Exogenous surfactant therapy in thirty-eight hour lung graft preservation for transplantation

Previous work in our laboratory has documented alterations in surfactant composition and function after prolonged lung graft storage and transplantation in dogs (Am Rev Respir Dis 1993;148:208-15). To determine whether exogenous surfactant therapy was beneficial, we pretreated 13 canine double lung blocks with prostacyclin, flushed them with 4° C modified Euro-Collins solution, and stored them at 4° C for 37 to 38 hours. After left lung transplantation and immediately before reperfusion, eight dogs were administered 50 mg of bovine lung lipid extract surfactant per kilogram (50 mg/ml) directly into the left main bronchus and five served as nontreated control animals. Blood gases, peak inspired pressures, and individual pulmonary artery blood flows were measured every 30 minutes during 6 hours of reperfusion. The native right and transplanted left lungs were then lavaged and surfactant large and small aggregates and protein yields were analyzed. All nontreated animals had physiologic evidence of severe ischemia-reperfusion lung injury during reperfusion. Three of eight dogs treated with bovine lung lipid extract surfactant had near normal lung function at 6 hours of reperfusion, as reflected by maintenance of an oxygen tension/inspired oxygen fraction ratio of more than 400 mm Hg and a normal carbon dioxide tension. Five of eight dogs did not respond to surfactant therapy and had decreases in gas exchange identical to those of the control animals. Blood flow through the left pulmonary artery was maintained in the three animals that responded to exogenous surfactant, whereas flow significantly decreased to the left lung in all other animals, reflecting the patterns of gas exchange. In addition, the ratio of poorly functioning small surfactant aggregates to the well-functioning large aggregates isolated from lung lavage after 6 hours of reperfusion was decreased in surfactant-treated animals, especially in those exhibiting a beneficial physiologic response to surfactant therapy. We conclude that therapy with bovine lung lipid extract surfactant can result in excellent preservation of lung grafts after prolonged storage and transplantation, but that the results are not consistent. Further investigations are required to determine the factors responsible for the differential response to surfactant therapy. (*J THORAC CARDIOVASC SURG* 1994;108:259-68)

Richard J. Novick, MD,^a Ruud A. W. Veldhuizen, PhD,^b Fred Possmayer, PhD,^c
John Lee, MD,^a David Sandler, MD,^a and James F. Lewis, MD,^b *London, Ontario, Canada*

From the Division of Cardiovascular-Thoracic Surgery, University Hospital, and the Robarts Research Institute^a; the Department of Medicine, Division of Respiriology, St. Joseph's Health Centre^b; and the Departments of Biochemistry and Obstetrics and Gynecology and the MRC Group in Fetal and Neonatal Health and Development, University of Western Ontario,^c London, Ontario, Canada. Supported by grants from the Canadian Cystic Fibrosis Foundation, the Heart and Stroke Foundation of Ontario, and the Medical Research Council of Canada.

Received for publication Dec. 17, 1993.

Accepted for publication April 7, 1994.

Address for reprints: Richard J. Novick, MD, Division of Cardiovascular-Thoracic Surgery, University Hospital, P.O. Box 5339, London, Ontario N6A 5A5, Canada.

Copyright © 1994 by Mosby-Year Book, Inc.

0022-5223/94 \$3.00 + 0 12/1/56620

During the past decade, surfactant replacement therapy has become well established in the treatment of premature infants with respiratory distress syndrome.¹ Furthermore, the alterations in endogenous surfactant that occur in a variety of experimental lung injury models have been characterized² and the changes in surfactant composition in patients with the adult respiratory distress syndrome (ARDS) have been documented.³⁻⁶ Preliminary work has shown the beneficial effect of exogenous surfactant therapy in patients with ARDS and sepsis.^{7,8}

Paralleling this progress in surfactant research has been the emerging role of lung transplantation in the treatment of end-stage respiratory failure. Since the initial clinical successes of a decade ago, donor and recipi-

Table I. Baseline physiologic data in control and surfactant-treated dogs

	Controls	Surfactant-treated
PO ₂ /FiO ₂ ratio (mm Hg)	534 ± 13	519 ± 18
PCO ₂ (mm Hg)	35 ± 3	38 ± 2
Peak airway pressure (cm H ₂ O)	18 ± 2	17 ± 1
Left PA blood flow (ml/min)	340 ± 48	315 ± 31
Right PA blood flow (ml/min)	698 ± 104	663 ± 42
Donor weight (kg)	22 ± 1	21 ± 1
Recipient weight (kg)	21 ± 1	21 ± 1
Warm ischemic time (min)	42 ± 2	46 ± 2
Cold ischemic time (hr)	37.4 ± 0.1	37.4 ± 0.1
Total ischemic time (hr)	38.1 ± 0.1	38.2 ± 0.1

