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INHALED NITRIC OXIDE AND PENTOXIFYLLINE IN RAT LUNG TRANSPLANTATION FROM NON-HEART-BEATING DONORS

Shinya Murakami, MD*
Emile A. Bacha, MD**
Philippe Hervé, MD
Hélène Détruit
Alain R. Chapelier, MD
Philippe G. Darteville, MD
Guy-Michel Mazmanian, MD
The Paris-Sud University Lung
Transplantation Group

Background: In non-heart-beating donor lung transplantation, postmortem warm ischemia poses a special challenge. Inhaled nitric oxide and pentoxifylline have been shown to attenuate ischemia-reperfusion injury after lung transplantation. We hypothesized that concomitant administration of inhaled nitric oxide and pentoxifylline would result in a synergistic effect on ischemia-reperfusion lung injury. **Methods:** Lungs were harvested from non-heart-beating donors after 30 minutes of in situ warm ischemia, flushed, and stored for 2 hours at 4° C before left lung transplantation in rats. Inhaled nitric oxide (30 ppm) was added during cadaver ventilation and reperfusion; pentoxifylline was given intravenously throughout reperfusion. The following groups were studied ($n = 8$ each): control, pentoxifylline, nitric oxide, and nitric oxide + pentoxifylline. Hemodynamic indices and arterial blood gases were obtained after ligation of the right pulmonary artery. Lung myeloperoxidase and wet/dry ratio were measured after death. **Results:** All rats that did not receive nitric oxide died within 10 minutes after ligation. Inhaled nitric oxide significantly decreased pulmonary vascular resistance and improved recipient survival. Nitric oxide + pentoxifylline improved pulmonary vascular resistance, arterial oxygen tension, and survival even further and reduced lung myeloperoxidase as compared with

From the Laboratoire de Chirurgie Expérimentale, the Department of Thoracic and Vascular Surgery, and the Department of Heart-Lung Transplantation, Centre Chirurgial Marie Lannelongue, Paris-Sud University, Le Plessis Robinson, France.

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Address for reprints: Guy-Michel Mazmanian, MD, Centre

Chirurgial Marie-Lannelongue, 133 Avenue de la Résistance, 92350 Le Plessis Robinson, France.

*Present address: Kanazawa University School of Medicine, Department of Surgery, 13-1 Takara-Machi, Kanazawa 920, Japan.

**Present address: General Surgical Services, Massachusetts General Hospital, Harvard Medical School, Boston, Mass.

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the group that received nitric oxide only. Preservation solution flush time was significantly decreased in both groups receiving nitric oxide, suggesting that inhaled nitric oxide used during cadaver ventilation allows for a more even distribution of the preservation solution. **Conclusion:** We conclude that treatment with inhaled nitric oxide + pentoxifylline results in a synergistic protection from ischemia-reperfusion injury after non-heart-beating donor lung transplantation. This is likely the result of a dual action on the graft vasculature and neutrophil sequestration. (*J Thorac Cardiovasc Surg* 1997; 113:821-9)

Although lung transplantation is an established treatment for end-stage lung disease, the shortage of donors is the most widely accepted problem facing widespread application. Use of organs harvested from non-heart-beating donors is one of the possibilities that are currently being explored.¹ Experimental lung transplantation from non-heart-beating donors was pioneered by Egan,¹ Roberts,² Ulicny,³ and their co-workers. The period of warm ischemia associated with non-heart-beating donor transplantation poses a special challenge, and gradual anoxic cell death and endothelial dysfunction have been documented to occur.^{4,5} Warm ischemia is generally seen as more harmful than cold ischemia.⁵ Severe ischemia-reperfusion injury occurs in about 10% to 20% of recipients of lungs from heart-beating donors⁶ and is characterized by acute lung injury and pulmonary hypertension, in association with endothelial dysfunction, polymorphonuclear neutrophil (PMN) activation and sequestration, and release of PMN- and non-PMN-derived reactive oxygen species.⁷ The central role of PMNs in mediating ischemia-reperfusion injury is well documented, and blockade of PMN adhesion or leukocyte depletion results in improved lung function after transplantation.⁸

