CARDIAC AND PULMONARY REPLACEMENT

ATTENUATION OF LUNG GRAFT REPERFUSION INJURY BY A NITRIC OXIDE DONOR

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Objective: **One of the primary features of ischemia-reperfusion injury is reduced production of protective autocoids, such as nitric oxide, by dysfunctional endothelium. Administration of a nitric oxide donor during reperfusion of lung grafts may therefore be beneficial through modulation of vascular tone and leukocyte and platelet function.** ***Methods:*** **Rat lung grafts were flushed with University of Wisconsin solution and reperfused for 1 hour in an ex vivo model incorporating a support animal. Group I grafts ($n = 6$) were reperfused immediately after explantation, group II ($n = 6$) and III ($n = 5$) grafts after 24 hours of storage at 4° C. In group III, glyceryl trinitrate, a nitric oxide donor, was administered during the first 10 minutes of reperfusion at a rate of 200 μg/min. In an additional group ($n = 5$), 200 μg/min hydralazine was administered instead, to assess the effect of vasodilation alone.** ***Results:*** **Graft function in group II deteriorated compared with that in group I, with significant reduction of graft effluent oxygen tension and blood flow and elevation of pulmonary artery pressure, peak airway pressure, and wet/dry weight ratio. In contrast, in group III, glyceryl trinitrate treatment improved graft function to baseline levels in all these parameters. Administration of hydralazine, meanwhile, produced mixed results with only two out of five grafts functioning at control levels.** ***Conclusions:*** **In this model, administration of glyceryl trinitrate to supplement the nitric oxide pathway in the early phase of reperfusion has a sustained beneficial effect on lung graft function after 24-hour hypothermic storage, probably through mechanisms beyond vasodilation alone. (J Thorac Cardiovasc Surg 1997;113:327-34)**

The susceptibility of lungs to ischemia continues to restrict storage times in clinical transplantation to about 6 hours. Even after such relatively short storage periods, graft dysfunction with increased pulmonary vascular resistance, impaired gas exchange, and pulmonary edema remains a significant cause of morbidity. This dysfunction may stem in part from ischemia-reperfusion injury.

A key feature in the pathophysiologic process of ischemia-reperfusion injury is a fall in available levels of nitric oxide (NO) within minutes of the onset of reperfusion.[1-4] NO has a number of regulatory functions including vasodilation and inhibition of platelet and leukocyte adhesion and activation.[5] Increasing the availability of NO during reperfusion may therefore be beneficial to graft function. There is now considerable evidence to support this concept in the setting of myocardial ischemia-reperfusion.[6, 7] Pulmonary endothelium, too, becomes dysfunctional after ischemia and reperfusion with impairment of NO-mediated responses.[8] The addition of glyceryl trinitrate (GTN; also known as nitroglycerin), a NO donor, to flush

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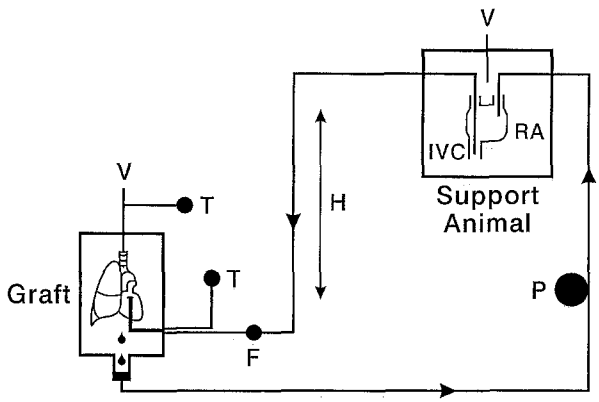

Fig. 1. Schematic representation of the reperfusion preparation. Deoxygenated blood from the inferior vena cava *(IVC)* of the support animal is hydrostatically delivered to the pulmonary artery of the graft, which is situated a vertical height *(H)* below. Graft effluent is returned to the right atrium *(RA)* of the support animal by a pump *(P)*. *V,* Ventilator, *T,* pressure transducer, *F,* flowmeter.

and storage solutions has recently been shown to enhance lung graft preservation in rodent models.[9-11] We hypothesized that administration of GTN during initial lung graft reperfusion, which is when the fall in NO levels is steepest, might attenuate progression of reperfusion injury. In a previous study we showed that protecting rat lung grafts for the first 10 minutes of reperfusion by reducing the reperfusion pressure to subphysiologic levels is beneficial to subsequent function.[12] Therefore GTN administration was limited to the initial 10 minutes of reperfusion on the basis that this might be sufficient to arrest development of reperfusion injury.

