Surfactant function in lung transplantation after 24 hours of ischemia: Advantage of retrograde flush perfusion for preservation

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Objective: Surfactant function was shown to be impaired in clinical and experimental lung transplantation. This study was designed to define the impact of retrograde flush perfusion on graft and surfactant function after an extended period of ischemia.

Methods: Left lung transplantation was performed after 24 hours of graft ischemia in 12 pigs. In half of the grafts antegrade cold flush perfusion (Perfadex) was used for preservation. In the second group grafts were flushed in a retrograde fashion via the left atrium. Graft function was monitored for 7 hours after transplantation. Before transplantation (basal) and after 2 hours of reperfusion, bronchoalveolar lavage fluid was obtained. Minimal surface tension of bronchoalveolar lavage fluid was determined and the ratio of small and large surfactant aggregates was calculated. Lung water content was analyzed online in the reperfusion period.

Results: Right-sided heart failure developed in 2 animals of group 1 (antegrade perfusion) within 2 and 4.5 hours of reperfusion, respectively. All other pigs survived the observation period. Po_2/Fio_2 (P = .001) and dynamic lung compliance (P = .001) were superior in retrogradely flushed grafts. A comparable increase of minimal surface tension was found after reperfusion in both groups. Small/large surfactant aggregate ratio after reperfusion (P = .03), as well as extravascular lung water content, was higher in the antegrade perfusion group.

Conclusion: Retrograde flush perfusion for 24-hour lung preservation with low-potassium dextran (Perfadex) solution led to better initial graft function than the standard antegrade perfusion technique. A moderate impairment of surfactant function was found in both groups, which was more pronounced in the antegrade perfusion group.

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Reperfusion injury is one of the most frequent causes of early death[1] after pulmonary allograft transplantation. The incidence of reperfusion injury has been reported to range from 20% to 40% in different lung transplant programs.[2] The requirement of extracorporal support was reported in 7.4% of 215 recipients after lung transplantation.[3] In addition, the problem of reperfusion injury led to a highly

selective acceptance of pulmonary grafts: only about 10% of organ donors are considered suitable donors for pulmonary transplantation. Therefore, numerous studies have been undertaken to enhance function of the reperfused graft to improve postoperative lung function and to widen acceptance criteria for pulmonary grafts. One of the findings is the importance of endothelial and alveolar type II cell integrity after transplantation.[4] A comparison of an extracellular preservation solution, such as low-potassium dextran (LPD) solution, with the currently used solutions of intracellular ion composition (Euro-Collins, University of Wisconsin), revealed less impairment of endothelial function[5,6] and improved metabolic activity of type II pneumocytes.[7] First clinical results favor the use of LPD solution for lung preservation.[8] A different approach to improve preservation technique was the development of retrograde perfusion. In this study both developments are combined for long-term preservation (24 hours). The study was designed to compare lung function and surfactant activity after preservation with LPD solution either as an antegrade or a retrograde flush perfusion during the reperfusion period. The hypothesis of this study is a further improvement of graft function by the retrograde perfusion technique.

Materials and Methods

Experimental Groups

Pigs were randomized into 2 groups of 6 animals each to compare the effect of 2 different preservation techniques. In the first group lungs were preserved by antegrade flush perfusion of LPD solution (Perfadex; Vitrolife, Göteborg, Sweden). In the second group lungs were perfused in a retrograde fashion with the same preservation solution. Twelve additional animals served as recipients of these grafts for left lung transplantation.

Surgical Preparation

Donor preparation

First group. Female pigs (22-30 kg) were anesthetized with sodium pentobarbital (10 mg/kg) and fentanyl (1 mg/kg followed by a fentanyl infusion of 1 $mg \cdot kg^{-1} \cdot h^{-1}$). The animals were intubated and ventilated with 100% of oxygen (inspiratory/expiratory ratio = 1:1, positive end-expiratory pressure = 5 mm Hg) in a pressure-controlled mode with a maximum airway pressure of 20 mm Hg and a ventilation rate of 10 breaths/min. A median sternotomy was performed and the pericardium was cut open. A catheter to infuse preservation solution was inserted into the main pulmonary artery. Heparin (3 mg/kg) was administered intravenously. Cardiac arrest was induced by clamping of the aorta and cardioplegic solution was infused into the aortic root. The venae cavae vere clamped. Thereafter, the auricle of the left atrium was incised and flush perfusion of the pulmonary artery induced. In 10 minutes 1000 mL of LPD solution were administered at a temperature of 8°C. No alprostadil (prostaglandin E) infusion was used in the donor or the recipient. Ventilation of the lung was maintained through the flush procedure. The heart and lungs were excised en bloc. Care was taken to clamp the main left bronchus, leaving the left lung in a mildly inflated state. The left lung was isolated from the heart-lung block with a generous atrial cuff and full length of both the pulmonary artery and main bronchus. The lung was stored at 4°C for 24 hours.

