CARDIAC AND PULMONARY REPLACEMENT

INHALED NITRIC OXIDE IMPROVES LUNG ALLOGRAFT FUNCTION AFTER PROLONGED STORAGE

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Morbidity caused by early allograft dysfunction, manifested by a progressive increase in pulmonary vascular resistance and a decrease in oxygenation, remains a serious problem in lung transplantation. Inhalation of nitric oxide, an essential homeostatic molecule, has been shown to have beneficial effects on a variety of acute lung injuries. The purpose of the present study was to investigate the effect of inhaled nitric oxide on posttransplant function of canine left lung allografts. Fourteen dogs underwent left lung allotransplantation. Donors received systemic heparin and prostaglandin E_1 followed by pulmonary artery flush with modified Euro-Collins solution. Donor left lungs were stored for 18 hours at 1° C and subsequently implanted. Immediately after reperfusion, the contralateral right main pulmonary artery and bronchus were ligated. The chest was closed and recipients turned to the supine position for the 6-hour assessment period. Hemodynamic and arterial and venous blood gas analyses were made at 15-minute intervals at an inspired oxygen fraction of 1.0 and 5 cm of water positive end-expiratory pressure. Animals were killed at the end of the assessment. Allograft myeloperoxidase activity assays and wet/dry weight ratios were done. In group I ($n = 5$), nitric oxide gas was administered continuously at concentrations of 60 to 70 ppm before reperfusion and throughout the 6-hour assessment period. In group II ($n = 5$), nitric oxide administration was initiated at the same concentration after reperfusion injury had developed. Group III animals ($n = 4$) received no nitric oxide. Significant improvement in gas exchange was apparent in group I. At the end of the 6-hour assessment period, mean arterial oxygen tension was 253.8 ± 44.7 mm Hg and 114.9 ± 25.5 mm Hg in groups I and III, respectively ($p < 0.05$). Group II animals had no improvement in oxygenation with nitric oxide. Systemic hemodynamics were unaffected by nitric oxide. However, an immediate decrease in pulmonary vascular resistance was noted. Group I myeloperoxidase activity was significantly lower than that in control group III (0.24 ± 0.06 versus 0.36 ± 0.04 units, respectively; $p < 0.05$). (J Thorac Cardiovasc Surg 1996;112:293-9)

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Lung transplantation has become a successful strategy for the treatment of a variety of end-stage lung diseases. The results of lung transplantation now equal those of other solid organs. However, early postoperative allograft dysfunction

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remains a common and unpredictable problem. This transient allograft dysfunction, sometimes severe, is accompanied by an increase in pulmonary artery pressure and poor gas exchange.[1]

Nitric oxide (NO) is a potent endogenous mediator produced by a variety of cells including vascular endothelium and is believed to be identical to the endothelium-derived relaxing factor.[2, 3] Nitric oxide has been shown to have many physiologic properties that play an important role in the maintenance of vascular homeostasis,[4, 5] neurotransmission, and immune function.[6] NO is known to be a potent vasodilator and a potent inhibitor of platelet aggregation[3, 7] and adhesion.[8] NO also has potent antineutrophil activity that includes the inhibition of neutrophil aggregation[9] and neutrophil adhesion.[10, 11] Furthermore, it was demonstrated recently that NO modulates microvascular permeability and that when NO production by the endothelium is diminished, vascular permeability is dramatically increased.[12]

Inhalation of NO gas in concentrations of 5 to 80 ppm has been shown, in animals and human beings, to produce significant pulmonary vasodilation without causing systemic vasodilation,[13] even in the presence of pulmonary endothelial injury.[14] NO is inactivated rapidly by binding with high affinity to hemoglobin. Therefore inhaled NO results in pulmonary vasodilation limited to ventilated regions of lung. In patients with acute lung injury such as acute respiratory distress syndrome, inhalation of low-concentration NO selectively improves the perfusion of ventilated alveoli, resulting in an improvement of pulmonary gas exchange.[15]

This study was undertaken to investigate whether inhalation of NO, administered before and after reperfusion injury at a concentration of 60 to 70 ppm, would decrease ischemia-reperfusion lung injury in an 18-hour preserved canine lung allograft model.

