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INHALED NITRIC OXIDE AFFECTS ENDOGENOUS SURFACTANT IN EXPERIMENTAL LUNG TRANSPLANTATION

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Background. **Inhalation of nitric oxide (NO) has been proposed as a therapy to improve lung transplantation outcome. We investigated the effect that inhaled NO has on the surfactant system in the context of ischemia-reperfusion injury.**

Methods. **Single left-lung transplantation was performed in weight-matched pairs of Landrace pigs. A double-lung block from the donor animal was flushed with University of Wisconsin solution at 4°C followed by immersion in cold University of Wisconsin solution for 22 hr. The left donor lung was transplanted into the recipient. Recipients were divided into two groups: (1) treated with inhaled NO (40 ppm) (n=6) immediately after initiating lung reperfusion and (2) without treatment (n=6). Lung function was measured during 2 hr of reperfusion. Surfactant components in small and large aggregates, isolated from cell-free bronchoalveolar lavages, and surfactant function were measured.**

Results. **NO inhalation significantly decreased arterial oxygenation. With respect to the surfactant system, NO inhalation worsened the surfactant adsorption rate to an air-liquid interface and affected levels of hydrophobic surfactant proteins (SPs), SP-B and SP-C, and phospholipids, which decreased in large surfactant aggregates but not in small surfactant aggregates. SP-A was reduced in large surfactant aggregates of transplanted lungs from both untreated and NO-treated groups.**

Conclusion. **A decreased level of SP-A, SP-B, and SP-C in large surfactant aggregates of transplanted lungs treated with NO is a marker of lung injury. We conclude that treatment with inhaled NO after lung transplantation is deleterious for the surfactant system and causes a parallel worsening of arterial oxygenation.**

Lung transplantation is the most promising option for selected patients with end-stage obstructive, restrictive, septic, or vascular lung diseases (*1*). Ischemia-reperfusion (I/R) injury is associated with the transplantation process, and it affects every graft recipient in some degree. Eventually, I/R injury may give rise to the onset of a severe dysfunction of the transplanted lung known as acute graft dysfunction or primary graft failure (*1*). I/R injury is an inflammatory process that apparently involves, among other things, the action of resident donor macrophages, the release of multiple inflammatory mediators, the recruitment and activation of circulating platelets and neutrophils (with the subsequent neutrophil-mediated lung injury), and the death of pulmonary cells (*2*, *3*). As a result, the vascular endothelium and the alveolar type I and II cells are injured. The characteristic I/R injury manifestations related to the lung are hypertension, vascular permeability, edema, and hypoxemia (*1*, *3*). A further complication of this scenario is the alteration and dysfunction of

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the pulmonary surfactant, as it has been described in experimental (*4*, *5*) and clinical (*6*) lung transplantations.

One of the clinical therapeutic approaches that have been considered to minimize I/R injury is the inhalation of nitric oxide (NO) (*7*). The rationale for the use of NO in lung transplantation relies on its capacity to reduce pulmonary hypertension. On the other hand, NO can produce significant cellular and surfactant damage through the generation of toxic reactive species (*8*).

We studied the effects of NO therapy after lung transplantation on the composition and interfacial adsorption rate of pulmonary surfactant. Weighing the positive and negative effects of NO inhalation on surfactant may provide a new approach to questions about widespread NO use in lung transplantation.

MATERIALS AND METHODS

Experimental Groups

We performed single left-lung transplantation procedures in weight-matched pairs of large (17–27 kg) Landrace white pigs, as described previously for canine lung transplantation (*5*). A total of 24 pigs were divided randomly into two groups: (1) untreated (n=12, six donors and six recipients), without NO administration and (2) treated with inhaled NO 40 ppm (n=12, six donors and six recipients). Donors and recipients were sedated with ketamine (20 mg/kg body weight), diazepam (0.1 mg/kg body weight), and atropine (0.02 mg/kg body weight). Anesthesia was induced with intravenous administration of propofol (2 mg/kg body weight), midazolam (0.6 mg/kg body weight), and fentanyl (5 μg/kg body weight). After endotracheal intubation, anesthesia was maintained with a continuous intravenous infusion of propofol (9 mg/kg/hr), midazolam (0.6 mg/kg/hr), fentanyl (5 μg/kg/hr), and pancuronium bromide (0.4 mg/kg/hr). A ventilator (Adult Star, Infrasonics Inc., San Diego, CA) was used for mechanical ventilation with 100% oxygen (FiO_2=1), tidal volume 10 to 15 mL/kg, 15 breaths/min, end-expiratory CO_2 35 to 45 mm Hg, and peak airway pressure 15 to 20 cm H_2O. All animals received humane care in compliance with the Spanish law and in accordance with the "Principles of Laboratory Animal Care" formulated by the Institute of Laboratory Animal Resources and the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health publication number 85–23, revised in 1985).