Methods

Isolated rat lung grafts were reperfused in a circuit incorporating a support animal. Male Sprague-Dawley rats (Charles River Laboratories, Kent, United Kingdom) weighing 350 to 420 gm were used and received humane care in compliance with the United Kingdom Government's Animals (Scientific Procedures) Act of 1986. All procedures were done with the use of terminal general anesthesia. Halothane inhalation and intraperitoneal pentobarbital sodium, 100 mg/kg, were used for anesthetic induction and maintenance, respectively. Animals' lungs and isolated grafts were ventilated with the use of Harvard rodent respirators (Harvard Apparatus, Kent, United Kingdom).

Graft procurement. Anesthetized lung donors were intubated through a tracheostomy with a 16-gauge cannula and the lungs ventilated with room air at 60 breaths/min, 10 ml/kg tidal volume, and 3 cm H_2O positive end-expiratory pressure. All donors were given methylprednisolone, 30 mg/kg, through the femoral vein 45 minutes before lung explantation in keeping with clinical practice. Median sternotomy was done, the pleurae and pericardium opened, and the thymus excised. A ligature was passed through the transverse sinus to encircle the aorta and pulmonary artery trunk. After heparinization (500 units), the inferior vena cava was clamped, the left atrial appendage amputated, and the right ventricular outflow tract opened. A primed, olive-tipped 20-gauge cannula was passed into the pulmonary artery trunk and secured with the previously placed ligature. The lungs were flushed through this cannula with a 60 ml/kg dose of 4° C University of Wisconsin (UW) solution (DuPont Pharmaceuticals, Letchworth Garden City, United Kingdom) delivered at 25 cm hydrostatic pressure. The tracheal cannula was then clamped with the lungs fully inflated and the heart-lung block was excised.

Reperfusion. Support animals were anesthetized and the lungs ventilated as described for lung donors. Through a median sternotomy, mediastinal structures were exposed and the brachiocephalic artery ligated. A 16-gauge cannula was passed through the right superior vena cava and right atrium into the inferior vena cava. Deoxygenated blood drawn through this cannula was delivered hydrostatically into the pulmonary artery of the grafts, which were situated at a level below the support animal (Fig. 1). The vertical distance between the support animals and grafts was designed to generate hydrostatic reperfusion pressure equivalent to the physiologic pulmonary artery pressure of these rats (18 to 20 mm Hg). The grafts, with the left lung and postcaval lobe removed, were suspended in an insulated chamber and ventilated with room air at 30 breaths/min, 10 ml/kg tidal volume, and 3 cm H_2O positive end-expiratory pressure. Blood that drained from the opened left atrium of the grafts was collected and returned by a pump (Variable Speed Peristaltic Pump, Harvard Apparatus) to the right atrium of the support animal through an 18-gauge cannula placed via the left superior vena cava. Thus the support animals continuously supplied deoxygenated blood to the grafts. The circuit tubing and reperfusion chamber were waterlogged to maintain blood temperature at 38° C and support animals were placed on a homeothermic warming blanket (Harvard Apparatus). Blood obtained from additional animals was used to prime the circuit and to replace losses together with 0.9% saline solution.

Experimental protocol. Grafts in group I ($n = 6$) were reperfused immediately after explantation to obtain baseline data. In groups II ($n = 6$) and III ($n = 5$), grafts were stored for 24 hours submerged in 4° C UW solution before reperfusion. All grafts were reperfused for a total of 60 minutes. In group III, GTN was infused into the reperfusion circuit just proximal to the graft at a rate of 200 μg/min during the first 10 minutes of reperfusion. This dose was selected after pilot experiments in which lower doses had been found to yield partial improvement in function.

