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Donor pretreatment with ambroxol or dexamethasone fails to ameliorate reperfusion injury in experimental lung transplantation

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Abstract Based on the known properties of ambroxol and dexamethasone to inhibit inflammation and increase endogenous surfactant levels, the potential advantage of donor pretreatment with either drug was investigated in an acute rat double-lung transplant model. Donor animals were randomly assigned to one of three treatment groups: an ambroxol group (AMB; 0.4 mg/kg), a dexamethasone group (DX; 2 mg/kg); or an untreated control group (CN). Drugs were given intraperitoneally 6 h prior to harvest. Following standard preservation and 16 h of cold ischemia, the donor double lung block was implanted into syngeneic recipients using custom-designed stents for the vascular anastomosis. During reperfusion, serial measurements of graft pulmonary vascular resistance and alveolar-arterial oxygen difference were obtained. Separate graft ventilation allowed determination of graft dynamic lung compliance. Final assessment included weight gain and histology. For phospholipid analysis, lung lavages were performed in the three study groups at the end of reperfusion and compared to levels before graft harvest. Donor pretreatment did not significantly affect preharvest phospholipid levels. Survival following graft ischemia and reperfusion was shortest after AMB (92 ± 5 min) and longest after DX (110 ± 5 min; DX vs AMB $P < 0.03$) and CN (116 ± 4 min; CN vs AMB $P < 0.02$). DX pretreatment provided better compliance ($P < 0.02$) and lower vascular resistance ($P < 0.0001$) than AMB treatment. Airway resistance was lower in the AMB and DX groups than in controls ($P < 0.04$ and $P < 0.02$, respectively). The alveolar-arterial oxygen difference was markedly similar in all groups. Graft weight gain amounted to 114 % ± 10 % in AMB, 88 % ± 12 % in DX, and 98 % ± 13 % in CN (P = NS). Thus, in this rat lung transplantation model, donor pretreatment with dexamethasone did not improve graft function compared to untreated controls and donor pretreatment with ambroxol was found to be potentially detrimental to graft function during reperfusion.

Key words Lung transplantation, reperfusion injury, ambroxol · Dexamethasone, lung transplantation · Ambroxol, lung transplantation

Introduction

Primary graft dysfunction is one of the major causes of early postoperative patient morbidity and mortality in clinical lung transplantation. Ischemia and reperfusion-related injury to the graft may alter endogenous surfactant levels and function with subsequent deterioration of pulmonary mechanics [12, 35]. Attempts to correct

Table 1 Comparison of demographic and pretransplant data. Donor pretreatment with ambroxol resulted in significantly reduced dynamic compliance in comparison to dexamethasone pretreatment or no treatment. Survival was shortest in the ambroxol pretreatment group

	Control	Ambroxol	Dexamethasone	Mann-Whitney *P* value
Donor animal dynamic compliance (ml/cmH_2O)	74 ± 3	39 ± 3	71 ± 4	CN vs AMB 0.001 CN vs DX 0.61 DX vs AMB 0.001
Donor weight (g)	356 ± 4	354 ± 8	367 ± 10	CN vs AMB 0.34 CN vs DX 0.38 DX vs AMB 0.21
Recipient weight (g)	363 ± 10	371 ± 9	366 ± 7	CN vs AMB 0.36 CN vs DX 0.22 DX vs AMB 0.26
Lung weight gain post-reperfusion (%)	98 ± 13	114 ± 10	88 ± 12	CN vs AMB 0.45 CN vs DX 0.44 DX vs AMB 0.06
Survival (min)	116 ± 4	92 ± 5	110 ± 5	CN vs AMB 0.02 CN vs DX 0.3 DX vs AMB 0.03

this have recently stressed the value of exogenous surfactant replacement therapy in the donor before organ preservation [26]. Experimental studies by Novick et al. [25] and Hausen et al. [17] have shown that replacement therapy with as little as 20–100 mg/kg of exogenous bovine surfactant can result in dramatic improvement in early postoperative graft function. However, the current high costs of surfactant may limit the practicality of this therapeutic option. Therefore, measures are needed that either prevent depletion of endogenous surfactant reserves or, alternatively, boost endogenous surfactant production before or during graft preservation.