Material and Methods

Adult conditioned mongrel dogs were studied. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Donor procedure. Donor animals were anesthetized with intravenous thiopental sodium (10 mg/kg), followed by atropine (0.5 mg), and intubated with an endotracheal tube (outer diameter 9 mm). Mechanical ventilation was established (Bennett MA1, Puritan Bennett, Inc., Overland Park, Kan.) with 100% oxygen at a tidal volume of 20 ml/kg, a rate of 12 breaths/min, and with a positive end-expiratory pressure of 5 cm H_2O. After a median sternotomy, the superior and inferior vena cavae, the ascending aorta, the main pulmonary artery, and the trachea were isolated. Animals were heparinized (400 U/kg) before insertion of a curved metal-tipped cannula (Sarns, Inc., Ann Arbor, Mich.) through a purse-string suture in the main pulmonary artery. Before the administration of flush solution, 250 μg prostaglandin E_1 (Prostin VR Pediatric, The Upjohn Company, Kalamazoo, Mich.) was injected directly into the main pulmonary artery. Cardiac inflow was occluded by ligation of the superior and inferior vena cavae 20 seconds after the infusion of prostaglandin E_1. The proximal inferior vena cava was cut and the tip of the left atrium was excised for decompression of the pulmonary artery flush. Immediately, the lungs were perfused at a pressure of 40 cm H_2O with 50 ml/kg of cold (1° C) Euro-Collins solution, modified by the addition of 4 mmol/L $MgSO_4$ and 32.7 gm/L glucose. During the flush the lungs were also topically cooled by flooding the chest with cold (1° C) saline solution. When the flush was completed, the trachea was clamped at end-inspiration and the heart-lung block excised. The harvested organs were placed in plastic bags containing cold (4° C) modified Euro-Collins solution and stored in ice slush. Lungs were removed from storage immediately before implantation at a time to coincide with a total ischemic time of 18 hours.

Recipient procedure. Recipient animals were anesthetized in the same manner as the donors and the lungs ventilated with an adjustable-rate Harvard pump respirator (model 613, Harvard Apparatus, South Natick, Mass.) with 98.5% oxygen and 1.5% halothane. A right femoral arterial line and Swan-Ganz catheter (Baxter Healthcare Corp., Edwards Division, Santa Ana, Calif.) were placed and continuously transduced (model 1290A, Hewlett-Packard, Andover, Mass.). After left thoracotomy, a wedge resection of the recipient's native left lung was taken as a control sample for myeloperoxidase activity assay (described later). The contralateral main pulmonary artery and upper and lower bronchus were mobilized and encircled separately with umbilical tapes. Left pneumonectomy was then done.

The donor left lung was separated from the heart-lung block. Left single-lung allotransplantation was done as previously described.[16] The lung was kept cold with crushed ice during implantation. The left atrial anastomosis was done first with a continuous everting mattress suture. Continuous over-and-over suture was used for both pulmonary artery and bronchus. After completion of these anastomoses, the left lung was reperfused after removal of air. A Millar pressure transducer (Millar Instruments, Inc., Houston, Tex.) was placed in the left atrium and two chest tubes were inserted. The contralateral right bronchial tapes were firmly tied, and airway occlusion was confirmed with a fiberoptic bronchoscope. Subsequently the contralateral right pulmonary artery was ligated. At this point, the ventilator was changed to a Bennett model MA1 (Puritan-Bennett, Carlsbad, Calif.) with 100% oxygen at a tidal volume of 550 ml, a rate of 20 breaths/min, and 5 cm of water positive end-expiratory

pressure. The chest was closed with skin staples and the animals turned to the supine position for a 6-hour assessment period.