Donor Operation

The donor chest was opened by median sternotomy, and the cavae venae, aorta, pulmonary artery, and trachea were prepared. After heparinization (3 mg/kg body weight), the aorta artery was clamped, and the heart was arrested with a cardioplegic crystalloid solution instilled into the aortic root. Lung perfusion was started with cold (4°C) University of Wisconsin (UW) solution (60 mL UW/kg body weight) in a retrograde fashion through the left atrial appendage and drained through an incision on the main pulmonary artery (*9*). Continuous ventilation was maintained through the perfusion procedure while both pleural cavities were opened and copiously irrigated with cold saline to further enhance graft preservation. This technique took approximately 5 min. Afterward, the heart was removed and the double-lung block was excised. Both lungs were immersed in UW solution at 4°C for 22 hr. Afterward, the donor left lung was transplanted into the recipient animal.

Recipient Operation and Nitric Oxide Treatment

Weight-matched recipients underwent a left posterolateral thoracotomy through the fourth intercostal space. The hemiazygos vein was ligated and divided. The pericardium was opened anterior to the pulmonary veins. The three anastomotic stumps were prepared after clamping of the left pulmonary artery at the point of its bifurcation and the left main bronchus proximal to the last branch. Last, the left atrial appendage was isolated with a side-biting clamp. The left lung was removed from recipient pigs and used as a control lung to obtain control surfactant.

The donor left lung was anastomosed to the recipient. The airway anastomosis was performed with a monofilament polypropylene 4–0 suture. Anastomosis of the pulmonary artery and atrial cuff were performed with nonabsorbable 5–0 running sutures, leaving the last one untied. The pulmonary artery clamp was removed first to eliminate air and the preservation solution through the venous anastomosis. Then the pulmonary artery clamp was closed, and the venous clamp was removed to allow for backflow. Finally the pulmonary artery clamp was progressively opened. Ventilation was resumed at a rate of 17±3 breaths/min (20 mL/kg body weight) and positive end-expiratory pressure of +5 cm H_2O.

NO was supplied as a mixture of 200 ppm diluted in nitrogen (Carburos Metálicos, Línea de Gases, Madrid, Spain). The mixture was delivered by an NO delivery device (SensorNOx, SensorMedics Co. Yorba Linda, CA) through the inspiratory limb of the respiratory circuit. The concentrations of NO and NO_2 were determined by the SensorNOx delivery device, using electrochemical cell analysis, in a continuous fashion. NO was administered at a concentration of 40 ppm, starting immediately after initiating reperfusion and continuing for 2 hr during the reperfusion period (approximate flow: 0.15 L/min; FiO_2=0.98). NO_2 levels did not exceed 2 ppm. A scavenging system was used in the operating room to reduce room pollution. Methemoglobin levels were measured, and no significant changes were found during the entire experiment.

Ventilatory and Hemodynamic Measurements

Ventilatory and hemodynamic parameters were measured in basal situation (presurgery) and after lung transplantation at 0, 1, and 2 hr of reperfusion. A 7-F double-lumen Swan-Ganz thermistor catheter (Edwards Swan-Ganz, Baxter Healthcare Corporation, Deerfield, IL) was introduced into the pulmonary artery through the right external jugular vein for direct measurement of cardiac output by thermodilution, central venous pressure, pulmonary artery pressure, and wedge pressure. A catheter was inserted into the left carotid artery for direct measurement of blood arterial pressure and heart rate, and for collection of arterial blood for analysis of arterial oxygen tension, carbon dioxide, arterial saturation, hemoglobin, and pH. The arterial catheter and Swan-Ganz catheter were connected to a Vitara PM6080 Monitor (Drager, Madrid, Spain). Arterial blood samples were analyzed by a 1306 pH/Blood Gas Analyzer (Instrument Laboratory, Milano, Italy).

