Nitroglycerin maintains graft vascular homeostasis and enhances preservation in an orthotopic rat lung transplant model

Transplanted lungs often fail during the peritransplantation period for poorly understood reasons. Because the nitric oxide pathway regulates pulmonary vascular tone, helps to maintain the integrity of the endothelial barrier, and modulates neutrophil adhesivity and activation, we hypothesized that perturbation of this pathway during the preservation and reperfusion of transplanted lungs might play a critical role in mediating early graft failure. To evaluate whether supplementing the preservation solution with the nitric oxide donor nitroglycerin enhances lung preservation for transplantation, we obtained hemodynamic measurements in a model of orthotopic left lung transplantation in the rat after ligation of the native right pulmonary artery. In these experiments, recipient survival and hemodynamics depended solely on the transplanted lung. The left lung was harvested from 22 rats, flushed with either lactated Ringer's solution alone (control, $n = 11$) or Ringer's solution supplemented with nitroglycerin (0.1 mg/ml, $n = 11$), preserved for 4 hours at 4° C, and then transplanted using a rapid cuff technique for bronchial and vascular anastomoses. Nitroglycerin significantly improved arterial blood oxygenation (339 ± 66 versus 130 ± 12 mm Hg, $p < 0.05$), increased pulmonary arterial flow (7.6 ± 1.9 versus 0.9 ± 0.2 ml/min, $p < 0.005$), decreased pulmonary vascular resistance (1.7 ± 0.4 versus $6.6 \pm 1.9 \times 10^3$ Wood units, $p < 0.05$), and enhanced recipient survival (64% versus 0%, $p < 0.05$). Control grafts had significantly greater neutrophil accumulation (50% greater as quantified by myeloperoxidase activity, $p < 0.05$) than grafts preserved in the presence of nitroglycerin. These studies show that supplementation of the preservation solution with the nitric oxide donor nitroglycerin maintains graft vascular homeostasis and significantly improves pulmonary function and recipient survival after transplantation. (J THORAC CARDIOVASC SURG 1995;109:206-11)

Yoshifumi Naka, MD, PhD^a (by invitation), Nepal C. Chowdhury, MD^b (by invitation), Mehmet C. Oz, MD^b (by invitation), Craig R. Smith, MD,^b Osvaldo J. Yano, MD^b (by invitation), Robert E. Michler, MD^b (by invitation), David M. Stern, MD^a (by invitation), and David J. Pinsky, MD^c (by invitation), *New York, N.Y.*

From the Departments of Physiology,^a Surgery,^b and Medicine,^c Columbia University College of Physicians and Surgeons, New York, N.Y.

Supported in part by grants from the American Heart Association, the Cystic Fibrosis Foundation, and the Public Health Service (HL42507 and HL50629).

Read at the Seventy-fourth Annual Meeting of The American Association for Thoracic Surgery, New York, N.Y., April 24-27, 1994.

Copyright © 1995 by Mosby-Year Book, Inc.
0022-5223/95 \$3.00 + 0 12/6/60330

The use of lung transplantation as a therapeutic option in patients with end-stage pulmonary disease has increased in recent years,¹ but the lungs remain extremely vulnerable to ischemia-reperfusion injury

Address for reprints: Yoshifumi Naka, MD, PhD/David J. Pinsky, MD, Department of Physiology, College of Physicians and Surgeons 11-518, Columbia University, 630 W. 168th St., New York, NY 10032.

during the transplantation procedure. Because levels of endothelium-derived relaxing factor (nitric oxide), which modulates important vascular homeostatic properties,²⁻⁴ plummet after reperfusion in coronary vessels,⁵ we hypothesized that a similar decline in nitric oxide levels after reperfusion in the lungs might have adverse vascular consequences that could be reversed by supplementing the preservation solution with the nitric oxide donor nitroglycerin. Using an orthotopic model of lung transplantation in the rat, in which the recipient depends entirely on the transplanted lung, we designed these experiments to establish the effects of nitroglycerin added to the preservation solution on pulmonary hemodynamics, gas exchange, graft leukostasis, and recipient survival during the reperfusion period.