Values are expressed as means ± standard error of the mean. $p > 0.05$ for all values between groups. *Warm ischemic time*, The time to complete the anastomoses during the lung transplant operation; *Cold ischemic time*, the storage interval of the lung graft.

ent selection, operative technique, and postoperative care have been refined, and a significant improvement in survival has resulted.⁹ At present the major limitation to clinical lung transplantation is the shortage of suitable donor organs. Graft preservation times are also limited and pulmonary dysfunction after transplantation is not uncommon.¹⁰ Just as in the lung injury in patients with ARDS, ischemia-reperfusion pulmonary injury is due to a complex interplay of various pathogenic mechanisms. These include leukocyte and platelet activation, oxygen-derived free radical formation, the complement cascade, and the generation of inflammatory mediators.¹⁰ Techniques that increase the duration of lung preservation or improve the posttransplantation function of lung grafts could have a major impact on clinical lung transplantation.

We¹¹ have previously characterized alterations in surfactant composition and function after prolonged lung graft storage and transplantation in dogs. These changes were similar to those recently reported in patients with ARDS.^{2,5,6} The purpose of the present study was to determine whether instillation of exogenous surfactant into the airway after a prolonged interval of lung preservation prevented the deterioration in pulmonary function typically observed during reperfusion. In addition, the physiologic responses noted during reperfusion were correlated with the changes in alveolar surfactant structural forms and alveolar lavage protein levels measured after surfactant treatment.

Methods

Animal preparation. Left single lung transplantation was performed in conditioned, 20 to 23 kg mongrel dogs, as de-

scribed previously.^{11,12} Donor and recipient animals were premedicated with xylazine, 1 mg/kg, and atropine, 0.4 mg/kg. Anesthesia was induced with thiopental sodium, 12 mg/kg, and maintained with 1% halothane. Neuromuscular blockade was achieved with pancuronium, 0.1 mg/kg, which was repeated at necessary intervals. After intubation with a cuffed endotracheal tube, volume-cycled mechanical ventilation was commenced with a Bear ventilator (Bear Medical Systems Inc., Riverside, Calif.) with a tidal volume of 20 ml/kg, a rate of 16 breaths/min, a fraction of inspired oxygen (FiO₂) of 1.0, and a positive end-expiratory pressure (PEEP) of 5 cm H₂O. All operations were performed by the same surgeon and the total duration of the lung transplant procedure did not differ significantly between experimental groups. All animals received humane care in accordance with the "Principles of Laboratory Animal Care" (National Society for Medical Research), the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health Publication No 86-23, revised 1985), and the specifications of the Council on Animal Care of the University of Western Ontario.

Donor operation. After median sternotomy and anterior pericardiectomy, the superior and inferior venae cavae, ascending aorta, pulmonary artery (PA), and trachea were mobilized. Heparin sodium, 300 units/kg, was administered, and a 15-minute infusion of prostacyclin (Burroughs-Wellcome Inc., Kirkland, Quebec, Canada) was begun at 0.1 µg/kg per minute through a right atrial catheter. The dose of prostacyclin was subsequently adjusted to maintain a steady-state infusion that reduced the systolic arterial pressure by 40%.¹² The donor animals then underwent PA flushing with 4° C modified Euro-Collins solution, 60 ml/kg. The heart was excised, followed by the double lung block. The double lung block was stored inflated to total lung capacity immersed in a 4° C saline bath.

Recipient operation. Thirty-six to 37 hours after the donor operation, weight-matched recipient dogs underwent a left posterolateral thoracotomy through the fifth intercostal space. The right and left PAs were each encircled with heavy silk ties for subsequent snaring during blood gas measurements from isolated lungs.^{11,12} In addition, ultrasonic flow probes (Transonic Inc., Ithaca, N.Y.) were placed around both PAs so that the individual PA blood flows could be continuously monitored during and after the operation. The chest was then closed with towel clips, with a small opening left through which the PA snares could be manipulated. The animals were placed in the supine position and baseline arterial blood gases were measured at an FiO₂ of 1.0 on a Corning 178 pH/blood gas analyzer (Ciba Corning Diagnostic Ltd., Halstead, Essex, England). The PAs were then sequentially snared to obtain baseline blood gas values for the left lung (right PA snared) and right lung (left PA snared) after 10 minutes of occlusion. The chest was then reopened, and a left pneumonectomy was performed.