Bronchoalveolar Lavage Processing and Isolation of Pulmonary Surfactant Aggregates

Individual lung lavages were obtained from the left lung removed from recipients (control) and the donor left lung transplanted into the recipients in both NO-treated and untreated groups. Each lung was lavaged twice with 4°C saline (50 mL/kg body weight) as previously described (*10*). Bronchoalveolar lavages (BALs) from each lung were pooled and immediately centrifuged at 400*g* for 10 min at 4°C to remove cells and cell debris. The volume of the remaining cell-free BAL was recorded, and an aliquot was taken for protein determination. BAL was centrifuged for 1 hr at 48,000*g* to obtain a pellet of large surfactant aggregates (LA), which are the active form of surfactant (*4*). The small surfactant aggregates (SA), which have poor functional properties, remained in the 48,000*g* supernatant.

The content of total proteins was determined in LA and SA. LA was also analyzed for surfactant protein SP-A content and interfacial adsorption activity. In addition, lipid extracts of LA and SA were prepared by chloroform and methanol extraction (*10*). These LA and SA lipid extracts were analyzed for total phospholipid determination by phosphorus analysis and SP-B and SP-C content.

Measurement of Surfactant Protein-A by Western Blot Analysis

Electrophoretic analysis of LA was performed under reducing conditions (5% β-mercaptoethanol) by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis, using running gels of 12%. The same protein amount (5 μg protein) was applied for all LA samples. In addition, 2 μg of porcine SP-A, previously isolated from control lungs as described (*11*), was always applied in each gel as standard. After electrophoresis, samples were transferred to nitrocellulose using a Bio-Rad Transblot Cell (Bio-Rad, Hercules, CA). Transfer was performed at 100 V constant voltage, 100 mA total increment of intensity, using 25 mM Tris, pH 8.3, 192 mM glycine, and 20% (v/v) methanol as a transfer buffer. Blotting of SP-A was performed as previously described (*12*). For SP-A, an anti-(porcine-SP-A) polyclonal antibody was used. Quantification of SP-A was finally achieved by densitometric evaluation.

Measurement of Surfactant Proteins-B and C by Enzyme-Linked Immunosorbent Assay

SP-B content was determined in organic extracts of LA and SA using an enzyme-linked immunosorbent assay (ELISA) procedure developed in our laboratory (*13*). Each assay plate (Polysorp F96, Nunc A/S) included a standard curve of porcine SP-B isolated from control lungs as described previously (*11*). Polyclonal anti-porcine SP-B antiserum was applied as a primary antibody with peroxidase-labeled anti-rabbit immunoglobulin-G (Amersham International, Buckingshamshire, UK) as the secondary antibody. Colorimetric detection of antibody binding was performed at 570 nm in a Digiscan G010301 microtiter reader (Asys Hitech GmbH, Eugendorf, Austria). Values of unknown samples within the linear range of the standards curve were used to obtain the total SP-B content of each sample.

SP-C content was determined in organic extracts of LA and SA using an ELISA procedure recently published (*14*). Porcine SP-C, isolated from control lungs as described elsewhere (*11*), was used as the standard. For SP-C measurement, an anti-recombinant human SP-C antiserum (donated by Altana Pharma, Konstanz, Germany) was used. There was cross-reactivity of this antiserum with porcine SP-C. The detection limit for porcine SP-C was 180±2.5 ng/mL. The reproducibility of this ELISA procedure with lipid extracts of porcine surfactant was investigated by analyzing several aliquots of LA from control lungs. The coefficients of variance for intra- and interassay series were 5% to 7% (two different LA samples, n=12) and 3% to 8% (two different LA samples, n=3), respectively. Furthermore, serial dilutions of LA samples were analyzed. Variations of SP-C values of 5% to 9% were obtained when corrected for dilution. Values for recovery from 90% to 115% were obtained by adding different calibrators to samples and comparing observed values with expected ones.

Adsorption Assay

The ability of LA, isolated from control and transplanted lungs from NO-treated and untreated recipients, to adsorb onto and spread at the air-water interface was tested at 25°C in a Wilhelmy-like high-sensitive surface microbalance (*5*). The samples were injected into the hypophase chamber of the Teflon dish, which contained 6 mL of 5 mM Hepes buffer, pH 7.0, 150 mM NaCl, and 5 mM $CaCl_2$, with continuous stirring. The amount of surfactant phospholipids injected in the hypophase was the same for all LA samples. Interfacial adsorption was measured after the change in surface tension as a function of time. For each preparation, the analysis was repeated three times.