It is apparent that different anesthesia techniques were used for donors and recipients. For lengthy implantation procedures a gas anesthesia technique was required and the Harvard ventilator (Harvard Apparatus, Inc., Natick, Mass.) was connected to a gas blender. For the shorter donor procedure and for subsequent recipient assessments (described later), an intravenous anesthetic technique was sufficient. However, precise control of inspired oxygen fraction and positive end-expiratory pressure levels was required and, for that reason, a Bennett MA1 ventilator was used.

Study groups. Fourteen dogs were allocated randomly to three groups. In group I ($n = 5$), NO gas was administered continuously at concentrations of 60 to 70 ppm before reperfusion throughout the 6-hour assessment period. In group II ($n = 5$), NO was administered to the recipient dogs at the same concentration as that in group I. For group II animals NO therapy was initiated after reperfusion injury was established. For purposes of this study, this point occurred when the ratio of arterial oxygen tension (Pa_{O_2}) to inspired oxygen fraction fell below 150 or after 3 hours of assessment, whichever came first. Group III animals ($n = 4$) received no NO.

Gas administration. In group I animals NO administration was initiated 10 minutes before reperfusion. The NO/N_2 gas mixture (2200 ppm NO in pure N_2) (Scott Medical Products, Plumsteadville, Pa.) was administered directly into the inspiratory limb of the respirator circuit, about 50 cm proximal to the endotracheal tube. Inspired NO and nitrous dioxide (NO_2) concentrations were continuously measured just proximal to the endotracheal tube with the use of chemiluminescence analyzers (model AR-PRN-1, B & W Technologies, Calgary, Alberta, Canada) and were recorded every 15 minutes. NO_2 concentration was kept less than 4 ppm.

Measurements. After repositioning of the animals to the supine position just after skin closure, the 6-hour assessment period was started. During this time, anesthesia was maintained with intravenous administration of thiopental sodium. Systemic arterial, pulmonary arterial, central venous, and left atrial pressures were continuously recorded throughout the assessment period. Cardiac output was determined hourly (model 9520, Edwards Laboratories, Inc., Santa Ana, Calif.). Arterial and mixed venous blood were collected for gas analysis every 15 minutes. Sodium bicarbonate was infused intravenously as necessary to maintain pH in physiologic range. Intravenous Ringer's lactate solution was administered throughout the surgical procedure and assessment in the recipient. Alveolar arterial oxygen difference ($A\text{-}aD_{O_2}$) and pulmonary vascular resistance (PVR) were determined hourly as follows:

$A\text{-}aD_{O_2}$ (mm Hg) = barometric pressure − water vapor pressure − Arterial carbon dioxide tension (Pa_{CO_2}) − Pa_{O_2}

PVR (dynes/sec/cm^{-5}) = (mean pulmonary artery pressure − left atrial pressure)/cardiac index × 79.9

Airway edema fluid from the left lung was collected via fiberoptic bronchoscope 10 minutes before each arterial blood gas assessment, and the total suction volume for the 6-hour assessment period was measured. After the final measurement, the animals were killed and both lungs were submitted to histologic examination, tissue myeloperoxidase assay, and wet/dry weight ratio measurement. Quantitative morphometric assessment was not used.

At the final blood sampling, venous blood samples were obtained from three animals in each group for measurement of methemoglobin.