Nitric oxide (NO), the endothelium-dependent relaxing factor, is associated with endothelial homeostasis.⁹ It is a potent vasodilator and inhibits PMN adhesion to the endothelium.⁷ During early reperfusion, pulmonary endogenous NO levels plummet,⁹ and a recent study has shown that inhaled NO attenuates ischemia-reperfusion injury after porcine lung transplantation.¹⁰

Pentoxifylline (PTX), a methylxanthine derivative, has been shown to reduce PMN-dependent lung injury in several animal models.¹¹⁻¹⁴ Previous studies from our laboratory, as well as others, demonstrated that PTX reduces ischemia-reperfusion injury after experimental lung transplantation and in an isolated rat lung model.¹¹⁻¹³

Therefore we hypothesized that inhaled NO, given during cadaver ventilation and reperfusion, and PTX, given during reperfusion, might have a beneficial synergistic effect on ischemia-reperfusion injury and might improve survival after lung transplantation from non-heart-beating donors.

Material and methods

Thirty-two normal weight-matched pairs of male Sprague-Dawley rats (Iffa Credo, Paris, France) weighing 300 to 350 gm were used. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication No. 86-23, revised 1985).

Non-heart-beating donor preparation. The donor rats were anesthetized with sodium thiopental (50 mg/kg intraperitoneally). After median laparosternotomy, each animal was exsanguinated through the abdominal aorta and put to death with hypovolemia. Heparin was not given. Donor death was identified by cessation of cardiac activity. Immediately after being put to death, the animal was tracheostomized and its lungs were ventilated with a Harvard rodent ventilator (model 680, Harvard Apparatus Co., Inc., S. Natick, Mass.) at a rate of 60 breaths/min, a tidal volume of 10 ml/kg, and a positive end-expiratory pressure of 1 cm H₂O. Ventilation was performed with a gas mixture (60% oxygen, 40% nitrogen) supplemented or not with NO at 30 ppm. Non-heart-beating donor animals were maintained in the supine position and left at room temperature for 30 minutes.

Lung harvest. A polyethylene cannula was inserted into the pulmonary artery through the right ventricle and ligated. After the incision of the left atrium, both lungs were flushed with 25 ml of an extracellular type of preservation solution (Celsior solution, Pasteur-Mérieux, Lyon, France), cooled to 4° C, through the pulmonary arterial cannula at a pressure of 20 cm H₂O. The composition of Celsior solution was as follows: lactobionate, 80 mmol/L; mannitol, 60 mmol/L; reduced glutathione, 3 mmol/L; glutamate, 20 mmol/L; histidine, 30 mmol/L; potassium, 15 mmol/L; sodium, 100 mmol/L; chloride, 41.5 mmol/L; magnesium, 13 mmol/L; and calcium, 0.26 mmol/L. Reduced glutathione was stocked in a separate

Fig. 1. Experimental protocol. *NO*, Nitric oxide; *PTX*, pentoxifylline; *PA*, pulmonary artery; *MPO*, myeloperoxidase; *W/D*, wet/dry ratio.

air-proof syringe and added to the preservation solution immediately before use. The time required to perfuse the 25 ml of preservation solution at constant pressure (20 cm H₂O) was recorded as an index of pulmonary vascular resistance during harvest after postmortem warm ischemia and was considered the pulmonary artery flush time. The pulmonary artery and pulmonary vein were divided as proximally as possible, the bronchus was ligated with the lung partially inflated and then divided, and the lung was removed. The cuffs made from 16-gauge intravenous Teflon catheters (Critikon, Inc., Tampa, Fla.) were placed in the lumina of the pulmonary artery and pulmonary vein and fixed with 7-0 Prolene suture (Ethicon, Inc., Somerville, N.J.). The lung was then stored at 4° C for 2 hours in a container filled with saline solution. The average time required to harvest the lung was 15 minutes.