Statistical Analysis

All data represent the mean of individual measurements ± standard deviation. The means were normally distributed (Shapiro-Wilk test). For statistical analysis, a two-tailed, unpaired Student *t* test was used for comparison of the two groups (untreated and NO-treated recipients) in the analysis of hemodynamic and gasometric parameters. In the surfactant study, differences in means between the three lung types (control lungs, transplanted lungs without NO treatment, and transplanted lungs with NO treatment) were evaluated by one-way analysis of variance followed by Bonferroni post hoc analysis; a confidence level of 95% or greater ($P<0.05$) was considered significant.

RESULTS

Physiologic Responses

Gasometric and hemodynamic parameters were measured in recipients from the untreated and NO-treated groups before (basal values) and after lung transplantation (Table 1). NO inhalation worsened the arterial oxygenation. The NO group showed alveolar-arterial oxygen gradient ($[D(A\text{-}a)0_2]$) values significantly higher than those of the untreated group after 2 hr of reperfusion. On the other hand, we did not find statistical differences when the hemodynamic parameters of untreated and NO-treated groups were compared.

TABLE 1. Gasometric and hemodynamic parameters in lung transplant with and without nitric oxide treatment

Parameter	Group	Basal	Reperfusion time		
			0 hr	1 hr	2 hr
$D(A\text{-}a)O_2$ (mm Hg)	UNT	124±44	154±60	139±48	183±34
	NO	145±22	237±104	230±117	290±109[a]
PaO_2/FiO_2 (mm Hg)	UNT	488±44	458±63	473±53	429±29
	NO	468±21	373±101	391±119	332±110
MAP (mm Hg)	UNT	76.0±12.6	85.8±3.3	80.6±7.3	73.0±7.3
	NO	79.4±10.3	85.2±7.8	83.2±14.7	76.2±8.5
CO (L/min)	UNT	3.5±0.8	2.6±0.3	2.3±0.5	2.1±0.3
	NO	2.2±0.5	2.4±0.3	2.0±0.7	2.4±0.9
MPAP (mm Hg)	UNT	18.6±6.2	24.1±7.6	24.3±5.4	26.5±6.4
	NO	21.4±6.9	22.4±2.3	20.6±6.5	24.4±11.1
PVR (dyne/sec/cm^{-5})	UNT	250±191	414±370	519±362	559±303
	NO	541±200	476±146	704±689	690±586

[a] $P<0.05$ (NO vs. UNT group).

Basal: presurgery values.

$D(A\text{-}a)O_2$, alveolar-arterial oxygen tension gradient; PaO_2, arterial oxygen tension; FiO_2, inspiratory oxygen fraction; MAP, mean arterial pressure; CO, cardiac output; MPAP, mean pulmonary artery pressure; PVR, pulmonary vascular resistance; NO, nitric oxide; UNT, untreated.

Surfactant Analysis

In this study, cell-free BAL has been separated into LA and SA by differential centrifugation. This widely used method fails to sediment all of the heavy surfactant subtypes (*15*). Therefore, certain constituents of heavy and ultraheavy surfactant subtypes, such as surfactant apolipoproteins SP-B and SP-C, may be recovered in SA fraction. In consequence, surfactant analysis has been performed in both LA and SA. LAs represent freshly secreted surfactant from type II cells, whereas SAs are metabolic products of LA formed within the airspace. The increased reuptake of the SA fraction by epithelial cells serves the function of clearing weakly active surfactant (*16*).

Figure 1 shows the protein and phospholipid content of LA and SA isolated from normal and transplanted lungs. The protein levels of SA from transplanted lungs were significantly higher than those from control lungs, regardless of the group considered (NO-treated or untreated). Increased levels of proteins recovered in cell-free BAL or SA indicate leakage of serum proteins into the alveoli. Serum protein contamination was visible by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in SA but not in LA from transplanted lungs. The protein levels in LA were low and remained unchanged in all lungs studied. In contrast, phospholipid content significantly decreased in LA from untreated or NO-treated transplanted lungs. This decrease was remarkable in LA from the NO-treated group. The phospholipid SA-to-LA ratio was significantly higher in the lavage material from the NO-treated group. Either one mechanism or the confluence of several mechanisms could lead to this typical uneven distribution of surfactant subtypes, such as increased degradation of LA by macrophages and neutrophils present in the inflamed lung, decreased secretion of surfactant, or accelerated LA-to-SA conversion.

