the standard treatment and the surfactant-treated group. Baseline = explanted left recipient lung. The mean ± SEM of 6 independent experiments is given. The 0-hour values of the native lung represent the baseline values (excised lung from the recipient) and the 0 hours of the graft represent the postischemia values (obtained by bronchoalveolar lavage [BAL] of the nontransplanted lung at the end of the ischemia period).

* $P < .05$; † $P < .01$, comparison between treated and untreated organs.

minimum surface tension in LSA of non-surfactant-treated animals (Figure 3).

In contrast to the transplanted lung, the native lung displayed, at best, minor alterations of surfactant characteristics. There was, for example, a slight but nonsignificant increase in the minimum surface tension at 6 and 12 hours. The phospholipid profile, the fatty acid profile, and the molecular species pattern of PC, as well as the concentrations of SP-B and SP-C, remained within the normal range in the native lungs of the animals receiving a contralateral graft (Tables 1 to 4, Figures 1 and 2).

Transbronchial application of 50 mg/kg BW of a calf lung surfactant extract (Alveofact) only into the donor lung prior to ischemia and of another 200 mg/kg BW into both lungs directly after onset of reperfusion resulted in a pronounced improvement of gas exchange and lung compliance (data not shown), which was accompanied by a far-reaching restoration of the biochemical and biophysical surfactant properties. In parallel to a marked increase in the total BALF phospholipid content, reflecting the alveolar deposition of the exogenous surfactant, a normalization of the phospholipid/protein ratio and the relative amount of LSA (Table 1) was noted. The relative content of PC palmitic acid (Table 2), the molecular species profiles (Figure 1 and Table 4), and the relative content of the hydrophobic apoproteins in the LSA fraction (Table 3) in lungs post-surfactant administration approached those measured in the exogenous surfactant material (data not given in detail). The surface activity was largely improved (Figure 2): $\gamma_{\min}$ values of LSA originating from the graft at the end of the reperfusion period were ~ 5 mN/m after surfactant

therapy, as compared with ~ 16 mN/m in the absence of this intervention (Figure 2, *left*). In addition, due to the far-reaching restoration of physiological phospholipid/protein ratios, the additional impact of protein inhibition was far less pronounced, with graft $\gamma_{\min}$ values measuring ~ 15 mN/m instead of ~ 26 mN/m after 12 hours of reperfusion (Figure 2, *right*).

Discussion

As outlined in detail in the accompanying article,^{15a} transplantation and reperfusion of canine lungs that had been stored for 24 hours at 4°C provoked a severe reperfusion injury, with increased endothelial and epithelial permeability, plasma protein leakage into the alveolar space, increased neutrophil influx into this compartment, progressive deterioration of gas exchange, and loss of lung compliance. We show here that, in parallel, severe disturbances of the surfactant system are encountered, with loss of adsorption facilities and minimal surface tension-lowering properties. As with the physiological abnormalities, these surfactant alterations were largely restricted to the transplanted lung and were obvious within 6 hours of reperfusion. Lung storage at 4°C itself did not provoke substantial changes of the surfactant system. The transbronchial administration of overall 250 mg/kg BW calf lung surfactant extract normalized biochemical surfactant properties and nearly fully restored physiological surfactant function and at the same time prevented the loss of gas exchange properties and compliance of the transplanted lung.

The present study spent particular effort in the detailed analysis of the surfactant abnormalities occurring in the

TABLE 4. Molecular species of phosphatidylcholine

	Baseline	0 hours post ischemia	6 hours post reperfusion	12 hours post reperfusion
16:0/16:0 (% of total)				
Standard treatment				
Donor lung		40.5 ± 2.1 R	29.9 ± 2.9 L	28.4 ± 1.3 L
Native lung	43.3 ± 1.7			37.5 ± 2.3
Surfactant treatment				
Donor lung		40.9 ± 1.4 R	30.1 ± 1.6 L	29.4 ± 1.7 L
Native lung	44.5 ± 5.2			
16:0/16:0 PC (total, µg/mL)				
Standard treatment				
Donor lung		92.8 ± 14.2 R	22.6 ± 10.9 L	40.6 ± 6.0 L
Native lung	58.1 ± 8.1			49.8 ± 14.8
Surfactant treatment				
Donor lung		78.6 ± 17.5 R	113.9 ± 22.4 Lt	219.7 ± 65.5 L*
Native lung	62.3 ± 15.2			
16:0/16:1 (% of total)				
Standard treatment				
Donor lung		12.8 ± 0.8 R	9.9 ± 1.3 L	10.9 ± 0.7 L
Native lung	12.8 ± 0.5			12.1 ± 0.4
Surfactant treatment				
Donor lung		8.2 ± 1.0 R*	10.9 ± 1.7 L	10.4 ± 1.0 L
Native lung	11.3 ± 2.4			
16:0/18:1 (% of total)				
Standard treatment				
Donor lung		6.5 ± 0.2 R	12.5 ± 3.0 L	8.2 ± 0.8 L
Native lung	6.3 ± 0.3			6.3 ± 0.5
Surfactant treatment				
Donor lung		8.1 ± 0.9 R	22.4 ± 4.5 L	18.1 ± 3.8 Lt
Native lung	6.0 ± 1.4			
16:0/18:2 (% of total)				
Standard treatment				
Donor lung		4.7 ± 0.5 R	6.2 ± 0.7 L	6.0 ± 0.5 L
Native lung	4.5 ± 0.5			4.8 ± 0.4
Surfactant treatment				
Donor lung		4.2 ± 0.8 R	5.9 ± 0.6 L	5.3 ± 0.3 L
Native lung	4.1 ± 0.7			
16:0/trans 18:1 (% of total)				
Standard treatment				
Donor lung		10.8 ± 0.5 R	10.4 ± 3.0 L	16.7 ± 1.1 L
Native lung	10.4 ± 0.5			12.9 ± 1.5
Surfactant treatment				
Donor lung		12.2 ± 0.8 R	8.6 ± 2.6 L	7.2 ± 1.8 L*
Native lung	11.1 ± 0.9			
16:0/14:0 (% of total)				
Standard treatment				
Donor lung		9.2 ± 0.5 R	7.3 ± 0.6 L	7.5 ± 0.5 L
Native lung	9.4 ± 0.3			8.5 ± 0.3
Surfactant treatment				
Donor lung		8.5 ± 0.3 R	7.7 ± 0.8 L	9.1 ± 1.7 L
Native lung	9.1 ± 0.3			