Orthotopic right lung transplantation. The recipient rats were premedicated by intramuscular injection of atropine (1.25 mg/kg) and anesthetized with intraperitoneal administration of sodium thiopental (50 mg/kg). After tracheostomy, the animals' lungs were ventilated at a rate of 60 breaths/min, a tidal volume of 10 ml/kg, and a positive end-expiratory pressure of 1 cm H₂O. Ventilation was performed with a gas mixture (60% oxygen, 40% nitrogen). A left thoracotomy was performed at the fourth intercostal space. The left bronchus, pulmonary artery, and pulmonary vein were isolated, crossclamped, and divided as distally as possible, and the native lung was removed. The bronchus cuff was placed immediately before transplantation to allow the donor lung to be inflated as soon as possible. The cuffs were connected to their respective structures in the recipient and anchored with 6-0 Prolene suture.¹⁵ The bronchial crossclamp was released first to allow ventilation. The vascular clamps were then released to reestablish blood flow, and the rat lung grafts were reperfused up to 1 hour (or until recipient

death). Warm ischemic time during connection of the cuffs was maintained below 12 minutes.

Measurement of lung graft function. Lung graft function was measured as described by Naka and associates.¹⁶ After lung transplantation, the recipient was placed in the supine position and a transverse thoracosternotomy was performed in the fourth intercostal space. Polyethylene cannulas (external diameter 1 mm) were introduced into the main pulmonary artery and left atrium. A perivascular flow probe (type 4 RB, Transonic Systems Inc., Ithaca, N.Y.) was then placed around the main pulmonary artery. Pulmonary arterial pressure (PAP) and left atrial pressure (LAP), in millimeters of mercury, were continuously monitored with a P23 ID transducer (Statham, France), and pulmonary artery flow (PA flow) was measured with a model T106 small animal blood flowmeter (Transonic Systems Inc.). Pulmonary vascular resistance (PVR) was calculated as follows: $PVR = (\text{mean PAP} - \text{LAP}) / \text{PA flow}$ (millimeters of mercury per milliliter per minute). Arterial blood gas analyses were done with an ABL-2 gas analyzer (Radiometer A/S, Copenhagen, Denmark). Baseline measurements of hemodynamics and arterial gas analysis were taken 30 minutes after reperfusion. The native right pulmonary artery was then ligated, and serial measurements of hemodynamics were taken every 5 minutes until the animal was killed at 60 minutes after onset of reperfusion (or until recipient death). Thirty minutes after ligation of the native right pulmonary artery or immediately before cessation of cardiac mechanical activity, arterial gas analysis was done. Thirty minutes after ligation of the right pulmonary artery, or at the time of recipient death, the transplanted lungs were removed, rinsed briskly in physiologic saline solution, and divided into two parts. One part was snap frozen in liquid nitrogen until the time of myeloperoxidase assay (MPO), performed by means of the method described by Mullane, Kraemer, and Smith.¹⁷ The other was dried in an oven at 60° C and weighed daily

Fig. 2. Effect of nitric oxide (NO) on preservation solution flush time (time required to perfuse 25 ml at constant pressure). Data are shown as the mean $\pm$ standard error of the mean. * $p < 0.05$ versus control; † $p < 0.05$ versus pentoxifylline group (PTX).

until the dry weight was stable for more than 5 days, to allow determination of the wet/dry lung weight ratio.

Gas preparation. NO gas was purchased from CFPO Inc. (Paris, France) as a mixture of 450 ppm in pure nitrogen. Inspired gas was mixed at the concentration of 60% oxygen and 40% nitrogen, supplemented or not with NO (30 ppm), and stored in a balloon. The concentrations of NO and nitrogen dioxide were assessed by means of the electrochemical method (NOxBox, Bedfont Scientific Ltd., United Kingdom). The gas mixture was passed through the soda-lime bottle just before it entered the ventilator.