Figure 2 shows the SP-B and SP-C content in LA and SA isolated from healthy and transplanted lungs. The levels of both SP-B and SP-C in LA did not change in transplanted lungs from untreated recipients. However, NO inhalation

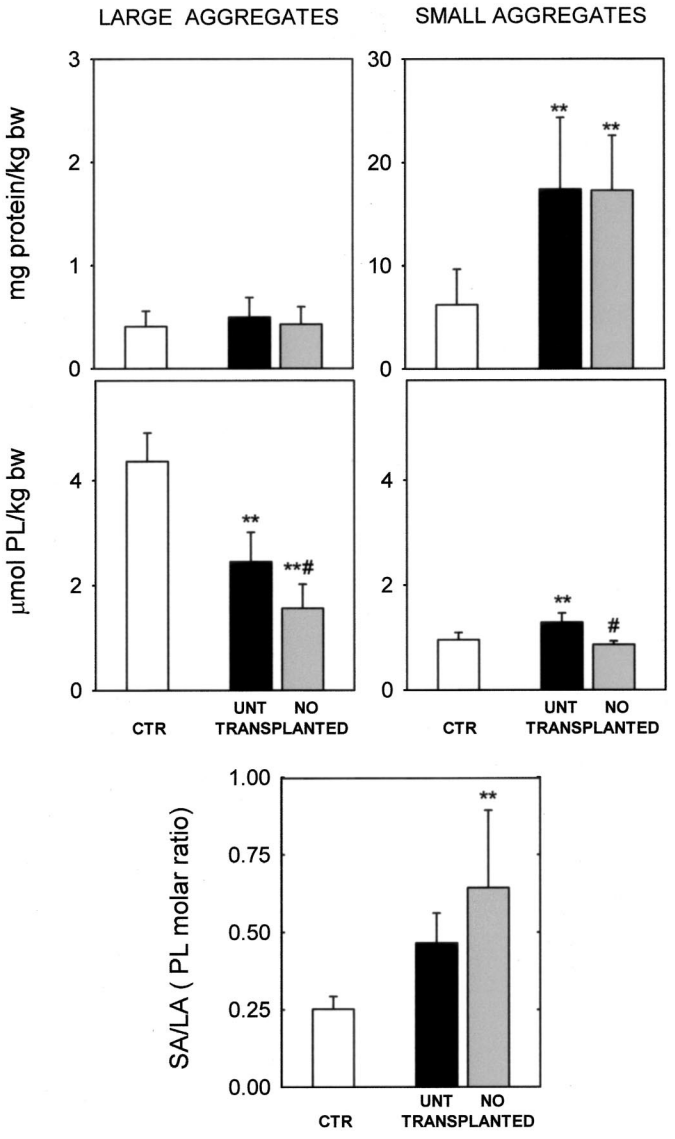

FIGURE 1. Protein and phospholipid content in large surfactant aggregates (LA) and small surfactant aggregates (SAs), and average SA-to-LA ratio in transplanted and control lungs. CTR, control (*open bars,* n=6); UNT, untreated transplanted lungs (*closed bars,* n=6); NO, transplanted lungs treated with nitric oxide (*gray bars,* n=6). **$P<0.01$ NO and UNT versus CTR; #$P<0.05$ NO versus UNT.