After a 37- to 38-hour storage interval, the double lung block was removed from its 4° C saline bath. The right lung was separated from the double lung block and lavaged through the main bronchus with 1 L of 0.9% sodium chloride containing calcium chloride, 1.5 mmol/L. This procedure was repeated three additional times and the total lavage volume was recorded and analyzed as described below.

Surfactant treatment and reperfusion. After lavage of the right lung, the left atrial cuff, PA, and bronchus of the donor left lung were anastomosed to the recipient. Just before completion of the bronchial anastomosis, a No. 16 Foley catheter was

inserted through the suture line just beyond the proximal left main bronchial clamp. Animals randomized to the surfactant treatment group ($n = 8$) were administered 50 mg bovine lipid extract surfactant per kilogram body weight (bLES, bLES Biochemicals, London, Ontario, Canada, 50 mg/ml) via the Foley catheter, followed by 20 ml of room air. Care was taken to insert the catheter only into the proximal left main bronchus, and after the instillation of air it was verified that all three lobes of the transplanted left lung had received exogenous surfactant. Control animals ($n = 5$) were administered only 20 ml of air. The Foley catheter was then removed and the bronchial suture was tied. Full reinflation of the left lung graft was timed with removal of the left bronchial clamp. Immediately thereafter, the left PA clamp was removed, initiating reperfusion. The chest was closed with towel clips, the animal placed in the supine position, and baseline postoperative values of arterial blood gases, peak inspired pressure, and individual PA blood flows were recorded. The tidal volume was adjusted to maintain an initial carbon dioxide tension (PCO_2) of 37 to 43 mm Hg, and the F_{IO_2} was adjusted to maintain an arterial oxygen tension (PO_2) in the 100 to 200 mm-Hg range. The ventilatory parameters were then kept constant throughout the experiment. PO_2/F_{IO_2} ratio, PCO_2 , peak inspired pressure, and individual PA blood flows were recorded at 30-minute intervals during 6 hours of reperfusion. At the end of reperfusion, the PAs were again sequentially snared while the animals were ventilated with an F_{IO_2} of 1.0. Isolated blood gases were obtained for the left lung (right PA snared) and right lung (left PA snared). The animals were then killed with an overdose of sodium pentobarbital. The heart was vented by incising the left atrial appendage, inferior vena cava, and main PA, and the trachea was clamped at end inspiration. The double lung block, consisting of the transplanted left lung and native right lung, was excised, and each lung was lavaged separately, similar to the donor lavage procedure described in the previous section.

Lung lavage analysis. Total lavage volume from each lung was centrifuged at 150 g for 10 minutes at 4° C to remove tissue and cellular debris. The 150 g supernatant was centrifuged for 15 minutes at 40,000 g (4° C) to obtain a pellet representing the large surfactant aggregates.¹³ The small aggregates remained in the 40,000 g supernatant. The pellet was resuspended in 0.9% sodium chloride containing calcium chloride, 1.5 mmol/L. Lipid extracts of the large and small surfactant aggregates were prepared by chloroform:methanol extraction according to the method of Bligh and Dyer.¹⁴ Total phospholipid was determined by phosphorus analysis of aliquots of the lipid extracts by the method of Rouser, Fleischer, and Yamamoto.¹⁵ The supernatant of the 40,000 g centrifugation was examined for total protein by the method of Lowry and associates¹⁶ with bovine serum albumin used as a standard.

Statistical analysis. Results were expressed as means $\pm$ standard error of the mean. Statistical analysis included the use of sequential analysis of variance with Dunnett's post hoc tests within each experimental group to determine the statistical significance of variables recorded during reperfusion, as compared with baseline values after transplantation. In addition, two-way analysis of variance testing was performed to compare results between experimental groups at each time interval. The Crunch Statistical Software Program (Oakland, Calif.) was used. A p value less than 0.05 was considered significant.

Results

All donor animals exhibited normal arterial blood gases before lung procurement. Baseline physiologic data for all experimental animals are shown in Table I. No statistically significant differences were detected between control and surfactant-treated dogs under baseline conditions. Immediately after transplantation, the PO_2/F_{IO_2} ratio was greater than 350 mm Hg in all animals, indicating that the transplant operation per se did not significantly impair gas exchange.