PTX administration. PTX (Hoechst, Paris, France) diluted in saline solution was given as an intravenous bolus of 1 mg/kg, which was followed by a continuous infusion as a dosage of 1.5 mg/kg per hour. PTX administration was started 15 minutes before reperfusion and continued throughout the reperfusion period.¹³

Graft tissue MPO activity. MPO, a marker enzyme specific to PMNs, can be used as an indirect measure of tissue PMN infiltration. The method described by Mul-lane, Kraemer, and Smith¹⁷ was used. In brief, lung tissue was homogenized in 10% weight/volume of hexadecyltri-methyl ammonium bromide phosphate buffer with a Poly-tron homogenizer (Kinematica, Luzern, Switzerland). The homogenate was sonicated, frozen at -70°C , thawed, and centrifuged. Supernatant was assayed for MPO activity spectrophotometrically. The change in absorbance was measured on a spectrometer (model 25, Beckman, Fullerton, Calif.). One unit of MPO activity is defined as the activity degrading 1 μmol of peroxide per minute at 25°C .

Experimental protocol (Fig. 1). The following four groups were studied ($n = 8$ each): control, PTX, NO, and NO+PTX. In the control and PTX groups, the lungs of non-heart-beating donors and recipients were ventilated without NO. In the NO and NO+PTX groups, the lungs of non-heart-beating donors and recipients were ventilated with the addition of inhaled NO.

Statistical analysis. Results are expressed as mean $\pm$ standard error of the mean. Statistical analysis was performed by nonpaired Student's t test, two-way analysis of variance, and Fisher's test for multiple comparison using SUPERANOVA (Abacus Concepts, Berkeley, Calif.). Comparisons of survival after ligation of the native right pulmonary artery were made by Kaplan-Meier and log-rank analysis. A p value < 0.05 was considered significant.

Results

Pulmonary arterial flush time (Fig. 2). Pulmonary arterial flush times of the groups treated with inhaled NO were significantly shorter ($p = 0.0001$) than those of the groups treated without NO.

Recipient survival (Fig. 3). All rats that did not receive NO died within 10 minutes after ligation of the right pulmonary artery (within 40 minutes of reperfusion). Survival was significantly improved in groups that received NO alone ($p = 0.02$) or NO+PTX ($p = 0.01$). Survival in the group that received NO+PTX was further enhanced ($p = 0.02$).

Hemodynamic measurements (Figs. 4 and 5). Five minutes after ligation of the right pulmonary artery, pulmonary arterial flow and pulmonary vascular resistance were significantly increased and decreased ($p = 0.001$ and $p = 0.0001$, respectively) by inhaled NO, given either alone or with the addition of PTX, as compared with the control and the PTX groups. Thus PTX alone had no effect on pulmonary arterial flow or pulmonary vascular resistance. However, pulmonary arterial flow was significantly increased ($p = 0.04$) by inhaled NO+PTX, as compared with inhaled NO alone, indicating that PTX had an additive effect on the action of NO. Because all recipients of groups that did not receive NO died within 10 minutes of the right pulmonary artery ligation, pulmonary arterial flow and pulmonary vascular resistance were compared again, between the group that received NO alone and the group that received NO+PTX, at a late phase, that is, at the final time at which recipients were alive. Again, similar to the results obtained at 5 minutes, treatment with inhaled NO+PTX resulted in significantly improved pulmonary arterial flow ($p = 0.01$) as compared with inhaled NO alone.

Gas exchange. Arterial blood gases were measured at the latest possible time, either at 30 minutes

Fig. 3. Recipient actuarial survival. * $p < 0.05$ versus control group; † $p < 0.05$ versus pentoxifylline group (PTX); ‡ $p < 0.05$ versus nitric oxide group (NO).