Second group. The preparation was identical but for the perfusion procedure. A 2-lumen catheter to infuse LPD solution and to measure left atrial pressure was placed at the auricle into the left atrium and tied. After cardiac arrest and clamping of the venae cavae, perfusion was started in a retrograde fashion and was drained through an incision of the main pulmonary artery. Care was taken not to exceed a left atrial pressure of 12 mm Hg. A 10-minute period was required to infuse 1000 mL of LPD solution. Ventilation was maintained though the perfusion procedure.

Recipient preparation. Female pigs (22-30 kg) were anesthetized with sodium pentobarbital (10 mg/kg) and fentanyl (1 mg/kg, followed by a fentanyl infusion of 1 $mg \cdot kg^{-1} \cdot h^{-1}$). The animals were intubated and ventilated with 50% oxygen (inspiratory/expiratory ratio = 1:1, positive end-expiratory pressure = 5 mm Hg) in a pressure-controlled mode (frequency = 10 breaths/min, maximum pressure = 20 mm Hg). A Swan-Ganz catheter (7.5F; Baxter Healthcare Corp, Irvine, Calif) and a catheter to monitor arterial pressure were placed into the right carotid artery and internal jugular vein, respectively. A catheter for monitoring of the extravascular lung water was inserted into a femoral artery. A left thoracotomy in the fifth intercostal space was performed, and the pericardium was opened. The left pulmonary artery, the tracheal bifurcation, and the pulmonary veins were dissected. Umbilical tapes were applied to the right and left pulmonary arteries and the right main bronchus. Heparin (3 mg/kg) was administered intravenously. After clamping of the left main bronchus and the left pulmonary artery, the left pulmonary veins were ligated. Pneumonectomy of the left lung was performed. A clamp was placed onto the left atrium so as to close the left pulmonary veins. The upper and lower pulmonary veins were incised with the atrial tissue between them to open an atrial cuff. Ventilation was continued and the donor lung was implanted starting with a bronchial end-to-end anastomosis with 4-0 running Prolene suture (Ethicon, Inc, Somerville, NJ). Anastomosis of the left atrial cuffs of the donor and recipient were performed followed by the left pulmonary arteries. Before reperfusion, the graft was deaired by retrograde perfusion. The pulmonary artery was unclamped and the graft ventilated. After 10 minutes of reperfusion, the right pulmonary artery and main right bronchus were clamped. Both umbilical tapes were tied. Experiments were terminated by means of a pentobarbital overdose after 7 hours of reperfusion or when systolic arterial pressure fell below 40 mm Hg. Use of inotropic substances was not permitted in the experiments.

Measurements of Lung Function and Extravascular Lung Water Index

In all experiments, atrial as well as arterial and pulmonary artery pressures were recorded online. Dynamic lung compliance was monitored continuously with a modified ventilator (Dräger, Lübeck, Germany). Arterial blood gas analyses were performed after placement of catheters and every 30 minutes during reperfusion. At these intervals pulmonary vascular resistance was calculated after measurement of cardiac output by means of the transfemoral thermodilution catheter, which was connected to the cardiac output computer

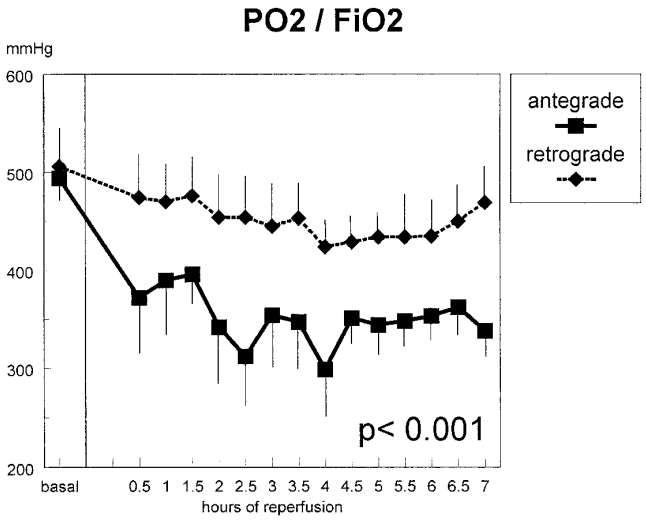

Figure 1. Po_2/Fio_2 of miniature pigs before single lung transplantation (basal) and after 0.5 to 7 hours of reperfusion after single lung transplantation and clamping of the contralateral lung. Grafts were preserved with LPD solution by antegrade versus retrograde flush perfusion and stored for 24 hours.