Methods

Orthotopic left lung transplantation. Orthotopic left lung transplantation was accomplished in inbred male Lewis rats (250 to 300 gm) with a modification of a previously described technique.⁶ In brief, after heparinization (500 U intravenously) and ligation of the superior vena cavae of the donor, 30 ml of 4° C preservation solution consisting of lactated Ringer's solution alone (control; Baxter Healthcare Corp., Edison, N.J.) or supplemented with nitroglycerin (0.1 mg/ml; DuPont Merck Pharmaceuticals, Manati, Puerto Rico) was infused at a constant pressure (<20 mm Hg). The pulmonary artery (PA) and pulmonary vein (PV) were divided, the bronchus was ligated with the lung partially inflated and then divided, and the lung was removed. A cuff made from 14-gauge grooved plastic cylinders was placed on each vascular stump, and a 16-gauge cylinder was inserted into the bronchus. The lung was then submerged in 4° C preservation solution for 4 hours. The recipient rat was anesthetized and intubated (the lungs ventilated with 100% oxygen), a left thoracotomy was performed, the left bronchus, PA, and PV were isolated, crossclamped, divided, and the native lung was removed. The cylinder (bronchus) and cuffs (PA and PV) were connected to their respective structures, with warm ischemic times maintained below 10 minutes. The hilar crossclamp was released, reestablishing blood flow, and the tie on the bronchus was removed, enabling gas exchange. A snare was then passed around the right PA, and Millar catheters (2F; Millar Instruments, Inc., Houston, Tex.) were introduced into the main PA and the left atrium (LA). A flow probe (Transonics, Ithaca, N.Y.) was then placed around the main PA.

Measurement of lung graft function. On-line hemodynamic monitoring was accomplished with a MacLab data interface module (ADI Instruments, Milford, Mass.) and a Macintosh IIfx computer (Apple Computer, Cupertino, Calif.). The hemodynamic parameters that were measured included PA pressure (millimeters of mercury), PA flow (milliliters per minute), LA pressure (millimeters of mer-

cury), and arterial oxygen tension (millimeters of mercury) during inspiration of 100% oxygen; oxygen tension was analyzed with a model ABL-2 gas analyzer (Radiometer A/S, Copenhagen, Denmark). Pulmonary vascular resistances (PVRs) were calculated as (mean PA pressure - LA pressure)/PA flow ($\times 10^3$ Wood units). Baseline measurements were taken, the native (right) PA was ligated, and serial measurements were then taken every 5 minutes for 30 minutes. Hemodynamic measurements were recorded at the final time at which the recipient was alive (gauged by continued regular cardiac mechanical activity viewed through the open thorax). Thirty minutes after restoration of blood flow, the transplanted lung was excised, rinsed briskly in physiologic saline solution, and snap frozen in liquid nitrogen until the time of myeloperoxidase assay, performed as described.⁷

Statistics. Data were evaluated by the Mann-Whitney *U* test or the χ^2 statistic. Values are expressed as means plus or minus standard error of the mean, with differences considered statistically significant if the *p* value was less than 0.05.

Results

Control lungs preserved for 4 hours in the absence of nitroglycerin demonstrated a marked increase in PVR as well as a decline in PA flow and arterial oxygenation (Fig. 1, *left panel*). Although PA pressures rose initially, the rise was followed by a rapid decline in PA pressure followed by recipient death. In sharp contrast, when nitroglycerin was added to the preservation solution, all of these parameters were stabilized and the recipient survived (Fig. 1, *right panel*).

When data from all 11 nitroglycerin-preserved lungs were compared with control data (*n* = 11), nitroglycerin added to the preservation solution significantly increased PA flow (0.9 ± 0.2 versus 7.6 ± 1.9 ml/min, *p* < 0.005) (Fig. 2, *left panel*), decreased PVR (6.6 ± 1.9 versus $1.7 \pm 0.4 \times 10^3$ Wood units, *p* < 0.05) (Fig. 2, *center panel*), and improved arterial oxygenation (130 ± 12 versus 339 ± 66 mm Hg, *p* < 0.05) (Fig. 2, *right panel*). Recipient survival was also increased significantly by the addition of nitroglycerin (64% versus 0% for control, *p* < 0.05) (Fig. 3).