Fig. 4. Pulmonary artery flow (PA flow) and pulmonary vascular resistance (PVR) 5 minutes after ligation of the native right pulmonary artery. Data are shown as the mean $\pm$ standard error of the mean. * $p < 0.05$ versus control group; † $p < 0.05$ versus pentoxifylline group (PTX); ‡ $p < 0.05$ versus nitric oxide group (NO).

after pulmonary artery ligation for the survivors or immediately before cessation of cardiac activity for the others. Rats treated with inhaled NO+PTX had the best oxygenation (175 ± 28 mm Hg, $p = 0.0006$) as compared with all other groups (79.5 ± 21 mm Hg in the NO group, 53 ± 2 mm Hg in the PTX group, and 49 ± 10 mm Hg in the control group). No difference was observed for arterial carbon dioxide tension among the different groups ($p = 0.6$).

Wet/dry weight ratio (Fig. 6). Only group NO+PTX exhibited a significantly decreased wet/dry ratio, a measure of lung permeability to fluid, as compared with all other groups ($p = 0.04$).

Graft PMN sequestration (Fig. 7). PMN sequestration into reperfused tissues is time-dependent.¹⁸ Inasmuch as all recipients in groups control and PTX died within 40 minutes of reperfusion, samples for MPO determination were frozen after less reper-

Fig. 5. Pulmonary artery flow (*PA flow*) and pulmonary vascular resistance (*PVR*) of nitric oxide (*NO*) and nitric oxide + pentoxifylline (*NO/PTX*) groups at the final time at which the recipients were alive. Data are shown as the mean $\pm$ standard error of the mean. $‡p < 0.05$ versus *NO* group.

Fig. 6. Grafted lung wet/dry weight ratio (*W/D*). Data are shown as the mean $\pm$ standard error of the mean. $*p < 0.05$ versus control group; $†p < 0.05$ versus pentoxifylline (*PTX*) group; $‡p < 0.05$ versus *NO* group.

fusion time than those in groups *NO* and *NO+PTX*. Therefore groups control and *PTX* (Fig. 7, right panel) and groups *NO* and *NO+PTX* (Fig. 7, left panel) were compared separately. Groups control

and *PTX* exhibited no differences in *MPO* activity. However, graft *PMN* sequestration was significantly inhibited after *NO+PTX* as compared with *NO* alone ($p = 0.01$).

Discussion

This study demonstrates that inhaled *NO*, given during cadaver ventilation and reperfusion, facilitates distribution of the flush solution, prevents ischemia-reperfusion-induced pulmonary vasoconstriction, and results in improved survival after transplantation of lungs from non-heart-beating donors. Addition of *PTX* to *NO* during reperfusion results in decreased *PMN* sequestration in the graft, decreased edema (wet/dry ratio), improved pulmonary hemodynamics and oxygenation, and further enhanced survival. In this model, *PTX* alone was not protective, but a synergistic effect of *NO* and *PTX* was observed.

The present protocol was chosen in an attempt to reproduce the conditions to be encountered in clinical lung transplantation from non-heart-beating donors. These conditions associate an in situ warm ischemic period followed by cold ischemia and transplantation. We used cadaver ventilation because it has been shown to reduce ischemic damage in experimental non-heart-beating donor lung transplantation.³ We did not heparinize the animals before putting them to death. This is in agreement

Fig. 7. Grafted lung myeloperoxidase (MPO) activity. Because PMN sequestration is time-dependent, values were compared separately for control and pentoxifylline (PTX) groups (*left panel*) and nitric oxide (NO) and NO+PTX groups (*right panel*). Data are shown as the mean $\pm$ standard error of the mean. ‡ $p < 0.05$ versus NO group.

with a recent study² showing that experimental non-heart-beating donor lung transplantation can be done without heparinization. Ligating the contralateral pulmonary artery keeps the animals to a rigorous functional standard, in which the entire cardiac output is directed toward the allograft and survival entirely depends on graft viability.¹⁶ Because of the ischemia-reperfusion-induced pulmonary hypertension, ligation of the right pulmonary artery places an acute strain on the right side of the heart, and death occurred less from hypoxia than from right ventricular failure.