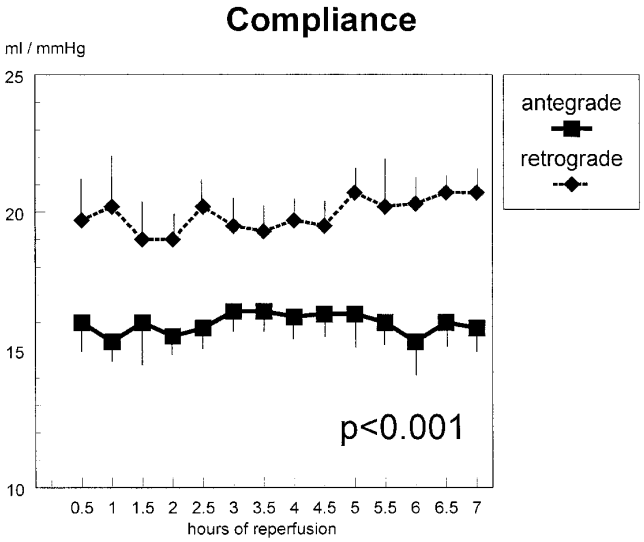

Figure 2. Dynamic lung compliance 0.5 to 7 hours after single left lung transplantation and clamping of the contralateral lung. Grafts were preserved with LPD solution by antegrade versus retrograde flush perfusion and stored for 24 hours.

(Picco; Pulsion Medical Systems AG, Munich, Germany). After being calibrated by injection of cold saline solution into the jugular vein, the system calculated cardiac output online by pressure curve analysis.[9] By means of the same thermal bolus injection that is distributed into intravascular blood volume and extravascular lung water, the extravascular lung water index (arithmetic value over kilograms of body weight) was computed.[10]

Surfactant Analysis

Bronchoalveolar lavage (BAL) fluid was obtained from the right lower lobe in all experiments after catheter placement with 100 mL of saline solution. A second BAL was performed after 2 hours of reperfusion of the left lower lobe. A differential cell count was carried out to determine the number of polymorphonuclear leukocytes as a percentage of the total cell count. The lavage was immediately centrifuged at 150*g* and the cell-free supernatant was frozen at –80°C. Pellet and supernatant were separated at 48,000*g* for 60 minutes. Protein and phospholipid content were determined. Phospholipids in the small aggregate–containing supernatant and in the large aggregate pellet were determined by phosphorus analysis and their weight expressed as a small aggregate/large aggregate (SA/LA) ratio. The surfactant pellet was resuspended in saline solution supplemented with calcium chloride, 1.5 mmol/L. Surfactant function was determined with a pulsating bubble surfactometer (Electronetics, inc, Buffalo, NY) according to the technique described by Enhorning[11]: 40 mL of large aggregate suspension, which had been adjusted to a phospholipid concentration of 1 mg/mL, was infused into the sample chamber. The surface tension at minimal bubble size (γ_{min}) was obtained after 5 minutes of bubble pulsation at a rate of 20 cycles/min and a temperature of 37°C. Before bubble pulsation was started, the initial surface tension after bubble formation was measured and the adsorption rate was determined as surface tension 10 seconds after formation of a bubble. All analog data were digitalized and recorded by a personal computer.

Animal Care

All animals received humane care in 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" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996.

Statistical Analysis

All data are expressed as mean ± SE. Continuous data were analyzed by repeated-measures analysis of variance. For data without repeated measurement, analysis of variance was applied. All data were analyzed with the Statistical Program of Social Sciences (SPSS for MS Windows Version 6.1; SPSS, Inc, Chicago Ill).