Dexamethasone has been shown to increase the surfactant protein A mRNA levels both in vivo and in vitro in adult rats [13, 38] and to increase the lipoprotein lipase activity with a resultant augmentation of the triacylglycerol-fatty acids needed for phospholipid production as the major component of surfactant [3, 16, 20]. In addition to its effect on endogenous surfactant levels and function, dexamethasone alters the chemotactic response of PMNs and their spontaneous migration [7]. Dexamethasone inhibits arachidonic metabolism and complement activation and has significant antioxidant activity [1]. The potential benefit of donor pretreatment with high steroids has been previously analyzed by Novick et al. [27]. In an acute canine lung transplant model in which oxygenation was the main functional outcome variable, this group was able to show only a moderate benefit from methylprednisolone bolus treatment in comparison to untreated controls. The novel expectorant ambroxol, a metabolite of the bronchial secretolytic bromhexine, can stimulate production and inhibit catabolism of endogenous surfactant [6, 34]. This compound is also known to have antioxidant properties and can inhibit the chemotactic response of PMNs and their spontaneous migration [28].

The present study was designed to investigate the value of short-term donor pretreatment with either dexamethasone or ambroxol. The hypothesis was that, due to their inhibitory effect on the nonspecific immune system and their potential to increase endogenous surfactant levels, either drug might help ameliorate graft dysfunction following lung transplantation as a result of ischemia-reperfusion injury. An acute, syngeneic, in vivo double-lung transplantation model in the rat was used for this investigation.

Materials and methods

The study was conducted following approval by the State Ethics Committee of Lower Saxony, Germany. Female Lewis rats (350–400 g) were obtained from Charles River (Salzfeld, Germany) and all animals received humane care in compliance with the "Principals 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 National Academy of Sciences and published by the National Institutes of Health (NIH Publication No.80–123, revised 1985).

Experimental groups

Donor animals were pretreated with a bolus of either 0.4 mg/kg ambroxol (group AMB; $n = 10$) or 2 mg/kg dexamethasone (group DX; $n = 10$; Fortecortin 100, Merck, Darmstadt, Germany) intraperitoneally 6 h before organ flush perfusion. The timing of administration and the dose chosen for both drugs was based on related studies performed by Phlebs et al. [30], Young et al. [38], Floros et al. [13] and Luisetti et al. [23]. The lungs of these two treatment groups and of the untreated controls ($n = 10$) were flushed with low-potassium dextran solution (Perfadex) at 4 °C using a perfu-

sion pressure of 20 mmHg and a perfusate volume of 60 ml/kg. After 16 h of cold ischemia, during which time the grafts were stored in the Perfadex solution, the explanted organs were reperfused in syngeneic recipients.

In order to determine the impact of donor pretreatment on pulmonary phospholipid levels and surfactant function in the bronchoalveolar lavage before graft harvesting and ischemia, lung lavages were performed in three additional control groups ($n = 6$) 6 h following drug treatment.

Experimental procedure

A detailed description of the model of acute, in vivo, double-lung transplantation has been previously published [18]. Following flush perfusion, the main pulmonary trunk was dissected from the right ventricle and the mitral valve was closed with an 8–0 Prolene suture. The left atrial appendage was incised to provide drainage of the donor left atrium during reperfusion. For storage, the lungs were inflated with 100% oxygen to reach an intratracheal pressure of 26 cm H_20. Static inflation has been previously shown to improve endogenous surfactant levels and to provide enhanced preservation [19]. For implantation into a syngeneic recipient, the donor pulmonary artery and left atrial appendage were connected to the left pulmonary artery and vein of the recipient rat using two specially designed T-shaped stents, placing the donor lungs partly in the left hemithorax of the recipient. One of these stents contained a custom-designed, built-in Doppler probe (H-2R probe, Transonic Systems, N.Y., USA). Both stents had a total length of 20 mm and were designed with side ports for blood retrieval and pressure measurements. The donor trachea was intubated with a 13-gauge cannula and the graft ventilated with a Harvard volume-controlled respirator (Harvard Rodent Ventilator Model 683; South Natick, Mass., USA) at a 14 ml/kg tidal volume and a positive end-expiratory pressure of 3 cm H_20. The inspiratory oxygen concentration was 1.0. After administration of heparin to the recipient, animal blood was allowed to flow through the graft. The respiratory rate of the donor lung was adjusted to maintain a left pulmonary venous $PC0_2$ of 30–40 mmHg. Fluid lost by sequestration or evaporation was replaced with either blood or crystalloid fluid to ensure a mean pulmonary artery pressure of 20 mmHg and a hematocrit of 30%–40%. The donor lung was kept moist by intermittent topical application of warm fluid. The pH measured in the pulmonary venous blood was titrated with 8.5% $NaHC0_3$ to maintain a pH of between 7.25 and 7.5.