In addition to increasing arterial oxygenation and improving pulmonary blood flow, nitroglycerin added to the preservation solution was associated with a decline in neutrophil infiltration into the reperfused graft. Control grafts had 50% more neutrophil infiltration than grafts preserved with supplemental nitroglycerin (*p* < 0.05), as quantified by the neutrophil-specific enzyme myeloperoxidase (Fig. 4).

Fig. 1. Representative hemodynamic tracing of a lung transplant after hypothermic preservation for 4 hours in lactated Ringer's solution (*left panel*) or lactated Ringer's solution supplemented with nitroglycerin (NTG; 0.1 mg/ml, *right panel*) after the native lung was removed from the pulmonary circulation as described. PAP, Mean pulmonary artery pressure (mm Hg); PAF, pulmonary arterial flow (ml/min); PVR, pulmonary vascular resistance ($\times 10^3$ Wood units), and arterial oxygenation (mm Hg). Reperfusion begins at time 0 in these tracings.

Fig. 2. Effects of nitroglycerin (NTG) on lung preservation in lactated Ringer's solution. All lung transplants were done after 4 hours of hypothermic preservation. Measurements were recorded after ligation of the native (right) pulmonary artery at the final time at which the recipient was alive (or before being put to death 30 minutes after reperfusion). Mean values $\pm$ standard error of the mean are shown. $n = 11$ for control; $n = 11$ for nitroglycerin. * $p < 0.05$; *** $p < 0.005$.

Discussion

Lung transplantation is being used increasingly as a therapeutic alternative for patients with end-stage lung disease.¹ Despite recent advances in methods of lung preservation,^{8,9} the lungs remain extremely vulnerable to ischemia-reperfusion injury. Because the endothelium appears to play a critical role in maintaining vascular homeostasis in preserved and transplanted hearts,⁷ we hypothesized that the vast capillary network of the lungs may become dysfunctional after preservation and reperfusion, contributing to graft failure. Because ischemia and reperfusion generates oxygen-derived free radicals, which

combine with nitric oxide to quench its biologic activity in other organs,^{5,10} it is likely that the burst of reactive oxygen intermediates produced by the reperfused aerated lungs also quench available nitric oxide. Because nitric oxide acts as a vasodilator^{2,4} and prevents neutrophil adherence to the endothelium,^{3,11} the loss of available nitric oxide could cause graft failure during the immediate postreperfusion period as a result of vasoconstriction and neutrophil accumulation. This suggested that supplementation of the nitric oxide pathway with a nitric oxide donor such as nitroglycerin might confer beneficial vascular effects,

Fig. 3. Recipient survival 30 minutes after ligation of the native (right) pulmonary artery demonstrating the importance of nitroglycerin (*NTG*; 0.1 mg/ml) added to the preservation solution. $n = 11$ for control; $n = 11$ for nitroglycerin. $*p < 0.005$.

thereby improving lung preservation for transplantation.

These studies showed that lungs preserved in the absence of nitroglycerin had a steep rise in PVR after reperfusion; although PA pressures initially rose after ligation of the native PA, blood flow and arterial oxygenation declined precipitously as the transplanted lung failed, leading to death of the recipient. These hemodynamic and functional parameters of pulmonary preservation were all stabilized by the addition of nitroglycerin to the preservation solution. The reduction in PVR is not surprising, given the fact that nitroglycerin is an endothelium-independent vasodilator capable of relaxing vascular smooth muscle. However, improvement was not limited to vascular resistance. Neutrophils are thought to play a significant role in reperfusion injury,¹² as well as in the no-reflow phenomenon after reperfusion.¹³ Inasmuch as nitric oxide donors are recognized to prevent neutrophil sequestration into reperfused tissues such as the heart,^{14,15} the beneficial effects of nitroglycerin may have been due largely to its ability to reduce neutrophil infiltration into the reperfused graft. This theory is supported by the work of others showing that depletion of neutrophils in the reperfusion solution can improve pulmonary function.¹⁶ In our experiments, it is likely that both reduced neutrophil infiltration and improved blood flow contributed to the improved arterial oxygenation and recipient survival in the nitroglycerin group.