Subsequently, an additional five grafts were reperfused after 24-hour storage in UW solution with administration of 200 μg/min hydralazine during the first 10 minutes of reperfusion. The objective was to establish whether the effects of GTN were mediated through vasodilation alone

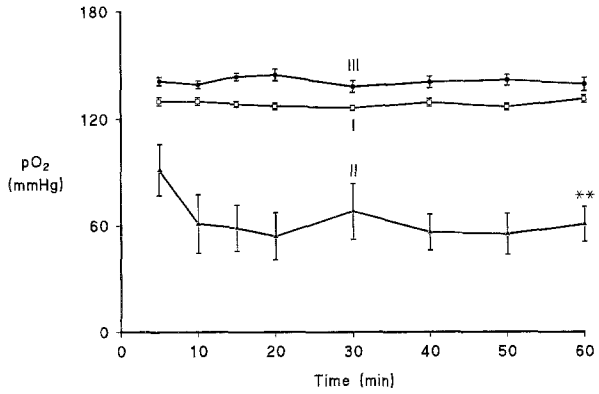

Fig. 2. Partial pressure of oxygen *(Po_2)* in graft effluent during 1 hour of reperfusion of grafts flushed with UW solution and reperfused immediately (group I) or after 24-hour storage (groups II and III). Group III received 200 μg/min GTN for the first 10 minutes of reperfusion. Data are shown as mean ± standard error of the mean. $^{**}p < 0.001$ versus group I.

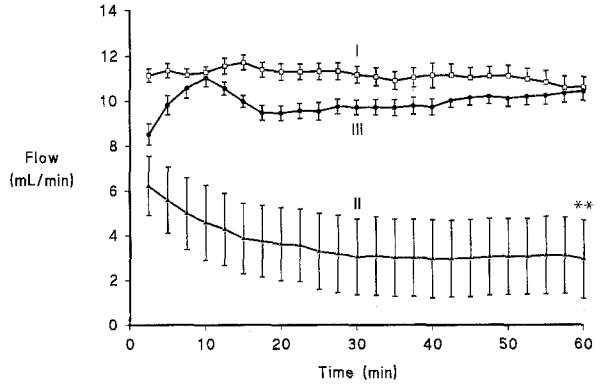

Fig. 3. Graft blood flow during 1 hour of reperfusion of grafts flushed with UW solution and reperfused immediately (group I) or after 24-hour storage (groups II and III). Group III received 200 μg/min GTN for the first 10 minutes of reperfusion. Data are shown as mean plus or minus the standard error of the mean. $^{**}p < 0.001$ versus group I.

or through additional mechanisms. In pilot experiments, this dose of hydralazine produced the same (maximal) increase in blood flow in nonischemic grafts as 200 μg/min GTN with no adverse effects on the support animal.

Measurements. Graft effluent was sampled at 5, 10, 15, and 20 minutes and every 10 minutes thereafter for measurement of gas tension levels. Blood samples were also taken from the reperfusion circuit proximal to the graft at the same times for gas tension and acid/base measurements. This allowed monitoring of the stability of the condition of the support animal and the consistency of (de)oxygenation of the reperfusate. An in-line ultrasonic flow probe (Transonic Systems, Ithaca, N.Y.) measured graft blood flow, and one lumen of the double-lumen reperfusion cannula was connected to a transducer for measurement of graft mean pulmonary artery (PA) pressure. Flow and PA pressure measurements were continuously digitalized and recorded onto a personal computer with the use of customized data acquisition software (Dataq Instruments, Akron, Ohio). These data were later analyzed for point measurements at 2.5-minute intervals. The graft ventilator was linked to another transducer for measurement of peak airway pressure, which, with fixed-volume ventilation, reflects changes in compliance. Lung tissue was dissected free and weighed at the end of the reperfusion period and again after drying to a constant weight at 120° C. Wet/dry weight ratio was calculated as (wet weight − dry weight)/dry weight.

Statistical analysis. For groups I to III, data are expressed as mean ± standard error of the mean, and means of values at the end of the 60-minute reperfusion period were compared by one-way analysis of variance. If differences were found, the Bonferroni post hoc test was used for significance testing; p values less than 0.05 were considered significant. Data for grafts treated with hydralazine were not distributed normally and are therefore presented individually.