Measurements

Serial measurements of pulmonary venous and arterial pressures and blood gases, graft blood flow, and graft dynamic compliance, as well as airway resistance, were performed every 20 min. Derived parameters were alveolar-arterial oxygen difference ($AaD0_2$), intrapulmonary ventilation and perfusion shunt (QSQT), and pulmonary vascular resistance (PVR). Immediately preceding each measurement, the donor lung was sigh-ventilated to remove atelectasis and secretions were suctioned from the airways and the amount recorded. The total reperfusion period was limited to 120 min. At the end of the reperfusion period or in the event of premature recipient death, the weight gain of the graft was determined and a histological analysis performed.

Bronchoalveolar lavage

A bronchoalveolar lavage (BAL) was performed in all treatment groups at the end of the reperfusion period and in three additional control groups 6 h after donor pretreatment and before graft harvest. For this procedure, only the right lung was lavaged with the left donor bronchus clamped to prevent alterations of left lung histology by the lavage fluid. The trachea was intubated with a 13-gauge cannula and 3 ml of 4 °C buffered saline was infused at a rate of 10 ml/min. This procedure was repeated a total of five times. Following retrieval, the lavage was immediately centrifuged at 270 *g* and the cell-free supernatant frozen at –80 °C.

Phospholipid and surface tension determination

The surfactant pellet was resuspended in 154 mmol/l saline supplemented with 1.5 mmol/l calcium chloride. After separation of pellet and supernatant at 27,000 *g* for 30 min, the phospholipid content of pellet (large aggregates) and supernatant (small aggregates) was determined from a 5 µl aliquot, according to the method of Bartlett [4] and then readjusted to a phospholipid concentration of 1 and 3 mg/l, respectively. Total phospholipid content then was calculated in micrograms and the relative contribution of large and small aggregates expressed as a percentage of total phospholipid content. Surface tension was measured in the Pulsating Bubble Surfactometer (Electronetics, Amherst, N.Y., USA) as described by Enhorning [11]. If the phospholipid content did not allow a concentration of at least 1 mg/l, the surface tension measurement could not be performed in these respective groups.

For surface tension measurements, 36 µl of the surfactant suspension was instilled into the sample chamber of the surfactometer. A bubble (diameter 0.4 mm) communicating with ambient air was created. Surfactant was allowed to adsorb to the air/liquid interface for 10 s, after which time the bubble pulsated at a frequency of 20 oscillations per minute between a minimal radius of 0.4 mm and a maximal radius of 0.55 mm. The pressure across the bubble was measured by a pressure transducer and the surface tension calculated using the LaPlace equation. The surface tension after 10 s of adsorption and the minimal surface tension after 5 min of cycling were determined and expressed in mN/m.

Protein levels of the BAL were determined according to the method described by Lowry et al. and expressed in mg/ml [22].

Histology

At the end of reperfusion, the lung vessels were rinsed with buffered saline to remove blood. The left pulmonary lobe was dissected, stored in formalin, and then cut and stained with hematoxylin-eosin. Evaluation was performed in a blinded fashion and the analysis was scored according to a semiquantitative scale. The evaluation protocol assessed the degree of interstitial edema, intra-alveolar edema, extravascular granulocyte infiltration, and pulmonary hemorrhage (score 1 = no, 2 = mild, 3 = moderate, 4 = severe).

Statistical analysis

Data were analyzed with the Statistical Program of the Social Sciences (SPSS for Windows, version 6.1.3, Birmingham, Ala., USA). All data are expressed as mean ± standard error. Analysis of continuous data, such as dynamic compliance, airway resistance, alveolar-arterial oxygen difference, and pulmonary vascular resistance, was performed by repeated measures ANOVA (r.m.A.) [24]. The

model used incorporated a fixed time effect, a fixed group effect, a time by group interaction effect, and a random animal effect. For multiple comparisons, the Bonferroni adjustment was incorporated. The change over time in each group was evaluated by one-way repeated measures ANOVA. Continuous data without repeated measurements, such as donor and recipient animal weights, weight increase of graft, donor compliance, and animal survival, as well as nonparametric data, such as the histological semiquantitative analysis, were compared with the Mann-Whitney test.