These experiments were based on an orthotopic rat lung transplant model described by Mizuta and

Fig. 4. Effect of nitroglycerin (*NTG*) on graft leukostasis, with graft neutrophil infiltration quantified by myeloperoxidase activity. Lungs were preserved in the absence ($n = 7$) or presence ($n = 4$) of nitroglycerin (0.1 mg/ml), after which they were transplanted/reperfused, snap frozen in liquid nitrogen, and the assay performed as described in the text. Mean values $\pm$ standard error of the mean are shown. $*p < 0.05$.

coworkers,⁶ modified in that a cuff technique was used for all vascular and bronchial anastomoses, which permitted extremely rapid anastomosis (<10 minutes). In addition, in our model, ligation of the PA to the native right lung effectively removed this lung from the pulmonary circulation, and hemodynamic and flow measurements were obtained, to more closely approximate human lung transplantation. Serial measurements were obtained for the first 30 minutes of reperfusion, because this period coincides with the early production of oxygen-derived free radicals by reperfused tissues.^{5,10}

Although lactated Ringer's solution is not used in clinical organ preservation, these experiments were undertaken in light of previous work in a cardiac transplant model demonstrating that it is an effective solution for evaluating the role of the endothelium and second messenger pathways in organ preservation.⁷ Pilot studies demonstrate that nitroglycerin likewise enhances pulmonary preservation when added to a clinically used preservation solution (Euro-Collins), with preservation times up to 8 hours possible (data not shown). Because nitroglycerin acts to increase intracellular cyclic guanosine monophosphate in target tissues, further studies are being undertaken to evaluate the role of the cyclic guanosine monophosphate second messenger pathway in lung preservation, especially in light of preliminary experiments in baboons; these experiments demonstrate that agents that stimulate second mes-

senger pathways may enhance both heart¹⁷ and lung¹⁸ preservation for transplantation.

In conclusion, adding nitroglycerin to a pulmonary preservation solution provides a simple, novel approach to enhancing lung preservation for transplantation. These studies add to the growing body of evidence that the endothelium plays an important role in maintaining vascular homeostasis during the critical early period after reperfusion of a transplanted organ.

REFERENCES

1. Kaye MP. The Registry of the International Society for Heart and Lung Transplantation: tenth official report—1993. *J Heart Lung Transplant* 1993;12:541-8.
2. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980;288:373-6.
3. Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci U S A* 1991;88:4651-5.
4. Ignarro LJ, Ross G, Tillisch J. Pharmacology of endothelium-derived nitric oxide and nitrosovasodilators. *West J Med* 1991;154:51-62.
5. Lefer AM, Tsao PS, Lefer DJ, Ma XL. Role of endothelial dysfunction in the pathogenesis of reperfusion injury after myocardial ischemia. *FASEB J* 1991;5:2029-34.
6. Mizuta T, Kawaguchi A, Nakahara K, Kawashima K. Simplified rat lung transplantation using a cuff technique. *J THORAC CARDIOVASC SURG* 1989;97:578-81.
7. Pinsky D, Oz M, Liao H, et al. Restoration of the cyclic AMP second messenger pathway enhances cardiac preservation for transplantation in a heterotopic rat model. *J Clin Invest* 1993;92:2994-3002.
8. Kirk AJB, Colguhoun IW, Dark JH. A review of current practice and future directions. *Ann Thorac Surg* 1994;54:990-1000.
9. Egan TM. Lung preservation. *Semin Thorac Cardiovasc Surg* 1992;4:83-9.
10. Zweier JL, Kuppusamy P, Lutty GA. Measurement of endothelial cell free radical generation: evidence for a central mechanism of free radical injury in postischemic tissues. *Proc Natl Acad Sci U S A* 1988;85:4046-50.
11. Lefer DJ, Nakanishi K, Johnston WE, Vinten-Johansen J. Antineutrophil and myocardial protecting actions of a novel nitric oxide donor after acute myocardial ischemia and reperfusion in dogs. *Circulation* 1993;88:2337-50.
12. Adkins W, Taylor A. Role of xanthine oxidase and neutrophils in ischemia-reperfusion injury in rabbit lung. *J Appl Physiol* 1990;69:2012-8.
13. Kloner R. No reflow revisited. *J Am Coll Cardiol* 1989;14:1814-5.
14. Nakanishi K, Vinten-Johansen J, Lefer DJ. Intracoronary L-arginine during reperfusion improves endothelial function and reduces infarct size. *Am J Physiol* 1992;263:H1650-8.
15. Johnson G III, Tsao PS, Mulloy D, Lefer AM. Cardioprotective effects of acidified sodium nitrite in myocardial ischemia with reperfusion. *J Pharmacol Exp Ther* 1990;252:35-41.
16. Hall TS, Breda MA, Baumgartner WA, et al. The role of leukocyte depletion in reducing injury to the lung after hypothermic ischemia. *Curr Surg* 1987;44:137-9.
17. Oz MC, Pinsky DJ, Koga S, et al. Novel preservation solution permits 24-hour preservation in rat and baboon cardiac transplant model. *Circulation* 1993;88(Suppl):II291-7.
18. Michler RE, Oz MC, Koga S, et al. cAMP preservation solution permits 24 hour baboon lung preservation. *Circulation* 1992;86(Suppl):I356.