Beneficial effects on non-heart-beating donor grafts have previously been reported with the use of free radical scavengers² and urokinase.¹⁹ NO, by maintaining endothelial homeostasis, vasodilating the pulmonary arterial bed, and inhibiting PMN and platelet adherence, exerts a protective effect in ischemia-reperfusion injury.⁷ Prevention of pulmonary ischemia-reperfusion injury after heart-beating donor lung transplantation has recently been achieved by stimulation of the distal NO pathway,¹⁶ NO donors in the flush solution,²⁰ or NO inhalation.¹⁰ Our choice to use NO inhalation as a means to prevent ischemia-reperfusion injury after non-heart-beating donor transplantation is based on the fact that gaseous NO can be delivered directly to the pulmonary endothelium by ventilation, even after

cessation of circulation. Furthermore, a study by our group¹⁰ showed that inhaled NO, applied at reperfusion, attenuates ischemia-reperfusion injury after porcine lung transplantation. We²¹ also found that it prevents endothelial dysfunction, pulmonary hypertension, and PMN sequestration after warm lung ischemia and reperfusion in an isolated porcine lung preparation. Nevertheless, the use of inhaled NO in ischemia-reperfusion injury remains controversial, because exogenous NO can combine with superoxide anions to generate toxic intermediates such as peroxynitrite.²² Eppinger and associates²³ reported this phenomenon in pulmonary ischemia-reperfusion injury. However, very high doses of inhaled NO (80 ppm) were used in this later study. In contrast to the present experiment, Naka and associates¹⁶ did not find an improved survival with inhaled NO after lung transplantation in the heart-beating donor rat model. Although experimental conditions (warm vs cold ischemia, flush solution, higher NO concentration) differed, we used inhaled NO throughout the period of in situ warm ischemia, because a recent study shows that ischemia-reperfusion injury in lungs from non-heart-beating donors is further reduced when NO is given during both warm ischemia and reperfusion, as compared with administration of NO during reperfusion only.²⁴ Possible mechanisms include inhibition of xanthine oxi-

dase,²⁵ which accumulates during ischemia and reacts with oxygen and hypoxanthine to produce a burst of superoxide and hydrogen peroxide at reperfusion. The beneficial effect seen in our study could also be explained by the vasodilator effect of NO during administration of the flush solution, resulting in shorter flush times seen in both groups in which inhaled NO was used and suggesting a more even distribution of the preservation solution during cold ischemia.

Several groups recently reported that PTX was beneficial after ischemia-reperfusion injury in an ex vivo lung model and improved survival in large-animal lung transplantation.^{6, 11-13} This effect was associated with reduced PMN sequestration. As opposed to these findings, PTX alone did not improve PMN lung sequestration, pulmonary vasoconstriction, and survival. PMN sequestration was relatively low in groups that received PTX but not significantly different from that in the control group. This is likely due to the lower pulmonary arterial blood flow in the transplanted lung and shorter survival of these groups, resulting in less reperfusion time and thus less PMN adhesion.¹⁸ PTX has been reported to have a vasodilatory effect, but this effect was seen at relatively higher concentrations.²⁶ Finally, PTX was given only during reperfusion in the present study and was not added to the flush solution. A recent study indicated in a canine lung transplantation model that PTX used during reperfusion did not improve survival as compared with PTX administration in the flush solution.¹¹

Although PTX alone did not reduce PMN sequestration, its addition to inhaled NO resulted in a further reduction in PMN sequestration. Analogous to the effect on PMN sequestration, a synergistic effect on the pulmonary vasculature was also observed, resulting in improved oxygenation and right ventricular function, decreased wet/dry ratio, and comparatively better survival in animals treated with NO and PTX. Possible mechanisms include inhibition of expression of adhesion molecules located on PMNs, either directly²⁷ or through a decrease in the production of various cytokines, such as tumor necrosis factor- α ,²⁸ radical oxygen species scavenging,²⁹ improvement of microcirculation,³⁰ and prevention of ischemia-reperfusion-induced endothelial dysfunction.⁵ Recent investigative work has shown that PTX-induced protection can involve different pathways than NO-induced protection.⁵ For example, a recent study concluded that PTX reversed tumor necrosis factor-induced endothelial

dysfunction, whereas L-arginine did not.²⁸ The noted synergistic effect could also be a reflection of the improved perfusion induced by inhaled NO, resulting in facilitated distribution of PTX to the microcirculation.²⁹ Further experiments should confirm the beneficial effects of combined NO and PTX treatment in a large animal, non-heart-beating donor model.