Results

Donor and recipient rats were randomly assigned to one of the three study groups and their demographic and pretransplant data, as well as animal survival and percent weight gain, are depicted in Table 1. The three study groups were comparable in terms of donor and recipient weight (Table 1). The type of pretreatment, however, had a significant effect on dynamic lung compliance assessed in the donor animal immediately before graft perfusion. Ambroxol pretreatment resulted in a decline in graft compliance in comparison to dexamethasone treatment and no treatment (Table 1). Following preservation, ischemia, and reperfusion, the overall quality of organ preservation was reflected in the mean survival, which was significantly lower in animals pretreated with ambroxol than in those with dexamethasone treatment (AMB vs DX: $P < 0.03$) and untreated controls (AMB vs CN: $P < 0.02$; Table 1).

During the experiments, considerable amounts of edematous fluid were suctioned from the airways, mainly in the AMB and DX groups. The average amount of fluid per animal and group was 0.81 ± 0.19 ml per measurement in the CN group, 1.16 ± 0.23 ml per measurement in the AMB group, and 1.67 ± 0.1 ml per measurement in the DX group. The difference between CN and DX was significant ($P < 0.006$). Despite the significant increase in the amount of edematous fluid retrieved from the airways in the DX group, the average weight increase of the graft during reperfusion was highest in the AMB group (114% ± 10%) compared to 98% ± 13% in the CN group and 88% ± 12% in the DX group. These differences were not significant (Table 1).

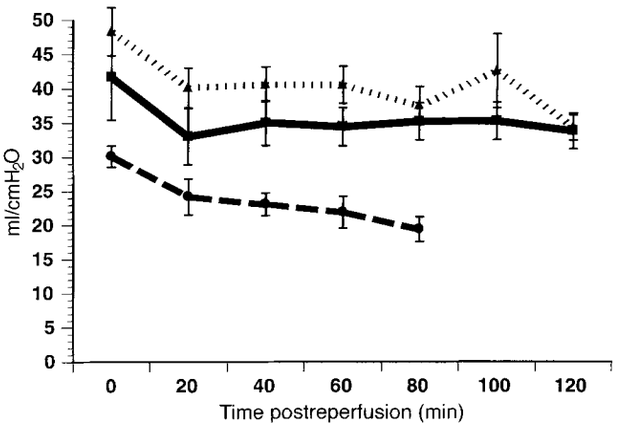

Fig. 1 Serial measurements of dynamic pulmonary compliance. In comparison to no treatment (controls) and dexamethasone pretreatment, ambroxol pretreatment resulted in significantly diminished compliance (Repeated measures ANOVA: AMB vs DX $P < 0.02$; AMB vs CN $P < 0.1$;DX vs CN $P < 0.23$). — Control; - - - ambroxol; '''' dexamethasone

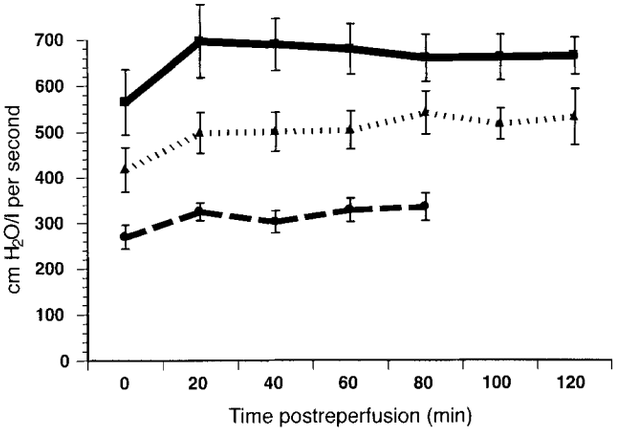

Fig. 2 Airway resistance, measured in cmH_2O/L per second, was significantly lower in untreated grafts than in grafts pretreated with either ambroxol or dexamethasone (Repeated measures ANOVA: AMB vs DX $P < 0.15$; AMB vs CN $P < 0.04$; DX vs CN $P < 0.02$). — Control; - - - ambroxol; '''' dexamethasone

Serial measurements

The dynamic pulmonary compliance (DLC) of the donor grafts during the 120-min reperfusion period is depicted in Fig. 1. Ambroxol pretreatment resulted in a poor initial compliance with subsequent further deterioration during the remainder of the reperfusion period (one-way ANOVA $P < 0.0001$). The compliance in this group was significantly lower than in the dexamethasone treatment group ($P < 0.02$). The difference in DLC between DX and CN and between AMB and CN was not significant.