Fig. 1 shows the PO_2/F_{IO_2} data for all experimental animals. All animals survived the transplant procedure and at least 270 minutes of reperfusion. Two surfactant-treated dogs died suddenly of ventricular fibrillation at 290 and 340 minutes of reperfusion, having had a significant decrease in PO_2 in the hours preceding their death. All 11 other animals survived until they were killed 6 hours after reperfusion. The PO_2/F_{IO_2} values of the five control animals were consistent and were expressed as mean values over time. The PO_2/F_{IO_2} ratio decreased significantly over time, starting 60 minutes after reperfusion ($p < 0.01$). Surfactant-treated dogs, on the other hand, followed two distinct patterns. Measurements in five surfactant-treated animals were similar to those in control animals, with a decrease in PO_2/F_{IO_2} that also reached statistical significance starting at 60 minutes compared with the initial posttransplantation value ($p < 0.05$). PO_2/F_{IO_2} values of these animals continued to decrease from 90 to 360 minutes of reperfusion ($p < 0.01$). Three other surfactant-treated dogs exhibited a significant decrease in PO_2/F_{IO_2} values relative to the initial posttransplantation value at 120 ($p < 0.05$), 150 ($p < 0.01$), and 180 minutes ($p < 0.05$). The PO_2/F_{IO_2} of these three dogs then increased significantly, and after 6 hours of reperfusion the mean PO_2/F_{IO_2} was 490 ± 46 mm Hg, which was not significantly different from baseline postoperative values. Because of these results, we have arbitrarily chosen to divide the surfactant-treated animals into two separate groups for subsequent analysis: the three "surfactant responders" and the five "surfactant nonresponders."

The individual oxygenation of each lung was assessed at the end of 6 hours of reperfusion by means of the PA snaring technique, as shown in Table II. Baseline values were similar among experimental groups. By 6 hours of reperfusion, the PO_2 in the transplanted lung had decreased significantly in the control animals and surfactant nonresponders, whereas it was not significantly different from pretransplantation baseline values in the surfactant responders. The PO_2 of the native right lung in the control animals and surfactant nonresponders also decreased significantly with reperfusion, whereas oxygen-

Fig. 1. PO_2/FiO_2 ratio of control dogs (mean $\pm$ standard error of the mean) and each surfactant-treated dog. Three surfactant-treated animals had near normal oxygenation by the end of reperfusion. TX, Transplantation.

Table II. Comparison of PO_2 between experimental groups with individual PA snaring (FiO_2 1.0)

	Experimental group		
	Control	Surfactant nonresponder	Surfactant responder
Transplanted lung (right PA snared)			
Pretransplantation PO_2 (mm Hg)	520 $\pm$ 27	513 $\pm$ 37	537 $\pm$ 26
Postreperfusion PO_2 (mm Hg)	65 $\pm$ 16*	57 $\pm$ 12*	425 $\pm$ 57†
Native lung (left PA snared)			
Pretransplantation PO_2 (mm Hg)	504 $\pm$ 11	533 $\pm$ 27	556 $\pm$ 43
Postreperfusion PO_2 (mm Hg)	227 $\pm$ 75‡	182 $\pm$ 63‡	477 $\pm$ 89

Values are expressed as means $\pm$ standard error of the mean.

* $p < 0.0001$ versus pretransplantation baseline.

† $p < 0.0001$ versus control and surfactant nonresponder groups.

‡ $p < 0.01$ versus pretransplantation baseline.

ation of the native lung in surfactant responders did not significantly change.

The ventilation efficiency of control and surfactant-treated animals is shown in Fig. 2. No significant differences in PCO_2 or peak airway pressure were observed under baseline conditions. The PCO_2 in control animals increased significantly compared with values immediately after transplantation starting at 150 minutes

($p < 0.01$), and it continued to increase thereafter. Similarly, the surfactant nonresponders showed a significant increase in PCO_2 by 90 minutes of reperfusion ($p < 0.05$), with a continued increase from 120 to 360 minutes ($p < 0.01$). On the other hand, the surfactant responders showed no significant change in PCO_2 over time. Starting at 210 minutes after reperfusion, the PCO_2 values were significantly lower in the surfactant responders than in the other two groups. Concomitant with these changes in PCO_2 , the peak airway pressure increased significantly, compared with the initial posttransplantation value, in control animals (starting at 150 minutes) and in the surfactant nonresponders (starting at 120 minutes). The peak airway pressure in the surfactant responders did not change significantly over time. Fig. 2, A and B indicates that the trend toward lower peak airway pressure in the surfactant responders occurred despite significantly lower PCO_2 values in this group from 210 to 360 minutes of reperfusion, implying more optimal ventilation efficiency in the surfactant responders.