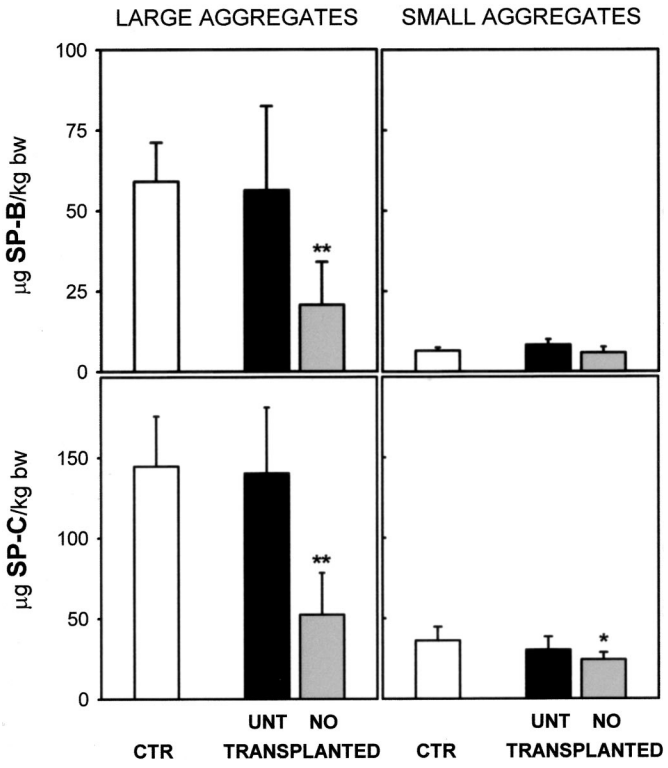

FIGURE 2. Surfactant protein SP-B and SP-C content in organic extracts of LA and SA from control and transplanted lungs. Quantitation of SP-B and SP-C was achieved by enzyme-linked immunosorbent assay (ELISA), using known amounts of porcine SP-B and SP-C (determined by amino acid analysis) to create a calibration curve. CTR, control (*open bars,* n=6); UNT, untreated transplanted lungs (*closed bars,* n=6); NO, transplanted lungs treated with nitric oxide (*gray bars,* n=6). **$P<0.01$ NO versus CTR and UNT; *$P<0.05$ NO versus CTR.

during reperfusion significantly decreased SP-B and SP-C levels compared with both untreated and control lungs. This decrease was not attributable to a parallel increase of SP-B and SP-C in SA because the levels of these hydrophobic proteins remained unchanged or slightly decreased in the SA fraction (Fig. 2). This is similar to the pattern of phospholipid content in SA (Fig. 1).

Figure 3 shows SP-B/phospholipid and SP-C/phospholipid molar ratios in the LA fraction of transplanted lungs from untreated and NO-treated recipients and control lungs. These molar ratios decreased in NO-treated lungs compared with untreated and control lungs, indicating that both SP-B and SP-C decreased in higher proportion than phospholipids. Notably, the SP-C/SP-B molar ratio in transplanted lungs from NO-treated recipients was similar to that found in untreated and control lungs (data not shown). This indicates that SP-B and SP-C decreased in identical proportion as a consequence of NO inhalation, so that the SP-C/SP-B molar ratio was maintained.

The SP-A content in LA from transplanted lungs was analyzed by Western blot, and the results are shown in Figure 4. All transplanted lungs demonstrated a significant decrease in SP-A levels in LA with respect to the control lungs. This decrease was more prominent in the NO-treated group ($\leq$75%).

Surfactant Function

Surfactant function was determined by measuring the ability of LA to adsorb onto and spread at an air-water interface

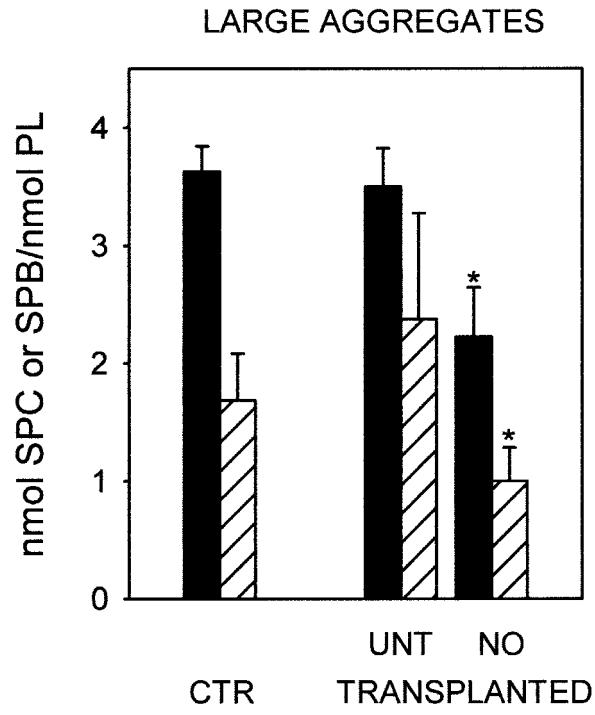

FIGURE 3. SP-B/phospholipid and SP-C/phospholipid molar ratio in LA from control and transplanted lungs. Phospholipid (PL), SP-B, and SP-C content was determined in LA lipid extracts. SP-B/PL (*diagonally hatched bars*), SP-C/PL (*closed bars*). CTR, control (n=6); UNT, untreated (n=6); NO, treated with inhaled nitric oxide (n=6). *P<0.05 NO versus CTR and UNT.