14:0, Myristic acid; 16:0, palmitic acid; 16:1, palmitoleic acid; 18:1, oleic acid; *trans*18:1, elaidic acid; 18:2, linoleic acid. R, right donor lung after ischemia; L, transplanted graft (left donor lung). Given are the relative (in % of all molecular species) or the absolute (in µg/mL BAL) concentration of major PC molecular species of the donor lung and the native lung in the standard treatment and the surfactant-treated group. Baseline = explanted left recipient lung. Mean ± SEM of 6 independent experiments is given. For reasons of complexity of the molecular species analysis, no measurements were undertaken for 6-hour reperfusion in the native lung (surfactant and standard treatment) and 12-hour reperfusion (surfactant treatment).

* $P < .01$, † $P < .05$, comparison between untreated and treated organs.

Figure 2. Minimum surface tension of large surfactant aggregates in absence (*left panel*) and in presence of proteins (*right panel*) from treated and untreated grafts and native lungs during reperfusion over 12 hours. Depicted are the mean $\pm$ SEM of 6 independent experiments. Reconstitution with bronchoalveolar lavage–derived proteins was performed as detailed in the Methods section. Surface activity was assessed in the Pulsating Bubble Surfactometer at 2 mg/mL phospholipid always. $^*P < .05$, $^{***}P < .001$, comparison between untreated and treated organs; $^{++}P < .01$, $^{+++}P < .001$, comparison between baseline and the untreated donor lung. The 0-hour values of the native lung represent the baseline values (excised lung from the recipient) and the 0-hour values of the graft represent the postischemia values (obtained by BAL of the nontransplanted lung at the end of the ischemia period).

Figure 3. Correlation of all data sets of relative palmitic acid concentration in PC and minimum surface tension, as assessed in the non-surfactant-treated lungs.

transplanted lungs, by measuring a variety of most relevant surfactant components hitherto unanalyzed in reperfused organs. The following mechanisms underlying the impaired surfactant function were identified:

1. Decrease in the large surfactant aggregate fraction. As shown in pulse chase studies,²³ the alveolar surfactant pool is composed of two major subfractions, LSA and the small surfactant aggregates (SSA), with the LSA representing the highly surface active precursor fraction of the interfacial surfactant film²⁴ and the SSA representing the much less surface active "degradation products."^{25,26} During reperfusion, a marked decrease of LSA was noted in the graft, nicely fitting observations in previous studies.^{10,13,14,27,28} However, in some of these studies a corresponding decline in the LSA fraction was similarly observed in the native lung of the recipient, which was not the case in the current investigation. The underlying reason for this difference may be the fact that separate, synchronized ventilation of each lung was performed, thus avoiding overinflation of the native lung due to the decreasing compliance of the transplanted organ. It is in accordance with such reasoning that recent studies provided direct evidence for accelerated conversion of LSA to SSA in vivo under conditions of high tidal volume ventilation strategies.²⁹ However, it may not be deduced from the present findings whether the decrease of LSA in the transplanted lungs was due to reduced surfactant synthesis in the type II pneumocytes or due to enhanced breakdown of this surface active fraction (eg, by proteolytic attack).
2. Altered surfactant composition. The biochemical alterations of the surfactant material retrieved from the graft after 6 and 12 hours of reperfusion included an impressive decline of the hydrophobic apoproteins SP-B and SP-C in the LSA fraction, an altered phospholipid profile with decreased percentages of phosphatidylglycerol and increased percentages of phosphatidylinositol and sphingomyelin, a pronounced reduction of the degree of palmitoylation of PC, and, even more prominent, a decrease of DPPC within the molecular species of PC. Only some of these variables, all of them being relevant for surfactant function, have previously been addressed in lung ischemia-reperfusion studies,^{12,13,28} and again changes were then encountered both in the graft and the native lung under conditions of combined mechanical ventilation, whereas the biochemical alterations were largely restricted to the transplanted lung in the present investigation. Overall, the profile of biochemical surfactant changes is reminiscent of "immature" surfactant in preterm infants, which may suggest but does not definitely prove loss of metabolic capacity of the post-