Pulmonary blood flow. No significant differences in preoperative right and left pulmonary blood flow were observed among the groups (see Table I). During reperfusion, there was a progressive decrease in left PA blood flow in control animals, which was statistically significant by 360 minutes of reperfusion compared with the initial posttransplantation value (Fig. 3). The left PA blood flow tended to decrease in the surfactant nonresponders, although the decrease was not statistically significant by

Fig. 2. Changes in PCO₂ (A) and peak inspired pressure (PIP) (B) during reperfusion (mean $\pm$ standard error of the mean). TX, Transplantation.

360 minutes compared with the initial posttransplantation value. Although surfactant responders also exhibited a trend toward a decreased left PA blood flow early after the operation, blood flow then tended to increase toward and beyond baseline values later in reperfusion. By 5 hours of reperfusion, left PA blood flow was significantly higher in surfactant responders than in the other two groups ($p = 0.007$).

Lung lavage analysis. Fig. 4 shows the protein yield in the 40,000 *g* supernatant fraction of the lung lavages. Donor lungs contained a relatively small amount of protein in this fraction, despite the prolonged storage interval. After transplantation, a large increase in protein occurred in the lavage of both the transplanted and native lungs in control animals. Surfactant therapy resulted in a trend toward a lower protein yield after 6 hours of reperfusion ($p = 0.13$, control versus surfactant-treated animals). Surfactant responders tended to have less protein than nonresponders in both the transplanted and native lungs after 6 hours of reperfusion.

Fig. 5 shows the ratio of small to large surfactant aggregates in stored donor lungs and in the transplanted left and native right lungs of recipient animals after 6 hours of reperfusion. The small/large surfactant aggregate ratio was low in donor lungs, and this ratio increased significantly after transplantation in control animals, consistent with the results of our previous work.¹¹ The exogenous surfactant that we used (bLES) consisted mainly of surface-active large aggregates (small/large

aggregate ratio = 0.04). Six hours after surfactant administration, the small/large aggregate ratio in the eight treated lungs averaged 0.34 ± 0.08 , compared with 1.21 ± 0.49 in control animals ($p = 0.05$). There was a trend toward a lower small/large aggregate ratio in the transplanted and native lungs of the surfactant responders ($p = 0.12$ and 0.10 , respectively) than in the nonresponders.

Discussion

The long-term preservation of lung grafts remains a major limiting factor in clinical lung transplantation. Moreover, the delicate alveolar-capillary membrane network of the lung is susceptible to ischemia-reperfusion injury.¹⁰ The suspected pathophysiologic mechanisms involved in this injury include leukocyte and platelet activation, with the generation of inflammatory mediators and oxygen free radicals.¹⁰ We¹¹ have previously characterized the alterations of the endogenous surfactant system in lung-injured dogs after pulmonary transplantation. In that study, lung grafts were stored for 12 hours before reperfusion. Significant impairment of gas exchange was present during reperfusion and was associated with abnormal composition of isolated alveolar surfactant. Specifically, sphingomyelin content was increased and phosphatidylglycerol and surfactant-associated protein A levels were decreased. These changes were similar to those observed in other animal models of lung injury, as well as in patients with ARDS.^{2,6,17}

Fig. 3. Changes in left pulmonary blood flow (as a percentage of pretransplantation baseline) during reperfusion (mean $\pm$ standard error of the mean). TX, Transplantation.

Therefore, because exogenous surfactant administration has been effective in various animal models of lung injury² and more recently has shown promise in the treatment of patients with sepsis and ARDS,^{7,8} we tested this therapeutic modality in experimental lung transplantation.

We chose a graft storage interval of 38 hours on the basis of a series of pilot experiments. These experiments evaluated the effects of various factors on graft survival. For example, when prostacyclin was administered to donor animals and harvested lungs were stored for 12 hours, significant improvements in oxygenation occurred after transplantation compared with responses noted when donor prostacyclin therapy was not used.¹² This modification in the experimental protocol, as well as using 5 cm H₂O PEEP in recipient animals and avoiding repetitive snaring of each PA during the 6 hours of reperfusion, resulted in adequate gas exchange in animals receiving transplanted lungs that had been stored for up to 24 hours. We therefore chose the prolonged storage interval of 38 hours to specifically assess the physiologic effects of exogenous surfactant in this model. The severity of the lung injury induced in these animals after prolonged graft storage was reflected by the marked deterioration in gas exchange over the 6 hours of reperfusion in all animals not receiving exogenous surfactant therapy. The eight animals that were administered exogenous

surfactant fell into two distinct groups. Five of the eight animals had a progressive deterioration in gas exchange and responded similarly to the nontreated, control animals. On the other hand, three animals, which had identical baseline physiologic parameters and surfactant instillation procedures, had virtually normal gas exchange at 6 hours of reperfusion. Although these three animals had an initial deterioration in oxygenation during the first 3 hours of reperfusion, gas exchange significantly recovered during the subsequent 3 hours.