Our findings are clinically relevant. Inhaled NO and PTX have both been used in clinical practice for a number of years, and widespread organ shortage would be lessened if lungs from non-heart-beating donors could be used. In conclusion, we found that PTX administration during reperfusion alone was not beneficial in this model, whereas inhaled NO was beneficial after non-heart-beating donor lung transplantation in terms of survival and prevention of ischemia-reperfusion-induced pulmonary vasoconstriction. Treatment with PTX and NO resulted in a synergistically enhanced protection from ischemia-reperfusion injury and further improved survival. This is likely the result of a dual vasculature and PMN sequestration.

REFERENCES

1. Egan TM, Lambert CJ, Reddick R, Ulicny KS, Keagy BA, Wilcox BR. A strategy to increase the donor pool: use of cadaver lungs for transplantation. *Ann Thorac Surg* 1991;52:1113-21.
2. Roberts CS, Hennington MH, D'Armini AM, Griffith PK, Lemasters JJ, Egan TM. Donor lungs from ventilated cadavers: impact of a free radical scavenger. *J Heart Lung Transplant* 1996;15:275-82.
3. Ulicny KS Jr, Egan TM, Lambert CJ, Reddick RL, Wilcox BR. Cadaver lung donors: effect of preharvest ventilation on graft function. *Ann Thorac Surg* 1993;55:1185-91.
4. D'Armini A, Tom EJ, Roberts CS, Henke DC, Lemasters JJ, Egan TM. When does the lung die? Time course of high energy phosphate depletion and relationship to lung viability after "death." *J Surg Res* 1995;59:468-74.
5. Normandin L, Herve P, Brink C, Chapelier A, Darteville P, Mazmanian G. L-Arginine and pentoxifylline attenuate endothelial dysfunction after lung reperfusion injury in the rabbit. *Ann Thorac Surg* 1995;60:646-50.
6. Novick RJ, Gehman KE, Ali IS, Lee J. Lung preservation: the importance of endothelial and alveolar type II cell integrity. *Ann Thorac Surg* 1996;62:302-14.
7. Cooke JP, Tsao PS. Cytoprotective effect of nitric oxide. *Circulation* 1993;88:245-50.
8. Schueler S, De Valeria PA, Hatanaka M, et al. Successful twenty-four-hour lung preservation with donor core cooling and leukocyte depletion in an orthotopic double lung transplantation model. *J Thorac Cardiovasc Surg* 1992;104:73-82.
9. Pinsky DJ, Naka Y, Chowdhury NC, Liao H, Oz MC, Michler RE, et al. The nitric oxide/cyclic GMP pathway in organ transplantation: critical role in successful lung preservation. *Proc Natl Acad Sci U S A* 1994;91:12086-90.