Discussion

Dr. Andrew S. Wechsler (*Richmond, Va.*). Have you considered testing the hypothesis further as regards the mechanism of nitroglycerin action by giving either arginine or an arginine antagonist to see if the mechanism you proposed was really the one that was operative?

Dr. Naka. We are currently testing L-arginine and L-arginine analogs in our model, but we have not yet obtained enough data to comment on their effects. We selected nitroglycerin in these experiments to enhance lung preservation for transplantation because nitroglycerin and other endothelium-independent vasodilators act as nitric oxide donors and do not require the presence of functional endothelium to exert their effects. These experiments clearly demonstrate that nitroglycerin enhances lung preservation, and our preliminary data suggest that stimulation of the nitric oxide/cyclic guanosine monophosphate pathway at the level of the cyclic guanosine monophosphate second messenger is also beneficial.

Dr. John Kennedy (*Cambridge, England*). You have ligated the PA of the native lung. Is there an autoregulation of the bronchial arterial circulation which, in the presence of hypoxia on that side, would increase flow and is that making a contribution to oxygenation in your model?

Dr. Naka. Although it is possible that the bronchial circulation contributes to systemic oxygenation to some extent, the contribution did not seem clinically important in our model of lung transplantation because all of the recipients that received control grafts had poor arterial oxygenation and died shortly after ligation of the native right PA.

Dr. Magdi H. Yacoub (*London, England*). My question is similar to Dr. Wechsler's. In trying to dissect out the mechanism of this protective effect, have you thought about comparing it to other vasodilators that act through different mechanisms, such as adenosine or prostacyclin, which increase cyclic adenosine monophosphate rather

than cyclic guanosine monophosphate and seeing what is causing this protective effect?

Dr. Naka. We are currently testing the effects of prostaglandins in our model, based on preliminary evidence that stimulating the cyclic adenosine monophosphate second messenger pathway also enhances lung preservation for transplantation. It is not surprising that both the nitric oxide and cyclic adenosine monophosphate pathways have similar effects. They both maintain vascular homeostasis by vasodilation, prevention of platelet aggregation, prevention of neutrophil adherence to the endothelial surface, and maintenance of the endothelial barrier. Therefore, we predict that agents that preserve

vascular homeostasis at multiple levels will be most effective at pulmonary preservation.

Dr. Yacoub. My question relates to whether you have compared the response to other vasodilators that increased cyclic adenosine monophosphate rather than guanosine monophosphate?

Dr. Naka. Yes, we have tested cyclic adenosine monophosphate analogs and have found them to be effective in preliminary studies.

Dr. Mehmet C. Oz (New York, N.Y.). We have done some of that work in the heart model, and we found it to be effective, although we have not gotten far enough in the lung model to say anything conclusive.

Bound volumes available to subscribers

Bound volumes of THE JOURNAL OF THORACIC AND CARDIOVASCULAR SURGERY are available to subscribers (only) for the 1995 issues from the Publisher, at a cost of \$91.00 for domestic, \$118.77 for Canadian, and \$111.00 for international subscribers for Vol. 109 (January-June) and Vol. 110 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue of the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby-Year Book, Inc., Subscription Services, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone 1 (800) 453-4351 or (314) 453-4351.

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