Results

Reperfusion was maintained for 1 hour in all experiments with no deterioration of the stability of the support animal's condition as assessed by visual inspection of heart rate and cardiac filling, core temperature, and venous blood oxygen tension and pH values. Volume replacement requirements did not vary significantly among groups. The oxygen tension of the graft reperfusate was constant during individual experiments, as well as within and between groups. This stability of the preparation has been documented in more detail in previous studies.[13]

GTN administration. Oxygenation in grafts reperfused after 24-hour storage (group II) was poor compared with that in control lungs (Fig. 2); at 1 hour, oxygen tension was 58 ± 8 versus 131 ± 2 mm Hg ($p < 0.001$). In contrast, stored grafts that received GTN during the first 10 minutes of reperfusion (group III) yielded oxygen tension values similar to those in group I (139 ± 4 mm Hg at 1 hour, p = not significant).

Deterioration of hemodynamic parameters occurred in group II with reduced blood flow (3.4 ± 1.4 ml/min at 1 hour vs 10.6 ± 0.5 ml/min in group I, $p < 0.001$) and elevated mean PA pressure (20.7 ± 1.2 mm Hg vs 15.6 ± 0.7 mm Hg, $p < 0.01$). In group III, during GTN administration, blood flow (Fig. 3) increased and PA pressure (Fig. 4) fell to group I levels. Both parameters subsequently stabilized at control levels and at 1 hour were 10.4 ± 0.4

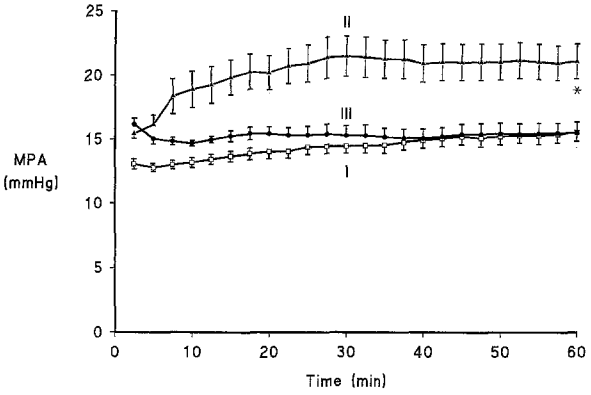

Fig. 4. Graft mean pulmonary artery pressure *(MPA)* during 1 hour of reperfusion of grafts flushed with UW solution and reperfused immediately (group I) or after 24-hour storage (groups II and III). Group III received 200 μg/min GTN for the first 10 minutes of reperfusion. Data are shown as mean ± standard error of the mean. $*p < 0.01$ versus group I.

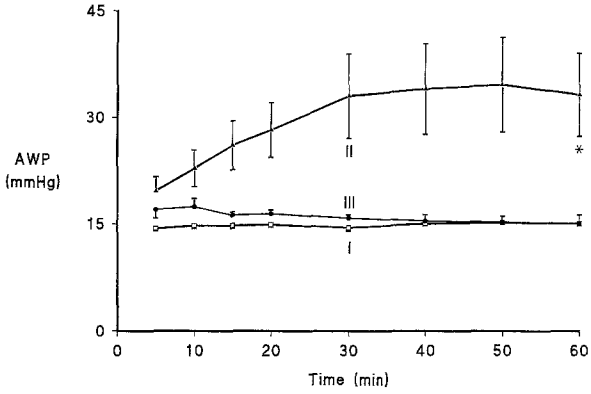

Fig. 5. Graft peak airway pressure *(AWP)* during 1 hour of reperfusion of grafts flushed with UW solution and reperfused immediately (group I) or after 24-hour storage (groups II and III). Group III received 200 μg/min GTN for the first 10 minutes of reperfusion. Data are shown as mean ± standard error of the mean. $*p < 0.01$ versus group I.

ml/min and 15.5 ± 0.8 mm Hg, respectively (p = not significant vs group I).

Peak airway pressure became elevated in group II whereas in group III it remained similar to that in control lungs (Fig. 5). At 1 hour, values were 15 ± 0 mm Hg in group I, 32 ± 5 mm Hg in group II ($p <$ 0.01 vs group I) and 15 ± 1 mm Hg in group III (p = not significant).

The wet/dry weight ratio in group II was elevated to 9.0 ± 0.6 compared with 5.4 ± 0.4 in group I ($p <$ 0.001), whereas in group III it was 7.1 ± 0.7 (p = not significant).