The observation that the surfactant response was variable despite similar physiologic parameters before treatment is not unique. Previous studies have shown that a significant number of neonates of similar gestational age with respiratory distress syndrome did not respond to exogenous surfactant.^{1,18} Moreover, several animal models of lung injury have also shown inconsistent results after the administration of exogenous surfactant.² Determining the factors causing the differential response to exogenous surfactant therapy in our study is of considerable importance. To understand the variability of surfactant response, it is helpful to consider the mechanism by which exogenous surfactant may result in physiologic improvement after lung injury. By reducing surface tension within the lung, exogenous surfactant administration results in an increase in lung volume at a lower distending pres-

Fig. 4. Protein yield in lung lavages from donor right lungs (*DONOR*), transplanted left lungs (*TRANSPLANT*), and native right lungs (*NATIVE*) of recipient animals.

sure. The three animals that responded to exogenous surfactant in our study presumably had adequate lung volumes during mechanical ventilation over the 6 hours of reperfusion. This was supported by the finding of increased blood flow to the lungs of these animals compared with the nonresponders (see Fig. 3). With more optimal ventilation-perfusion matching within the lung, oxygenation was maintained during reperfusion. On the other hand, ineffective exogenous surfactant (as in the nonresponders) would result in decreased pulmonary perfusion and, consequently, suboptimal ventilation-perfusion matching and poor gas exchange.

A clinically important issue, therefore, is to ascertain why the exogenous surfactant was ineffective in maintaining lung volume in the nonresponders. One potential factor could be that the lung injury was more severe in these animals than in the responders, despite there being no difference between animals in the measured physiologic parameters before treatment. It is possible that the nature of the lung injury became different only after 2 to 3 hours of reperfusion. For example, the initial deterioration in oxygenation in all animals over the first 3 hours of reperfusion may have been due to a certain degree of pre-existing lung damage from the prolonged storage interval. Inflammatory mediators and toxic oxygen radicals present within the lung before the administration of exogenous surfactant would then begin to exert their dele-

terious effects. Subsequently, the exogenous surfactant was able to mitigate the progression of lung injury in some animals and restore normal gas exchange. Recent work has confirmed that reperfusion after a prolonged interval of pulmonary ischemia results in significant cytokine release in lung effluent.¹⁹ Other studies have demonstrated that surfactant and its components can downregulate cytokine production and the inflammatory response.^{20, 21} Specifically, surfactant has been shown to inhibit cytokine release from activated monocytes²⁰ and alter the inflammatory activity of alveolar macrophages.²¹ It is possible that animals in this model of lung injury had variable expression of cytokines during the reperfusion period and that this would account for the differential response to exogenous surfactant. Further studies in which specific cytokines and inflammatory cells are measured over the course of reperfusion are required. In addition, the potential benefit of administering exogenous surfactant earlier, possibly into the donor lung before storage, should be investigated. Such an approach may prevent the deterioration in gas exchange that was seen early in reperfusion, even in the surfactant responder group.

One of the paramount factors determining the efficacy of surfactant administered for replacement therapy is the homogeneity of surfactant distribution.² Gattinoni and associates²² have documented the heterogeneity of lung

Fig. 5. Surfactant small/large aggregate ratio in donor right lungs (*DONOR*), transplanted left lungs (*TRANSPLANT*), and native right lungs (*NATIVE*) of recipient animals.

injury in patients with ARDS. Moreover, we^{2, 23, 24} have shown that the underlying pattern of lung injury may affect the distribution of exogenous surfactant. Further studies with radiolabeled surfactant preparations are currently being performed in our laboratory to document the individual lobar distribution of exogenous surfactant in a canine single lung transplant model.

Another potential factor limiting the response to surfactant in this model may have been how the exogenous surfactant was metabolized once deposited within the injured lung. Previous studies have shown that endogenous alveolar surfactant metabolism was altered in acute lung injury.^{2, 11, 25} The ratio of poorly functioning small surfactant aggregates to well-functioning large aggregates was significantly higher than in noninjured, control animals. In a subsequent study, when animals were treated with a large dose of exogenous surfactant, the ratio of isolated alveolar surfactant aggregates differed in normal versus injured animals.²⁶ These results suggested not only that the exogenous surfactant was differentially metabolized within the injured lung compared with the normal lung, but also that these metabolic differences significantly influenced lung function. In the present study, we found a trend toward an increased ratio of small to large surfactant aggregates in the nonresponders as compared with the responders (see Fig. 5). This increased ratio indicated increased small aggregate forms within the airway and potentially poorer functioning surfactant in the nonresponders. Although the difference in the

small/large aggregate ratio between responders and nonresponders was not statistically significant ($p = 0.12$), we believe that this difference may be physiologically important given the large quantity of exogenous surfactant instilled into the lungs (50 mg lipid per kilogram) compared with the endogenous surfactant pool size (10 mg lipid per kilogram). It is possible that a longer reperfusion time would have shown the difference in aggregate ratios to be statistically significant.