Myeloperoxidase assay. Each donor and recipient lung sample was immediately frozen by immersing it in dichlorodifluoromethane (CCl_2F_2) that had been precooled to the freezing point. Samples were subsequently stored at −70° C until assay. Quantitative myeloperoxidase activity was determined as previously described.[17] Briefly, 100 mg of frozen lung tissue was homogenized in 1 ml of 0.5% hexadecyltrimethylammonium bromide, 5 mmol/L ethylenediaminetetraacetic acid, and 50 mmol/L potassium phosphate buffer (pH 6.2) with a Broeck tissue grinder (Kontes Glass Co., Vineland, N.J.). Hexadecyltrimethylammonium bromide is a detergent that releases myeloperoxidase from the primary granules of the neutrophil. The homogenate was centrifuged at 10,000 *g* for 15 minutes at 4° C. The supernatant was subsequently assayed for total soluble protein by the method of Pierce Laboratories[18] and for myeloperoxidase activity. Myeloperoxidase activity was measured spectrophotometrically: 10 μl of fivefold supernatant was combined with 0.6 ml Hanks bovine serum albumin (0.255 bovine serum albumin added to Hanks solution), 0.5 ml of 100 mmol/L potassium phosphate buffer (pH 6.2), 0.1 ml 0.05% H_2O_2, and 0.1 ml of 1.25 mg/ml o-dianisidine. Color development was stopped by addition of 0.1 ml of 1% N after 5 minutes and after 20 minutes at room temperature. Then the optical density was measured at 460 nm with a spectrophotometer (model PMQ II, Carl Zeiss, Munich, Germany). The color development from 5 minutes to 20 minutes was linear. One unit of enzyme activity was defined as the amount of 1.0 optical density unit per minute per milligram of tissue protein at room temperature.

Statistical analysis. All values are given as the mean plus or minus standard error of the mean and were analyzed by means of the StatView 4.0 statistical system (Abacus Concepts, Berkeley, Calif.). One-way analysis of variance was used to compare dependent variables among three groups. Scheffe's probability testing was used for post hoc comparisons. Differences in blood gas and hemodynamic data were compared between groups at each time point by analysis of variance. Changes in blood gas and hemodynamic data within groups over time were assessed by repeated-measures analysis of variance. Differences were considered significant at the $p < 0.05$ level.

Results

As expected, there were no significant differences between groups regarding donor weight, recipient weight, flushing time, preservation time, and warm ischemic time (Table I). The mean concentrations of NO and NO_2 were similar in study groups I and II.

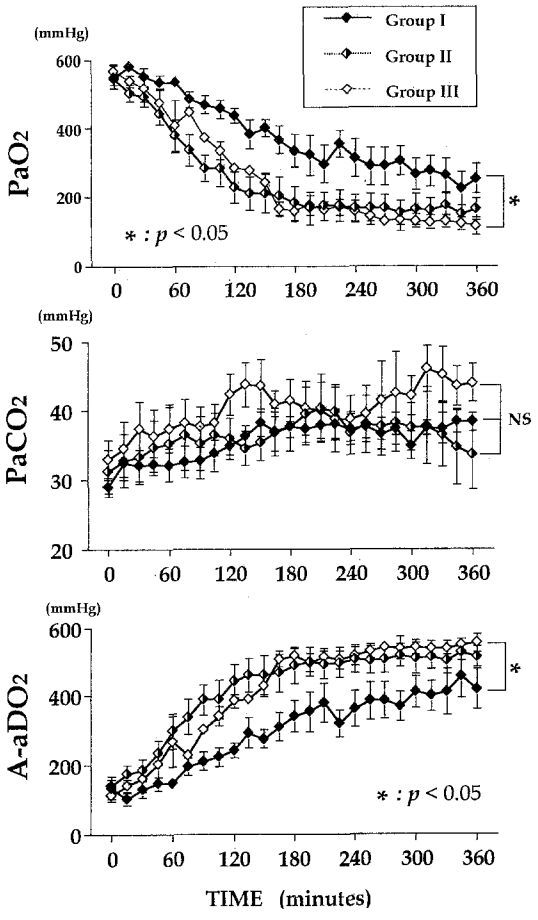

Fig. 1. Pa_{O_2}, Pa_{CO_2}, and A-aD_{O_2} for NO administration groups (immediate use for group I and delayed use for group II) and control group (group III) through 6-hour assessment period. There is significant difference ($p < 0.05$) between group I and III in Pa_{O_2} and A-aD_{O_2} exchange over time. *NS,* Not significant.