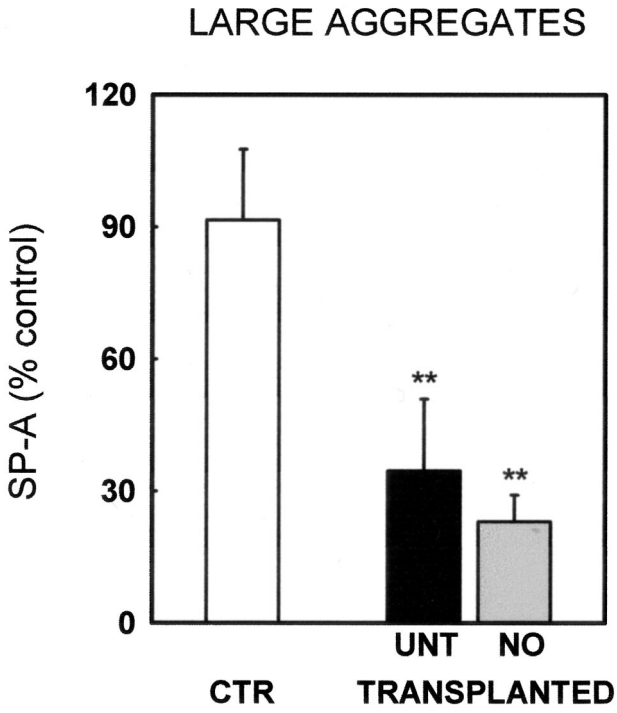

FIGURE 4. SP-A content in LA from control and transplanted lungs. Measurement of SP-A was achieved by densitometric evaluation of the 36 to 40 kDa SP-A bands obtained from Western blot analysis as described in *Materials and Methods*. A volume of LA corresponding to 5 μg of proteins was applied for all of the LA preparations. CTR, control (*open bars*, n=6); UNT, untreated transplanted lungs (*closed bars*, n=6); NO, transplanted lungs treated with inhaled nitric oxide (*gray bars*, n=6). **P<0.01 NO and UNT versus CTR

in a diffusion-independent system (Fig. 5A). The amount of surfactant phospholipids injected into the hypophase was the same for all samples from the control and transplanted groups. Adsorption is performed through (1) the transport of the material injected through the bulk liquid to accumulate at the air/liquid interface and (2) the spread of the material along the surface, producing the surface pressure we measured. The results indicate that the adsorption rate of LA from transplanted lungs decreased markedly with respect to LA from control lungs. We did not find differences in the rate of surfactant adsorption between NO-treated and untreated groups when assays were performed at low phospholipid concentrations (Fig. 5A). Schürch et al. (*17*) showed that this process depends on SP-A at low phospholipid concentrations. The SP-A content was markedly decreased in both NO-treated and untreated groups (Fig. 4). The effect of the reduction of SP-A content on the adsorption rate can be overcome by increasing surfactant concentration (*17*). Figure 5B shows that the surfactant adsorption rate of LA from the untreated group was significantly greater than that of the NO-treated group when assays were performed at higher surfactant phospholipid concentrations. The decrease of both SP-B and SP-C content in LA of the NO-treated group could explain the decreased surface activity of surfactant isolated from the NO-treated group. Surfactant peptides SP-B and SP-C are the major surfactant components responsible for facilitating the adsorption of surfactant phospholipids (*18*).

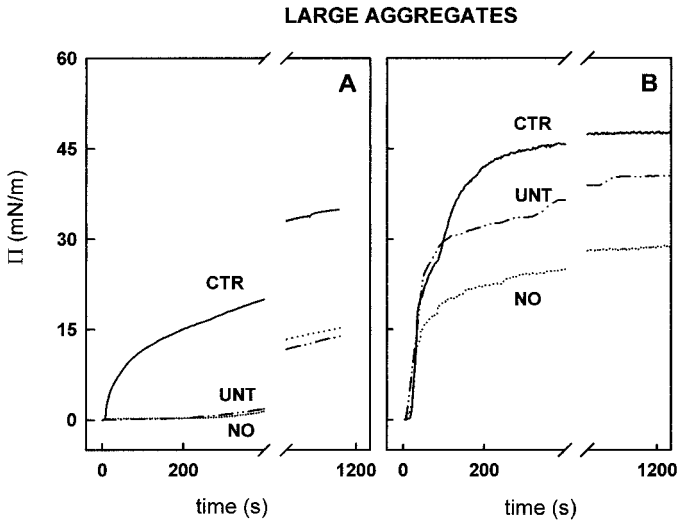

FIGURE 5. Interfacial adsorption kinetics of LA from control and transplanted lungs. The final concentration of phospholipids in the hypophase was 60 nmol/mL (A) or 100 nmol/mL (B) for all surfactant preparations; π: surface pressure; CTR, control (*solid line,* n=6); UNT, untreated transplanted lungs (*dash-dot-dot line,* n=6); NO, transplanted lungs treated with inhaled nitric oxide (*dotted line,* n=6). Differences between UNT and NO lungs versus control lungs (A) and between CTR and UNT lungs versus NO lungs (B) were significant ($P<0.01$).