A final factor that may have compromised the response to exogenous surfactant in this experiment was the specific surfactant preparation used to treat these animals. Although bLES has been beneficial in multicenter trials of neonates with respiratory distress syndrome,^{27, 28} it is possible that it may not be optimal for the complicated injury in adult lungs. bLES does not contain the major surfactant-associated protein, SP-A. This protein has been shown to decrease serum protein inhibition of surfactant²⁹ and to alter the uptake and secretion of surfactant from type II cells.^{30, 31} Furthermore, we¹¹ have previously shown that SP-A was decreased in donor lungs that had been stored for 12 hours before reperfusion. Future work characterizing SP-A levels in lungs stored for prolonged intervals is necessary. In addition, studies evaluating the effectiveness of administering bLES supplemented with SP-A would be informative.

In summary, we have shown that storage of lung grafts for 38 hours followed by transplantation and reperfusion results in severe lung dysfunction in animals not treated

with surfactant. When exogenous surfactant was administered into the transplanted lung immediately before reperfusion, we were able to restore normal gas exchange in three of eight dogs. Although the surfactant response was inconsistent and the factors responsible for the inconsistencies remain to be determined, we believe that these results are provocative and require further study. Issues including the optimal timing of surfactant therapy, the ideal mode of surfactant delivery, the distribution and metabolism of the exogenous surfactant, and the optimal surfactant preparation to use in this model will be investigated. Understanding these issues will lead to the possibility of prolonged graft storage intervals and the mitigation of ischemia-reperfusion injury in lung transplantation.

We thank Jenifer Duplan, Lynn Denning, Simon Ledingham, Kevin Inchley, and Kathy McDougall for their excellent technical assistance. The prostacyclin used in the lung donors was supplied by Mr. Sylvain Rocheleau of the Burroughs-Wellcome Company, Montreal, Quebec, Canada. The surfactant administered to treated animals was supplied by bLES Biochemicals (London, Ontario, Canada). Heather Motloch provided expert assistance in manuscript preparation.