Airway resistance (Fig. 2) was significantly lower in the AMB and DX groups than in the untreated controls (repeated measures ANOVA, $P < 0.04$ and $P < 0.02$, respectively). There was no significant difference between AMB and DX treatment.

The alveolar-arterial oxygen difference was similar in all study groups (Fig. 3). During the reperfusion period, all groups showed a significant rise in $AaD0_2$, starting at values between 96 and 190 mmHg and ending be-

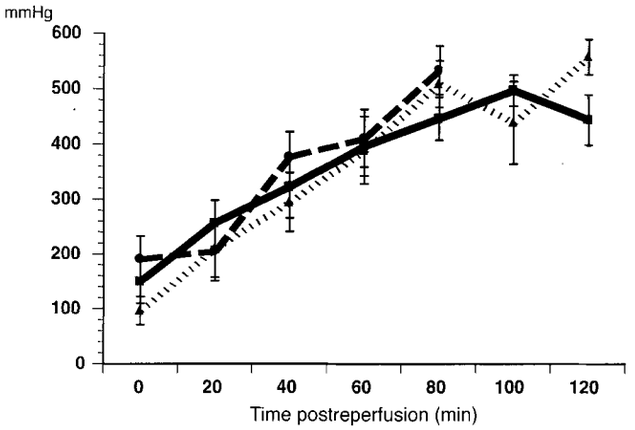

Fig. 3 Comparison of alveolar-arterial oxygen difference ($AaD0_2$). Donor pretreatment with either dexamethasone or ambroxol did not effect the $AaD0_2$. The average $AaD0_2$ increased significantly in all three groups over the 2-h reperfusion period (Repeated measures ANOVA: AMB vs DX $P < 0.56$; AMB vs CN $P < 0.65$;DX vs CN $P < 0.55$). — Control; - - - ambroxol; '''' dexamethasone

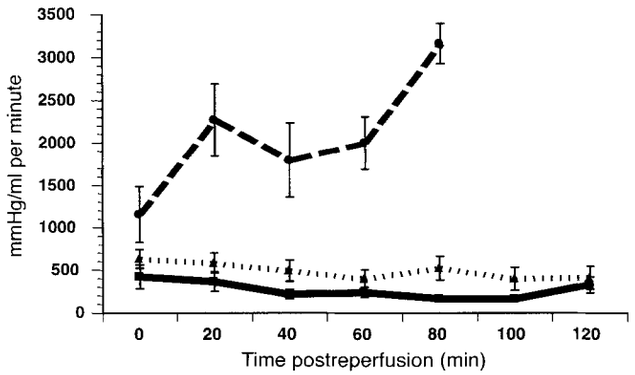

Fig. 4 Resistance to pulmonary blood flow (PVR) measured in mmHg min/ml was markedly elevated in the AMB group with almost no blood flow through the graft by the end of the reperfusion period (Repeated measures ANOVA: AMB vs DX $P < 0.0001$; AMB vs CN $P < 0.0001$; DX vs CN $P < 0.13$). — Control; - - - ambroxol; '''' dexamethasone

tween 443 and 617 mmHg (one-way ANOVA; CN group $P < 0.001$; AMB group $P < 0.001$; DX group $P < 0.00001$).

The type of donor pretreatment had a significant effect on the pulmonary vascular resistance (Fig. 4). Ambroxol pretreatment resulted in a drastically elevated PVR (1157 + 330 mmHg/ml per minute) that increased even further, resulting in minimal graft perfusion by the end of the study period (PVR 3264 + 1924 mmHg/ml per minute;one-way ANOVA $P < 0.009$). Comparison to the DX and CN groups by r.m.A. showed significant differences ($P < 0.0001$ and $P < 0.0001$, respectively). The PVR in the CN and DX groups was not significantly different.