Gas exchange. Throughout the 6-hour assessment period, oxygenation in group I animals was superior to that in other groups (Fig. 1). This difference between group I and control group III reached statistical significance after 90 minutes. The mean Pa_{O_2} at 120 minutes in group I was 435.6 ± 22.2 mm Hg versus 285.2 ± 16.0 mm Hg ($p < 0.01$) in the control group. At 360 minutes mean Pa_{O_2} for group I was 253.8 ± 44.7 mm Hg versus 114.9 ± 25.5 mm Hg in group III. Pa_{O_2} of the delayed NO_2 administration group (group II) did not have any statistical difference from that in the control group at any time. There was no statistical significance in Pa_{CO_2} values between groups. The A-aD_{O_2} followed the same time course as Pa_{O_2} and revealed statistical significance between groups I and III (Fig. 1).

Table I. *Characteristics of experimental groups*

	Group I	*Group II*	*Group III*
Donor weight (kg)	28.6 ± 1.1	27.7 ± 2.1	28.1 ± 0.8
Recipient weight (kg)	28.9 ± 1.9	28.3 ± 0.9	28.5 ± 0.8
Flushing time (sec)	87.8 ± 14.7	95.2 ± 10.1	94.9 ± 13.0
Warm ischemic time (min)	53 ± 0.9	55 ± 2.1	53 ± 4.1
Preservation time (hr)	17:30 ± 0:33	17:25 ± 0:12	17:28 ± 0:17
Mean NO concentration (ppm)	66.3 ± 2.1	64.9 ± 3.0	—
Mean NO_2 concentration (ppm)	3.8 ± 0.2	3.1 ± 0.4	—

Hemodynamics. There were no significant differences among the three groups in aortic pressure and cardiac index throughout the 6-hour study period. There were significant differences regarding mean pulmonary artery pressure and PVR during the assessment period between groups I and III. Although group II PVR did decrease with NO therapy, there was no significant difference between group II and other groups (Fig. 2).

Wet/dry weight ratio. Allograft wet to dry weight ratios were similar among the three groups (Fig. 3).

Suction fluid volume. There was no significant difference among the three groups as to total volume of allograft edema fluid aspirated during the assessment period (Fig. 3).

Myeloperoxidase activity assay. There was a significant difference in myeloperoxidase activity between group I and group III (0.245 ± 0.027 units versus 0.365 ± 0.03 units; $p < 0.05$). However, there was no difference between group II (0.308 ± 0.037 units) and group III (Fig. 4).

Methemoglobin. Although the sampling number was small ($n = 3$ in each group), there were no significant differences between groups with respect to whole blood methemoglobin levels (1.06% ± 0.55%, 1.12% ± 0.11%, and 0.91% ± 0.24% for groups I, II, and III, respectively).

Histology. No significant difference in histologic findings was noted among the grafts in the three groups. The lung showed varying degrees of edema formation and emphysematous changes after the 6-hour assessment period.

Discussion

Numerous studies have been undertaken to improve the quality of lung allograft preservation and limit reperfusion injury. Pathophysiologic mechanisms of reperfusion injury still are unclear, but the studies regarding the role of leukocytes, platelets,