REFERENCES

1. Jobe A, Ikegami M. Surfactant for the treatment of respiratory distress syndrome. *Am Rev Respir Dis* 1987; 136:1256-75.
2. Lewis JF, Jobe AH. Surfactant and the adult respiratory distress syndrome. *Am Rev Respir Dis* 1993;147:218-33.
3. Hallman M, Spragg R, Harrell JH, Moser KM, Gluck L. Evidence of lung surfactant abnormality in respiratory failure: study of bronchoalveolar lavage phospholipids, surface activity, phospholipase activity, and plasma myoinositol. *J Clin Invest* 1982;70:673-83.
4. Rauvala H, Hallman M. Glycolipid accumulation in bronchoalveolar space in adult respiratory distress syndrome. *J Lipid Res* 1984;25:1257-62.
5. Pison U, Seeger W, Buchhorn R, et al. Surfactant abnormalities in patients with respiratory failure after multiple trauma. *Am Rev Respir Dis* 1989;140:1033-9.
6. Gregory TJ, Longmore WJ, Moxley MA, et al. Surfactant chemical composition and biophysical activity in acute respiratory distress syndrome. *J Clin Invest* 1991;88:1976-81.
7. Weg J, Reines H, Balk R, et al. Safety and efficacy of an aerosolized surfactant (Exosurf^R) in human sepsis-induced ARDS. *Chest* 1991;100:137S.
8. Wiedemann H, Baughman R, de Boisblanc B, et al. A multicenter trial in human sepsis-induced ARDS of an aerosolized synthetic surfactant (Exosurf). *Am Rev Respir Dis* 1992;145:A184.
9. Trulock EP, Cooper JD, Kaiser LR, et al. The Washington University-Barnes Hospital experience with lung transplantation. *JAMA* 1991;266:1943-6.
10. Novick RJ, Menkis AH, McKenzie FN. New trends in lung preservation: a collective review. *J Heart Lung Transplant* 1992;11:377-92.
11. Veldhuizen RAW, Lee J, Sandler D, et al. Alterations in pulmonary surfactant composition and activity after experimental lung transplantation. *Am Rev Respir Dis* 1993; 148:208-15.
12. Novick RJ, Reid KR, Denning L, Duplan J, Menkis AH, McKenzie FN. Prolonged preservation of canine lung allografts: the role of prostaglandins. *Ann Thorac Surg* 1991;51:853-9.
13. Veldhuizen RAW, Inchley K, Hearn SA, Lewis JF, Possmayer F. Degradation of surfactant-associated protein B (SP-B) during in vitro conversion of large to small surfactant aggregates. *Biochem J* 1993;295:141-7.
14. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959;37: 911-7.
15. Rouser G, Fleischer S, Yamamoto A. Two dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. *Lipids* 1970;5:494-6.
16. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin reagent. *J Biol Chem* 1951;193:265-75.
17. Holm BA, Matalon S. Role of pulmonary surfactant in the development and treatment of adult respiratory distress syndrome. *Anesth Analg* 1989;69:805-18.
18. Horbar JD, Soll RF, Sutherland JM, et al. A multicenter randomized, placebo-controlled trial of surfactant therapy for respiratory distress syndrome. *N Engl J Med* 1989; 320:959-65.
19. Palace GP, del Vecchio PJ, Horgan MJ, Malik AB. Release of tumor necrosis factor after pulmonary artery occlusion and reperfusion. *Am Rev Respir Dis* 1993;147:143-7.
20. Speer CP, Götze B, Curstedt T, Robertson B. Phagocytic functions and tumor necrosis factor secretion of human monocytes exposed to natural porcine surfactant (Curosurf). *Pediatr Res* 1991;30:69-74.
21. Thomassen MJ, Meeker DP, Antal JM, Connors MJ, Wiedemann HP. Synthetic surfactant (Exosurf) inhibits endotoxin-stimulated cytokine secretion by human alveolar macrophages. *Am J Respir Cell Mol Biol* 1992;7:257-60.
22. Gattinoni L, Pesenti A, Bombino M, et al. Relationships between lung computed tomographic density, gas exchange and PEEP in acute respiratory failure. *Anesthesiology* 1988;69:824-32.
23. Lewis JF, Ikegami M, Jobe AH, Absolom D. Physiological responses and distribution of aerosolized surfactant (Surfanta^R) in a nonuniform pattern of lung injury. *Am Rev Respir Dis* 1993;147:1364-70.
24. Lewis JF, McCaig L. Aerosolized versus instilled exoge-

- nous surfactant in a nonuniform pattern of lung injury. *Am Rev Respir Dis* 1993;148:1187-93.
25. Lewis JF, Ikegami M, Jobe AH. Altered surfactant function and metabolism in rabbits with acute lung injury. *J Appl Physiol* 1990;69:2303-10.
 26. Lewis JF, Ikegami M, Jobe AH. Metabolism of exogenously administered surfactant in the acutely injured lungs of adult rabbits. *Am Rev Respir Dis* 1992;145:19-23.
 27. Enhorning G, Shennan A, Possmayer F, Dunn M, Chen CP, Milligan J. Prevention of neonatal respiratory distress syndrome by tracheal instillation of surfactant: a randomized clinical trial. *Pediatrics* 1985;76:145-53.
 28. Dunn MS, Shennan AT, Possmayer F. Single versus multiple-dose surfactant replacement therapy in neonates of 30 to 36 weeks' gestation with respiratory distress syndrome. *Pediatrics* 1990;86:564-71.
 29. Cockshutt AM, Weitz J, Possmayer F. Pulmonary surfactant-associated protein A enhances the surface activity of lipid extract surfactant and reverses inhibition by blood proteins in vitro. *Biochemistry* 1990;29:8424-9.
 30. Wright JR, Wager RE, Hawgood S, Dobbs L, Clements JA. Surfactant apoprotein $M_r = 26,000$ -36,000 enhances uptake of liposomes by type II cells. *J Biol Chem* 1987;262:2888-94.
 31. Kuroki Y, Mason RJ, Voelker DR. Pulmonary surfactant apoprotein A structure and modulation of surfactant secretion by rat alveolar type II cells. *J Biol Chem* 1988;263:3388-94.

Bound volumes available to subscribers

Bound volumes of THE JOURNAL OF THORACIC AND CARDIOVASCULAR SURGERY are available to subscribers (only) for the 1994 issues from the Publisher, at a cost of \$82.50 for domestic, \$106.28 for Canadian, and \$100.50 for international subscribers for Vol. 107 (January-June) and Vol. 108 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue of the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby-Year Book, Inc., Subscription Services, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone 1 (800) 453-4351 or (314) 453-4351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.