In all groups, the amount of ventilation-perfusion shunting increased significantly during the reperfusion period, with less than 15% shunt at 20 min of reperfusion (CN 14.8% ± 2.7%, AMB 9.9% ± 3% DX 9.2% ± 2.4%), followed by a gradual increase in all groups to 25%–30% shunt at 80 min (CN 25.7% ± 2.3%, AMB 25.2% ± 3.7% DX 26.3% ± 3.2%; ANOVA CN vs AMB $P < 0.43$; CN vs DX $P < 0.32$; AMB vs DX $P < 0.1$).

Phospholipid aggregates, surfactant function, and alveolar protein content

The analysis of phospholipids obtained from the BAL of lungs pretreated with either ambroxol or dexamethasone in comparison to untreated controls prior to flush perfusion, graft ischemia, and reperfusion is depicted in Table 2. The total phospholipid content per BAL and the percentage of functional surfactant, as expressed by the amount of large aggregates in the 27,000 *g* pellet, were almost identical in the pretreatment groups compared to the untreated controls. Surfactant function, as expressed in surface tension as well as in protein content, was also similar in these three groups.

The type of donor pretreatment did not effect the phospholipid content in the BAL of the three study groups at the end of the reperfusion period (Table 2). The amount of functional surfactant expressed as the percentage of large phospholipid aggregates was significantly lower in the AMB and DX groups than in untreated controls ($P < 0.02$ and 0.03, respectively). The total amount of phospholipids retrieved in the BAL of these groups was not sufficient to allow surface tension measurements. Table 2 also depicts protein concentration in the BAL, revealing no significant differences.

Histology

The results of the semiquantitative evaluation of the specimens are listed in Table 3. In the statistical analysis, the amount of interstitial or intra-alveolar edema, pulmonary hemorrhage, or extravascular granulocyte infiltration was similar in all three study groups.

Discussion

Preservation, ischemia, and reperfusion in experimental lung transplantation can significantly affect endogenous surfactant levels and related graft function [2, 35]. According to studies by Erasmus and colleagues, the sur-

Table 2 Biochemical analysis of bronchoalveolar lavage

	Control (CN) (no treatment)	Ambroxol (AMB) (0.4 mg/kg)	Dexamethasone (DX) C2 mg/kg)	Mann-Whitney *P* value
a Bronchoalveolar lavage following donor pretreatment without graft preservation, ischemia, and reperfusion				
Total phospholipid content in lavage (μg)	1052 ± 134	1305 ± 248	1467 ± 244	CN vs AMB 0.33 CN vs DX 0.2 DX vs AMB 0.9
Large aggregate phospholipid content in 27,000 *g* pellet (%)	56 ± 6	56 ± 6	59 ± 4	CN vs AMB 0.81 CN vs DX 0.59 DX vs AMB 0.67
Small aggregate phospholipid content in 27,000 *g* pellet (%)	44 ± 6	44 ± 6	41 ± 4	CN vs AMB 0.81 CN vs DX 0.59 DX vs AMB 0.67
Minimal surface tension at 1 mg/ml	17 ± 3	20 ± 2	16 ± 3	CN vs AMB 0.31 CN vs DX 0.75 DX vs AMB 0.18
Minimal surface tension at 3 mg/ml	16 ± 2	10 ± 9	14 ± 3	CN vs AMB 0.5 CN vs DX 0.58 DX vs AMB 0.7
Adsorption surface tension at 1 mg/ml	34 ± 2	38 ± 5	34 ± 2	CN vs AMB 0.51 CN vs DX 0.69 DX vs AMB 0.51
Adsorption surface tension at 3 mg/ml	30 ± 1.5	32 ± 1	29 ± 1	CN vs AMB 0.51 CN vs DX 0.41 DX vs AMB 0.09
Protein concentration (mg/ml)	0.2 + 0.04	0.1 + 0.01	0.2 + 0.02	CN vs AMB 0.09 CN vs DX 0.94 DX vs AMB 0.1
b Bronchoalveolar lavage at the end of reperfusion				
Total phospholipid content in lavage (μg)	1561 ± 132	1579 ± 250	1555 ± 56	CN vs AMB 0.12 CN vs DX 0.44 DX vs AMB 0.13
Large aggregate phospholipid content in 27000 G pellet (%)	30 ± 5	14 ± 3	14 ± 2	CN vs AMB 0.02 CN vs DX 0.03 DX vs AMB 0.93
Small aggregate phospholipid content in 27000 G pellet (%)	70 ± 5	86 ± 3	86 ± 2	CN vs AMB 0.02 CN vs DX 0.03 DX vs AMB 0.93
Protein concentration (mg/ml)	0.9 + 0.05	1.6 + 0.6	1.1 + 0.2	CN vs AMB 0.17 CN vs DX 0.17 DX vs AMB 0.94

factant pool is inversely proportional to the time of cold ischemia [12]. Depletion may be due to activation of various phospholipases with subsequent metabolism of the phospholipid component of endogenous surfactant. Finally, increased membrane permeability with leakage of serum proteins into the alveoli further deactivates endogenous surfactant [33].