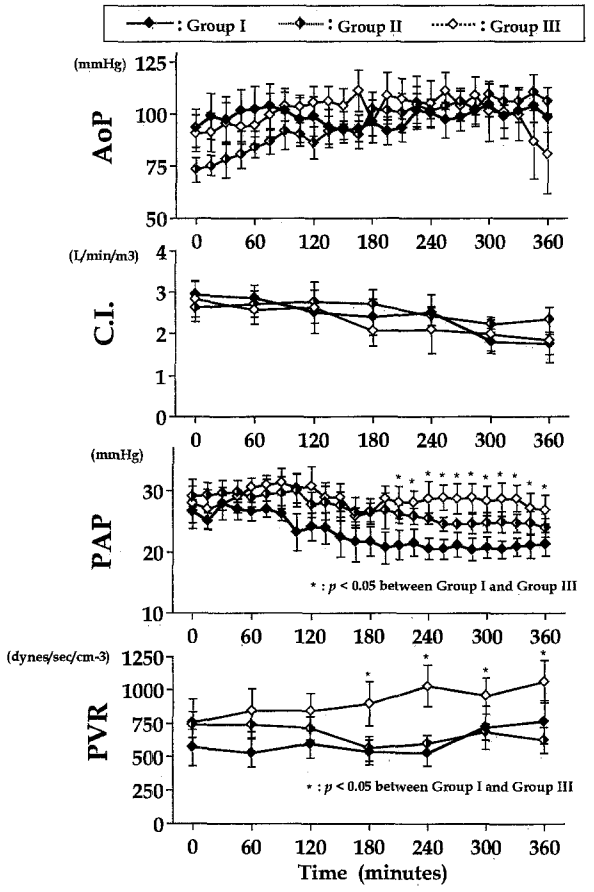

Fig. 2. Aortic pressure *(AoP)*, cardiac index *(C.I.)*, mean pulmonary arterial pressure *(PAP)*, and PVR for NO inhalation groups (immediate use for group I and delayed use for group II) and control group (group III) through 6-hour assessment. There are no differences in systemic hemodynamics (aortic pressure and cardiac index) between three groups. However, there is statistical significance between groups I and III in pulmonary hemodynamics (pulmonary arterial pressure and PVR) over time.

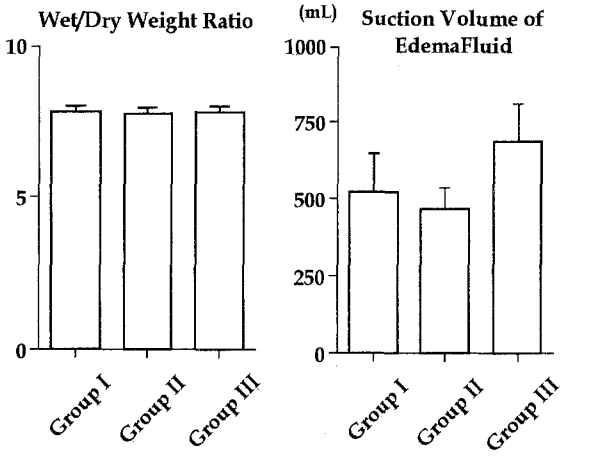

Fig. 3. Wet/dry weight ratio after 6-hour assessment and total suction volume of edema fluid collected through fiberoptic bronchoscope. There are no differences among three groups.

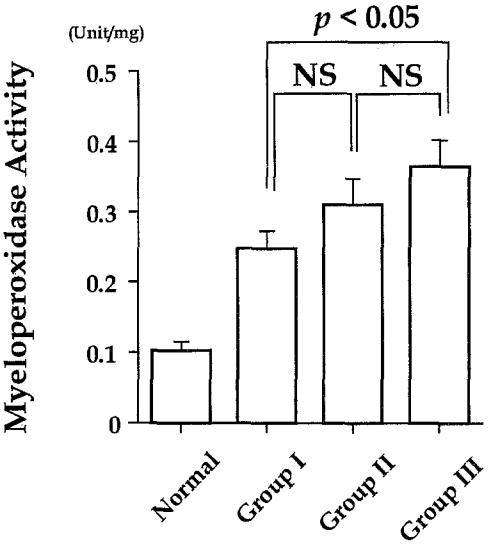

Fig. 4. Results of myeloperoxidase activity assay of lung tissue after 6-hour assessment. *Normal* means fresh left lung tissue sampled just after thoracotomy. There is significant difference between groups I and III. However, there is no statistical difference between groups II and III. *NS,* Not significant.