ischemic type II pneumocytes. The relevance of the biochemical surfactant alterations for the noted disturbances in surfactant function is underscored by the fact that surface activity and the degree of palmitoylation of PC were found to be significantly correlated (Figure 3), as were relative DPPC, SP-B, and SP-C levels and surface tension lowering properties (data not shown).

3. Inhibition of surfactant function by proteins. As previously shown in ARDS patients,⁴ inhibition of surfactant function by BALF-derived proteins was directly demonstrated by reconstitution of LSA with the proteins of the individual BALF supernatant, at the same ratio as observed in the original BALF. Under these conditions, the surface tension properties further deteriorated, both regarding minimum surface tension and adsorption facilities. Thus, protein inhibition is apparently a potent surfactant inhibitory mechanism in lungs undergoing ischemia-reperfusion injury. Interestingly, increased minimum surface tension values have recently also been described for clinically asymptomatic lung transplant recipients in the further time course after transplantation, suggesting a persisting disturbance of the alveolar surface tension regulation.³⁰

The endobronchial administration of 50 mg/kg BW of Alveofact, an organic solvent extract with excellent surface activity when tested in vitro or in animal studies, into the donor lung prior to ischemia, followed by another 200 mg/kg BW being bronchoscopically distributed in both lungs after reperfusion onset, resulted in a significant improvement of arterial oxygenation (see companion article^{15a}). The present study demonstrates that this is paralleled by an impressive restoration of surfactant properties, with respect to both the biochemical and the biophysical variables. In parallel to a marked increase in total BALF phospholipid content, reflecting the alveolar deposition of the exogenous surfactant, a normalization of the phospholipid/protein ratio and the relative amount of LSA was noted, and the fatty acid profiles, the molecular species pattern, and the percentages of the hydrophobic apoproteins in the LSA fraction in the lungs post-surfactant administration approached those measured in the exogenous surfactant material (especially in view of the low percentage of DPPC in Alveofact (~33%; own unpublished observations), which reflects the overall low abundance of this molecular species in calf lung surfactant.³¹

The surface activity was largely improved but not fully normalized in the presence of the surfactant inhibitory proteins originating from the individual lavage sample. Despite the fact that a marked improvement of surfactant properties was achieved by the surfactant replacement regimen, two aspects deserve particular consideration:

1. There was a three- to fivefold increase in the lavagable phospholipid pool in response to surfactant administration. However, the amount of administered surfactant material (250 mg/kg BW) surpassed the endogenous surfactant pool (10-15 mg/kg BW) by at least a factor of 15! In healthy adult rabbits, an alveolar turnover time of PC of only 5 to 10 hours has previously been reported³² and, upon intratracheal injection of labeled surfactant material, the overall loss from the airspaces and the lung reached 90% and 70%, respectively, after 24 hours.³³ Although clearance studies have not been conducted in lungs undergoing ischemia-reperfusion injury, it thus seems reasonable to assume that a substantial quantity of the exogenously administered surfactant material was cleared from the lung within a few hours. It may be speculated that some of this material may reenter the alveolar compartment due to recycling via type II cells, which might explain the surprising finding that both in the graft and in the recipient native lungs, the 12-hour values of total lavagable phospholipids surpassed the 6-hour values.
2. The minimum surface tension values in the presence of the individual BALF proteins were markedly improved but not fully normalized in the surfactant-treated grafts, though all investigated biochemical variables and the phospholipid/protein ratio were in physiological ranges. A possible explanation for this finding is the fact that SP-A is lacking in the bovine surfactant preparation used in this study, and this hydrophilic apoprotein is known to enhance protein resistance of natural surfactant preparations.³⁴ In addition, changes in the neutral lipid profile, which was not analyzed in this study, might contribute to impairment of surface activity.

In conclusion, severe abnormalities of the alveolar surfactant system were noted after single dog lung transplantation. These abnormalities were restricted to the graft with virtually normal surfactant function in the recipient native lung under conditions of separate but synchronized ventilation of each lung to attenuate ventilator-induced lung injury. The surfactant changes in the graft included loss of the surface active large aggregate fraction, reduction of most relevant surfactant components within this fraction (surfactant apoproteins [SP]-B and SP-C, dipalmitoyl-phosphatidylcholine [DPPC], phosphatidylglycerol), and surfactant inhibition by proteinaceous material. Bronchoscopic surfactant administration fully normalized the biochemical and largely improved the biophysical surfactant properties, alongside maintenance of lung gas exchange properties. Surfactant therapy may thus offer protection against reperfusion injury in lung transplantation.

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