In order to prevent deterioration of graft function following clinical lung transplantation, supplementation of the donor graft with exogenous surfactant has been proposed [26]. Studies by Novick et al. [25] and Hausen et al. [17] have emphasized the importance of early donor treatment as the preferred treatment modality, with surfactant given in a dose of 100–200 mg/kg. At the present unit cost of exogenous surfactant, however, this type of treatment strategy may not be financially feasible for clinical use. Alternatives must be sought that may include the use of drugs that can trigger production or inhibit metabolism of endogenous surfactant.

The drugs used in the present study – dexamethasone and ambroxol – have both been shown to be anti-inflammatory as well as capable of inducing endogenous surfactant production [7, 14, 36]. Ambroxol can inhibit free radical-mediated processes in lung tissue [28]. By protecting the alpha-1 proteinase from oxidative inacti-

Table 3 Semiquantitative histological assessment of lung specimens at the end of reperfusion showing no significant differences in the three treatment groups. Score 1 = no, 2 = mild, 3 = moderate, 4 = severe

Group	Interstitial edema	Intra-alveolar edema	Granulocyte infiltration	Pulmonary hemorrhage
Control	2.0 ± 0.5	1.9 ± 0.2	1.5 ± 0.2	2.8 ± 0.3
Ambroxol	1.8 ± 0.8	2.3 ± 0.8	1.2 ± 0.1	2.5 ± 1.0
Dexamethasone	2.0 ± 0.2	2.2 ± 0.3	1.1 ± 0.1	2.6 ± 0.3
Mann-Whitney *P* value	CN vs AMB $P = 0.32$ CN vs DX $P = 0.61$ DX vs AMB $P = 0.22$	CN vs AMB $P = 0.07$ CN vs DX $P = 0.11$ DX vs AMB $P = 0.44$	CN vs AMB $P = 0.09$ CN vs DX $P = 0.12$ DX vs AMB $P = 0.54$	CN vs AMB $P = 0.32$ CN vs DX $P = 0.48$ DX vs AMB $P = 0.65$

vation [37], ambroxol can also inhibit the chemotactic response and spontaneous migration of human polymorphonuclear cells [5, 29]. Ambroxol can increase the incorporation of labeled precursors into phospholipids of rat type II pneumocytes in culture, isolated perfused rat lungs, rat lung tissue in vivo, and rat bronchoalveolar lavage [9].Ambroxol is also known to increase phospholipid concentrations in the lung by inhibiting catabolism through reduced phospholipase activity in alveolar macrophages [31]. Steroids have been shown to reduce lysosomal enzyme release, oxygen radical production, and neutrophil aggregation [32]. In addition, dexamethasone has been shown to increase surfactant lipid pools of adult rat lungs by changing the phospholipid content and total protein levels of lamellar bodies [39]. Eik-Nes and collaborators [10] used dexamethasone to accelerate production of lung surfactant after lung injury. Finally, Floros et al. reported a significant increase in surfactant protein A mRNA and protein in adult rats treated with a single dexamethasone bolus ranging from 2 μg/kg to 20 mg/kg [13].

In the setting of experimental lung transplantation, steroids have been used by various authors to improve graft function during reperfusion with varying success. In an ex vivo lung preservation model, Hall et al. noted significantly improved lung compliance and histology following donor steroid treatment [15]. Novick et al. have investigated the effect of donor pretreatment with methylprednisolone in a canine in vivo lung transplant model on graft function after extended ischemia and reperfusion [27]. Steroid pretreatment demonstrated only a moderate improvement in oxygenation as the primary functional outcome variable in their study. In their interpretation of the data, both groups focused mainly on the anti-inflammatory effect of steroid treatment.

Based on the data from this study, donor pretreatment with dexamethasone failed to improve graft function during reperfusion, while administration of ambroxol resulted in an adverse outcome compared to untreated controls.