Results

Right-sided heart failure developed in 2 animals of group 1 (antegrade perfusion) within 2 and 4.5 hours of reperfusion, respectively. All other pigs survived the observation period of 7 hours. After 30 minutes of reperfusion, a decrease in Po_2/Fio_2 from 490 ± 35 mm Hg (before transplantation) to 372 ± 45 mm Hg (Figure 1) was found in group 1. Thereafter, the Po_2/Fio_2 values remained in the range of 300 to 400 mm Hg. In group 2 (retrograde perfusion) initial Po_2/Fio_2 was comparable with pretransplantation values (475 ± 40 mm Hg) and remained between 400 and 480 mm Hg during the reperfusion period. Po_2/Fio_2 was higher in

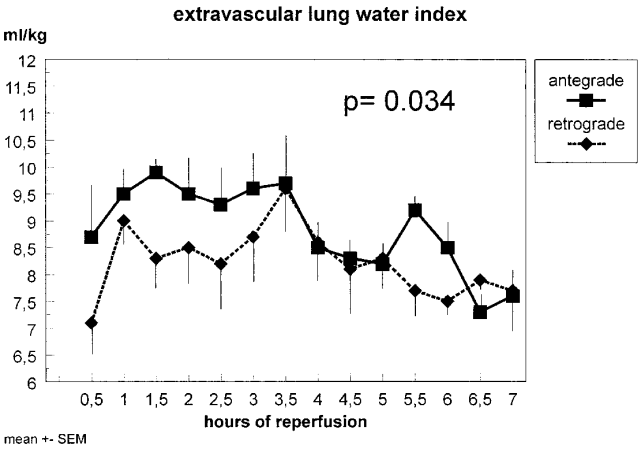

Figure 3. Extravascular lung water index 0.5 to 7 hours after single left lung transplantation and clamping of the contralateral lung. Grafts were preserved with LPD solution by antegrade versus retrograde flush perfusion and stored for 24 hours.

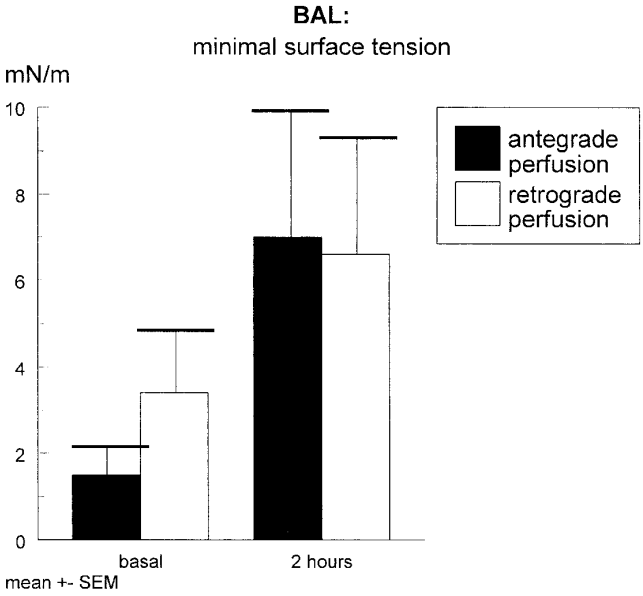

Figure 4. Minimal surface tension of the BAL fluid in milliNewtons per meter, as determined with a bubble surfactometer. Results are shown before flush perfusion of the right lung (basal) and after 2 hours of reperfusion of the transplanted left lung.

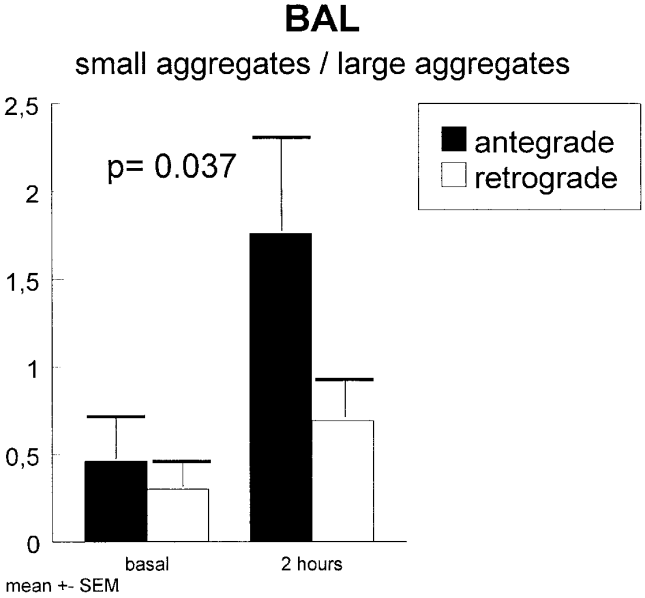

Figure 5. SA/LA ratio of BAL fluid. Results are shown before flush perfusion of the right lung (basal) and after 2 hours of reperfusion of the transplanted left lung. A significant increase (*P* = .037) was found after reperfusion (2 hours) in the antegrade group.