DISCUSSION

We found some unmistakable negative effects of inhaled NO on pulmonary surfactant isolated from lung transplant recipients and a parallel deterioration of arterial oxygenation. The significant decrease that NO inhalation caused in SP-B and SP-C levels in LA compared with untreated transplanted and control lungs is relevant and has not been reported. SP-B and SPC are highly hydrophobic peptides inserted in surfactant membranes. These proteins are required for the reduction of surface tension in the alveolus; they also increase the rate of surfactant adsorption to an air-liquid interface and participate in the regulation of intracellular and extracellular processes critical for the maintenance of respiratory structure and function (*18*). We found that the amount of SP-B and SP-C decreased in higher proportion than that of phospholipids, which were also significantly reduced in LA of the NO-treated group. Notably, SP-B and SP-C levels decreased in the same proportion. As a result, the SP-C-to-SP-B molar ratio did not change when compared with untreated and control lungs. These results indicate that the mechanism of NO-induced injury affected SP-B and SP-C equally. Two possible mechanisms could be involved: a decrease in SP-B and SP-C gene expression by tumor necrosis factor-α (*19*, *20*) or an increase in clearance of LA by macrophages and neutrophils present in inflamed lungs (*21*). An increased release of inflammatory cytokines and other inflammatory mediators occurs in I/R injury after lung transplantation (*1*, *2*), and inhaled NO could aggravate alveolar inflammation (*22*). The reduction of SP-B and SP-C levels in LA of the NO-treated group could explain the significant decrease of the surface adsorption rate of surfactant from the NO-treated group when compared with the untreated group. On the other hand, surfactant adsorption rate depends highly on SP-A at low phospholipid concentrations (*17*). SP-A levels were significantly reduced in LA from transplanted lungs as reported elsewhere (*4*, *5*), although this decrease was more prominent in NO-treated lungs. When surfactant adsorption rate was studied at low phospholipid concentrations, this process markedly decreased in both untreated and NO-treated groups, and there was no significant difference between both groups. This supports the role of SP-A on surfactant adsorption under limited conditions of surfactant phospholipid concentration (*17*).

A decreased level of SP-A, SP-B, SP-C, and phospholipids in LA of transplanted lungs treated with NO is a marker of lung injury. The various clinical disorders caused by mutations in the genes encoding SP-B and SP-C (*18*) serve as examples of the critical role of these proteins in the pathogenesis of pulmonary diseases. Decreased levels of SP-B and SP-C in the air spaces may render the lung susceptible to atelectasis and injury caused by a loss of surfactant function. On the other hand, the simultaneous reduction of SP-A may further decrease adsorption facilities of surfactant, increase the rate of LA-SA conversion, and enhance the susceptibility of surfactant-to-plasma protein inactivation (*16*). Furthermore, surfactant membranes and their apolipoproteins SP-A, SP-B, and SP-C function not only as a protective layer against alveolar collapse but also as the primary antimicrobial defense in the alveolar fluid (*23*). Decreased levels of SP-A, SP-B, and SP-C were also recently found in patients with acute respiratory distress syndrome (*14*, *16*).

Inhaled NO is used to alleviate pulmonary hypertension and hypoxemia. However, the usage of inhaled NO therapy has been moderated by an increase in morbidity and decrease in the percentage of patients alive and off ventilatory support in a large multicenter trial of patients with acute lung injury (*24*). Moreover, a "rebound" increase in pulmonary artery pressure on discontinuing the gas is common and may result in cardiovascular collapse (*25*). Remarkably, inhaled NO was inefficient in human lung transplantation outcome when prophylactically applied (*26*, *27*). O_2-rich environments such as the lung are particularly predisposed to NO toxicity (*28*). NO administration might trigger oxidative events that damage alveolar cells and specifically surfactant, especially in the presence of high O_2 concentrations (*29*, *30*). The edematous and inflammatory state after reperfusion in lung transplantation may provide a breeding ground for NO oxidant exacerbated effects (*8*, *28*).

CONCLUSION

Our work indicates that inhaled NO after lung transplantation can be deleterious for the surfactant system. Future studies should try to differentiate the possible underlying mechanism.

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