The total phospholipid levels in the lavage of donor rats did not rise significantly within 6 h after administration of either ambroxol or dexamethasone. The percentage of surface active phospholipids represented as large phospholipid aggregates was not affected by the type of drug treatment, and surface tension measurements were similar, irrespective of the treatment group. The lack of a significant effect on donor surfactant levels and function may have been related to the method of administration, since drug absorption may be incomplete after intraperitoneal administration. Also, the timing of administration may have been critical. Floros et al. have shown a significant rise in SP-A mRNA 24 h after administration. The 6-h interval used in this study was chosen to simulate a realistic therapeutic option in potential lung donors, a situation often providing only short intervals for treatment between organ donation and harvest. The use of static lung hyperinflation with 100% oxygen may have preserved endogenous surfactant levels to a point where drug therapy may not have been able to improve levels any further. Finally, assessment of surfactant levels was limited to the quantitative determination of overall phospholipid levels with special emphasis on surface-active large and surface-inactive small aggregates. Overall surfactant levels may remain unchanged despite significant alterations in the different phospholipid components [35]. Therefore, the similar phospholipid levels in the three control groups does not rule out a significant difference in surfactant function.

Repeated, standardized BAL was used to attempt evacuation of all surfactant from the airways for quantitative assessment. This method has a high rate of variability that may limit the interpretation of this particular data [21]. In addition, specific surfactant proteins could not be measured due to the absence of rat-specific antibodies for quantitative protein determination.

Despite similar surface tension and total phospholipid measurements in donor rats immediately prior to graft harvest, assessment of the dynamic pulmonary compliance in the donor animal before organ perfusion and ischemia demonstrated a significant, negative impact of ambroxol therapy on lung compliance. As donor lung compliance is also dependent on interstitial water content, these changes may be totally unrelated to ambroxol's effect on endogenous surfactant.

In order to evaluate the impact of drug pretreatment on lung function following prolonged graft ischemia, it is important to compare donor function before harvest with donor function following ischemia and during reperfusion. In a comparison of the mean baseline compliance before preservation to the initial measurements after ischemia, ambroxol pretreatment resulted in a 23% drop, dexamethasone pretreatment in a 32% drop, and lungs without drug pretreatment demonstrated a 43% drop in dynamic compliance. During reperfusion, the use of ambroxol for donor pretreatment resulted in a further decline in compliance while the DX and CN groups remained stable. In contrast, the resistance to airflow was reduced more by ambroxol than by dexamethasone treatment, which can perhaps be ascribed to the known secretolytic properties of these compounds. While oxygenation was similar in all three study groups, the use of ambroxol dramatically increased the pulmonary vascular resistance to a point where the donor grafts were almost not perfused by the end of the reperfusion period. How ambroxol could possibly affect endothelial function with increased obstruction to blood flow cannot be explained from the data derived from this and other studies.

An explanation of why a single bolus treatment with ambroxol for donor animals resulted in such poor performance during reperfusion cannot be limited to its effect on endogenous surfactant. Despite its described function as a free radical scavenger, very little is actually known in detail about the potential negative effects of ambroxol therapy on the nonspecific immune system. This study was not designed to specifically address the impact of donor pretreatment on the nonspecific immune system. Distinct assays of interleukin and cytokine levels in the future may help find answers to these questions.

It is important to note that the ischemic insult on the donor lung following extended ischemia of 16 h results in immense damage to the graft. This, in itself, may be so severe that positive effects of drugs with only moderate efficacy on ischemia/reperfusion injury may not be reflected in measurements of graft physiology. In addition, the relatively short follow-up period of 2 h may also affect the ability of this model to detect subtle modulations of reperfusion injury.

The majority of experimental studies on ischemia/reperfusion injury utilize perfectly healthy donor organs without prior injury, a major shortcoming of these studies. In the clinical setting, with lung trauma due to the effects of brain death, prolonged ventilation, hyperhydration, or general activation of the nonspecific immune system, the use of drugs such as dexamethasone or ambroxol may be more beneficial.

In conclusion, this study has shown that donor pretreatment with dexamethasone does not improve graft function when compared to untreated controls during ischemia/reperfusion injury. In contrast, donor pretreatment with ambroxol resulted in a significant, functional impairment of the graft, as reflected in a decreased lung compliance in the donor before harvest with subsequent further impediment of graft function after ischemia and during reperfusion.

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