retrogradely perfused grafts (group 2; $P < .001$). Initial dynamic lung compliance of group 1 revealed stable values of 15 to 16.5 mL/mm Hg throughout the reperfusion period. Animals with retrogradely preserved grafts were found to have slightly increasing compliance at a higher level (Figure 2; $P < .001$). In animals of group 2 (retrograde perfusion), at basal conditions (before clamping of the left lung for pneumectomy) the pulmonary vascular resistance was 657 ± 630 dynes · s^{-1} · cm^{-5}. At early reperfusion of the transplanted lung and ligation of the contralateral pulmonary artery, a 2-fold increase of pulmonary vascular resistance to 1365 ± 573 dynes · s^{-1} · cm^{-5} was calculated. The pulmonary vascular resistance of this group remained stable for the observation period. The data of group 1 (antegrade) were similar at basal conditions (454 6 293 dynes · s^{-1} · cm^{-5}) and at early reperfusion (1565 ± 722 dynes · s^{-1} · cm^{-5}). They also remained at the same level throughout the reperfusion period. The initial extravascular lung water index was lower ($P = .034$) in the retrogradely preserved grafts (Figure 3). There was an increasing tendency in both groups until comparable values were calculated after 3.5. hours of reperfusion. Thereafter, the extravascular lung water index decreased in both groups during the later follow-up period. The analysis of surfactant function revealed low minimal surface tension before transplantation in both groups. After 2 hours of reperfusion a moderate but comparable ($P = .07$) increase of surface tension was seen (Figure 4). The SA/LA ratio was at a low level in the 2 groups before transplantation (Figure 5). A more than 3-fold increase ($P = .037$) after 2 hours of reperfusion was found in the recipients of antegradely perfused grafts. In contrast, the values in the retrogradely preserved animals remained in the range of pretransplant findings. The protein content of the BAL fluid (Table 1) revealed a tendency toward higher values after reperfusion in both groups, which was more pronounced in the antegrade perfusion group. However, this finding did not reach statistical significance

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TABLE 1. Protein content and neutrophil count of BAL fluid (mean ± SEM)

	Protein count (mg/mL)		Neutrophil count (%)	
	Before perfusion	After transplantation	Before perfusion	After transplantation
Basal	188 ± 33	152 ± 27	1.0 ± 0.45	26.8 ± 11.4
Two hours of perfusion	971 ± 595	452 ± 136	5.5 ± 2.5	11.0 ± 5.0

Protein count (mg/mL) and number of neutrophils (percent of total cell count of BAL fluid). Results are shown before flush perfusion (basal) and after transplantation and 2 hours of reperfusion. In the antegradely perfused group, increasing (P = .009) neutrophil count was obtained after reperfusion.

(P = .1 antegrade; P = .5 retrograde). A similar pattern was seen with regard to neutrophil count: the relative number of neutrophils in the BAL fluid increased after reperfusion in the antegradely preserved group (P = .009) but not in the retrogradely perfused group (P = .54).

Discussion

Since lung transplantation became a clinical reality, hundreds of clinical and experimental studies have been conducted to increase ischemic time and enhance primary graft function. The introduction of flush perfusion with Euro-Collins solution allowed for clinical lung preservation 6 to 8 hours after organ harvest.[12] It has been successfully applied for more than a decade and has become the most often used method of lung preservation. However, reperfusion injury in the early post-transplant period remains a significant problem in lungs preserved with Euro-Collins solution.[13] Recently, experimental work showed that use of an LPD solution ameliorates reperfusion injury and improves primary graft function in lung transplantation.[14] Furthermore, the surfactant function of reperfused grafts was found to be well preserved, in contrast to results with other crystalloid preservation solutions. This finding is of major importance, because surfactant function is impaired in clinical lung transplantation[15] and exogenous surfactant substitution may be required. Clinical use of LPD solution supported the laboratory findings of less reperfusion injury[8] and improvement of primary graft function.[16]

In contrast to hundreds of articles dealing with modified composition of preservation solutions, little effort has been given to the route of administration: topical cooling was used for the first successful single lung transplants by the Toronto group.[17] Thereafter, antegrade pulmonary perfusion was established for the majority of clinical procedures.[18] However, only a few data exist regarding retrograde flush perfusion. Such a route of preservation was established for other solid organs, such as use of retrograde cardioplegia in heart surgery.[19] In an experimental study, Varela and coworkers[20] found an improvement of distribution of pulmonary flush perfusion, especially to the tracheobronchial wall, when flushing Euro-Collins solution retrogradely via the left atrium.