oxygen free radicals, and cytokines have contributed to our understanding of the complex mechanisms involved.[19-24] It is now recognized that the neutrophil plays an important role in acute lung injury associated with ischemia and reperfusion. We have previously reported that pentoxifylline, which has not only microcirculatory benefits but also antineutrophil effects, reduces canine lung allograft reperfusion injury.[25] We have also reported that a combination of adhesion molecule monoclonal antibodies designed to limit neutrophil adhesion attenuates the reperfusion injury in rat lung isografts.[26]

In this report, we have demonstrated a significant improvement in Pao_2 and neutrophil sequestration of the lung allograft when NO was administered before reperfusion. However, delayed NO administration after reperfusion injury was established had no effect. Additionally, immediate administration of NO gas resulted in significant reduction of pulmonary artery pressure and resistance. It is generally

recognized that inhaled NO will modify ventilation-perfusion relationships by dilating vessels of ventilated regions of the lung. Apart from the documented effect of inhaled NO as a pulmonary vasodilator, there are three other possible mechanisms by which immediate inhalation of NO enhanced the function of reperfused canine lung allografts.

First, NO has been shown to be an endogenous inhibitor of leukocyte chemotaxis, adherence, and activation.[9-11] Adherence of neutrophils to the endothelium is thought to be the essential step in diapedesis of leukocytes from the vasculature. Because normal endothelial cells release NO and this NO may prevent leukocytes from adhering to endothelial cells, it is possible that reduced basal NO release after reperfusion may promote or allow neutrophil adherence to endothelial cells, resulting in neutrophil-induced reperfusion injury. Kubes and Granger[27] have shown that infusion of specific inhibitors of NO synthesis, such as N^G-monomethyl-L-arginine (L-NMMA) or $N\omega$-nitro-L-arginine methyl ester (L-NAME), promotes neutrophil adhesion in postcapillary venules through a CD11/CD18–dependent mechanism. Lefer, Nakanishi, and Vinten-Johansen[28] recently demonstrated that infusion of the NO precursor L-arginine at the time of reperfusion dramatically reduces the extent of neutrophil-mediated myocardial and endothelial cell injury in dogs.

Second, NO maintains vasomotor function and may decrease pulmonary edema in lung injury. Lung ischemia and reperfusion are associated with a decreased release of endothelial NO and prostacyclin synthesis. Both of these molecules are potent modulators of pulmonary vasomotor tone. In a lung injury model Kavanagh and associates[29] demonstrated that pretreatment with inhaled NO (90 to 120 ppm) reduced pulmonary artery pressure and precapillary PVR in addition to decreasing pulmonary capillary filtration coefficient. Xiong and associates[30] reported that maintaining NO levels by administration of the NO precursor L-arginine abolished the increase in microvascular permeability in isolated rat lungs.

In addition, NO has a strong antiplatelet effect. NO has been shown to block platelet aggregation and to reduce platelet adhesion to endothelial cell monolayers.[3, 7, 8] The platelet activating factor antagonist CM3988 has been shown to improve post-transplant lung allograft function.[31] Although we did not measure platelet function in these experiments, it is possible that NO may have a similar antiplatelet effect on preserved lung allografts.

Unfortunately, delayed administration of NO could not reverse the severe lung allograft reperfusion injury. Gas exchange and the degree of neutrophil sequestration in the allograft was not affected by delayed inhalation of NO. It is possible that in the presence of such severe injury inhaled NO may be rapidly inactivated by interstitial or alveolar edema fluid. This might explain its lack of effect apart from modest vasodilation. In human lung allografts we have recently demonstrated that inhaled NO does improve gas exchange and pulmonary hemodynamics of early allograft dysfunction.[32] It is possible that the injury in this model (18-hour ischemic time) was so severe as to enable any beneficial effect as observed in human allografts having a shorter ischemic time (4 to 8 hours).

In summary, we have demonstrated that inhaled NO when administered from the onset of reperfusion reduces the severe lung injury expected after prolonged allograft storage. The mechanism of this effect is uncertain but likely is a result of a combination of the known vasodilatory and antineutrophil adhesion effects of NO.

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