10. Bacha EA, Hervé P, Murakami S, Chapelier A, Mazmanian G-M, de Montpreville V, et al. Lasting beneficial effect of short-term inhaled nitric oxide on graft function after lung transplantation. *J Thorac Cardiovasc Surg* 1996;112:590-8.
11. Yamashita M, Schmid RA, Okabayashi K, Ando K, Kobayashi J, Cooper JD, et al. Pentoxifylline in flush solution improves early lung allograft function. *Ann Thorac Surg* 1996;61:1055-61.
12. Reignier J, Mazmanian GM, Détruit H, Chapelier A, Weiss M, Libert JM, et al. Reduction of ischemia-reperfusion injury by pentoxifylline in the isolated rat lung. *Am J Respir Crit Care Med* 1994;150:342-7.
13. Chapelier A, Reignier J, Mazmanian M, Dulmet E, Libert JM, Darteville P, et al. Amelioration of reperfusion injury by pentoxifylline after lung transplantation. *J Heart Lung Transplant* 1995;14:676-83.
14. Mandell GL. ARDS, neutrophils and pentoxifylline. *Am Rev Respir Dis* 1988;138:1103-5.
15. Mizuta T, Kawaguchi A, Nakahara K, Kawashima Y. Simplified rat lung transplantation using a cuff technique. *J Thorac Cardiovasc Surg* 1989;97:578-81.
16. Naka Y, Roy DK, Smerling AJ, Michler RE, Smith CR, Stern DM, et al. Inhaled nitric oxide fails to confer the pulmonary protection provided by distal stimulation of the nitric oxide pathway at the level of cyclic guanosine monophosphate. *J Thorac Cardiovasc Surg* 1995;110:1434-41.
17. Mullane KM, Kraemer R, Smith B. Myeloperoxidase activity as a quantitative assessment of neutrophil infiltration in ischemic myocardium. *J Pharmacol Methods* 1985;14:157-67.
18. Milazzo VJ, Ferrante RJ, Sabido F, Silva MB Jr, Hobson RW, Duran WN. Time course of leukocyte adhesion to endothelium in ischemia-reperfusion. *J Surg Res* 1996;61:139-42.
19. Umemori Y, Date H, Uno K, Aoe M, Ando A, Shimizu N. Improved lung function by urokinase infusion in canine lung transplantation using non-heart-beating-donors. *Ann Thorac Surg* 1995;59:1513-8.
20. Naka Y, Chowdhury NC, Oz MC, Smith CR, Yano OJ, Michler RE, et al. Nitroglycerin maintains graft vascular homeostasis and enhances preservation in an orthotopic rat lung transplant model. *J Thorac Cardiovasc Surg* 1995;109:206-11.
21. Barbotin-Larrieu F, Mazmanian GM, Baudet B, Détruit H, Chapelier A, Darteville P, et al. Prevention of ischemia reperfusion lung injury by inhaled nitric oxide in neonatal piglets. *J Appl Physiol* 1996;80:782-8.
22. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci U S A* 1994;87:1620-4.
23. Eppinger MJ, Ward PA, Jones ML, Boiling SF, Deeb GM. Disparate effects of nitric oxide on lung ischemia-reperfusion injury. *Ann Thorac Surg* 1995;60:1169-76.
24. Murakami S, Bacha EA, Hervé P, Détruit H, Chapelier A, Darteville P, et al. Prevention of reperfusion injury by inhaled nitric oxide in lungs harvested from non heart beating donors. *Ann Thorac Surg* 1996;62:1632-8.
25. Hassoun PM, Yu FS, Zulueta JJ, White AC, Lanzillo JJ. Effect of nitric oxide and cell redox status on the regulation of endothelial cell xanthine dehydrogenase. *Am J Physiol* 1995;268:L809-17.
26. Kamphuis J, Smith P, Thien T. Vascular effects of pentoxifylline in humans. *J Cardiovasc Pharmacol* 1994;24:648-54.
27. Sullivan GW, Carpenter HT, Mandell GL. Pentoxifylline modulates activation of human neutrophils by amphotericin B in vitro. *Antimicrob Agents Chemother* 1992;36:408-16.
28. Greenberg S, Xie J, Wang Y, Cai B, Kolls J, Nelson S, et al. Tumor necrosis factor-alpha inhibits endothelium-dependent relaxation. *J Appl Physiol* 1993;74:2394-403.
29. Pasquier C, Franzini E, Abedinzadeh Z, Kaouadji MN, Hakim J. Gamma and pulse radiolysis study of pentoxifylline, a methylxanthine. *Int J Radiat Biol* 1991;60:433-47.
30. Berens KL, Duke DR. Pentoxifylline in the isolated perfused rat kidney. *Transplantation* 1990;49:876-9.