The aim of the present study is 2-fold: (1) to determine the effect of long-term preservation (24 hours) with LPD solution on surfactant function and (2) to define possible advantages by retrograde perfusion technique with respect to early graft and surfactant function.

A limitation of this study is the short post-transplant observation time. Results of BAL fluid and surfactant analysis represent only the early reperfusion period. The reason for early bronchoscopy was to evaluate a high number of animals, since a high survival was not expected. However, single lung transplantation with clamping of the contralateral lung is a challenging task for the preservation procedure and reveals the quality of the graft during the 4- to 6-hour reperfusion period.[21] In addition, compared with other preparations of ex vivo perfused lungs, the interaction of pulmonary circulation, reperfusion, and right-sided heart function resembles the clinical situation of single lung transplantation.

Our findings suggest that antegrade flush perfusion with LPD solution does not offer safe long-term lung preservation in the miniature pig model. Primary graft failure followed by right-sided heart failure was present in 2 of 6 experiments. In the retrogradely perfused group, oxygenation and compliance were better than in the antegradely perfused grafts. In addition, no sign of respiratory or cardiac insufficiency was seen throughout the observation period. In an earlier study, Steen and associates[21] demonstrated safe 24-hour preservation with LPD solution in a similar model using a different perfusion technique: flush perfusion with warm solution was carried out through the pulmonary and bronchial arteries followed by topical cooling of atelectatic lungs. A possible explanation of the advantage of both warm antegrade perfusion and retrograde flush is a more homogeneous distribution of the perfusion solution. Another study showed that flush perfusion via the pulmonary and bronchial arteries was associated with higher flow rates than isolated pulmonary artery perfusion or retrograde perfusion.[22] This study emphasized that distribution to the airway tissues was improved by combined pulmonary and bronchial artery perfusion. However, data on graft function were not available.

In terms of surfactant function, our study revealed a moderate impairment of surface tension in the BAL fluid of transplanted and reperfused grafts of both groups. In the

antegradely perfused grafts this finding was associated with a reduction of dynamic lung compliance. In addition, SA/LA ratio was increased after reperfusion in antegradely preserved lungs, indicating a decrease of surface-active large aggregates and an increase of inactive small aggregates. In contrast, the SA/LA ratio in the retrogradely preserved group was remarkably lower. The increase of SA/LA in the antegradely perfused group reflects an accelerated conversion of surfactant aggregates into less active subtypes. This might be promoted by enzymes leaking into the alveoli during the protein-rich reperfusion edema. This hypothesis is supported by the findings of increased lung water index in the early reperfusion period and the tendency toward higher protein content of BAL fluid in this group. In addition, the finding of higher inflow of polymorphonuclear leukocytes into the airways suggests higher inflammatory activity in the reperfusion period of antegradely preserved lungs associated with an increased SA/LA conversion. The slight reduction of surfactant function in the retrogradely preserved group without concomitant increase of SA/LA ratio is suggestive of a better preservation of the surfactant system after lung transplantation. Type II pneumocytes are very susceptible to ischemic injury.[7] Therefore, a reduction of surfactant release or enhanced conversion of surfactant large aggregate to small aggregate forms may be sequelae of long-term ischemia. Additional measurements of surfactant protein levels would further enlighten our understanding of surfactant composition and function in the early reperfusion period. In animal experiments,[23,24] as well as in clinical cases,[25] early postoperative graft dysfunction was successfully improved by administration of surfactant preparations.

In summary, cold antegrade flush perfusion with LDP solution, which is the most frequent clinical application, does not reveal safe 24-hour preservation of graft and surfactant function in our experiments. In contrast, retrograde application of cold LPD solution led to appropriate initial graft function after 24 hours of ischemia with less vascular leakage and inflammatory response in the pulmonary circulation compared with antegradely perfused grafts. In addition, in this group the disturbance of surfactant was less pronounced.

Since a body of evidence has been established to prefer LPD solution in lung preservation, retrograde perfusion of this solution should be considered. Long-term preservation of the lung may impair surfactant function by ischemic injury to pneumocytes and should be avoided in clinical lung transplantation.

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