Hydralazine administration. Of the five stored grafts to which hydralazine was administered during the initial 10 minutes of reperfusion, two functioned at baseline levels, one was moderately impaired, and two performed poorly, with reduced oxygenation and blood flow and elevated PA and peak airway pressures (Fig. 6). The data are not presented as means or medians because of this distribution.

Discussion

This study has shown that isolated rat lung grafts reperfused after 24-hour storage in UW solution perform poorly in terms of oxygenation, blood flow, PA pressure, peak airway pressure, and weight gain, but that when GTN is administered during the first 10 minutes of reperfusion, sustained improvement of function to baseline levels is achieved in all of these parameters. Further, this effect does not appear to have been caused by vasodilation alone inasmuch as hydralazine did not afford protection as consistently.

One of the physiologic functions of the endothelium is production of autocoids such as prostacyclin, adenosine, and NO, which maintain local homeostasis by inhibiting vascular tone, platelet aggregation, and leukocyte activation and adhesion. A key feature of the progression of ischemia-reperfusion injury is reduced endothelial production of these substances.[14] In models of myocardial ischemia-reperfusion, significant falls in NO-mediated, endothelium-dependent vasomotor responses in artery segments have been demonstrated after only 2.5 minutes[1] and 10 minutes[2] of reperfusion. Direct measurements have also shown a decline in myocardial NO levels early during reperfusion.[3] Pulmonary ischemia-reperfusion, too, produces endothelial dysfunction. In a canine model of lung transplantation with the use of Euro-Collins solution for flush perfusion, relaxation of pulmonary artery rings dependent on endothelial production of NO was normal after 3-hour hypothermic storage but was significantly impaired after 1 hour of reperfusion.[8] Similarly, in pulmonary artery ring studies in a rabbit model, endothelium-dependent vasomotor responses were unaltered after up to 48 hours of cold storage in Ringer's lactate solution but were significantly reduced after 5 hours of storage and 4 hours of reperfusion.[15] There may, however, be some species-related or flush solution–related variation in these observations, because porcine lungs

flushed with low-potassium dextran solution exhibited impaired NO-dependent relaxations after flushing alone and after hypothermic storage without reperfusion.[16] In studies in which direct measurements of NO were made in rat lungs with use of a porphyrinic microsensor, 6-hour storage in Ringer's lactate resulted in some reduction but after 10 minutes of reperfusion levels of NO were considerably depressed.[4] Addition of superoxide dismutase resulted in restoration of NO levels, and the authors concluded that increased consumption of NO by reactive oxygen species may be more important than reduced production.[4]

NO has a wide spectrum of effects,[5] loss of which may contribute to the development of ischemia-reperfusion injury. It is a powerful vasodilator; this effect is mediated through increased levels of cyclic guanosine monophosphate (cGMP) in vascular smooth muscle cells. It inhibits platelet aggregation and adhesion to endothelium, again through stimulation of cGMP.[5] Adhesion of neutrophils to endothelium, which occurs after ischemia-reperfusion, is prevented by NO donors.[2, 17] This adherence is central to the activation and accumulation of neutrophils in reperfused tissue in which they release destructive free radicals and enzymes. Increased endothelial permeability as assessed by albumin leakage from venules is inhibited by NO donors.[17] NO also reduces availability of oxygen-derived free radicals by inhibiting production of superoxide by neutrophils[18] and directly scavenging reactive oxygen species.[19]

Considerable interest has therefore been directed toward strategies that supplement the NO/cGMP pathway in an effort to attenuate ischemia-reperfusion injury. Providing supplemental L-arginine, the substrate for endogenous production of NO, during myocardial ischemia-reperfusion can improve myocardial performance and reduce endothelial dysfunction, infarct size, and neutrophil accumulation.[20, 21] Blocking production of NO with inhibitors of NO synthase, on the other hand, can be detrimental to postischemic myocardial function.[21, 22] Solutions of NO, organic nitrates, and a number of other compounds that act as NO donors have been found to attenuate myocardial ischemia-reperfusion injury when administered during reperfusion (references 6 and 7 provide reviews). However, not all studies have found NO supplementation to be beneficial; blocking NO synthesis can also reduce postischemic myocardial injury.[23, 24] It has been postulated that NO may contribute to such injury by reacting with superoxide, with the production of peroxynitrite and hydroxyl radicals.[23, 24]

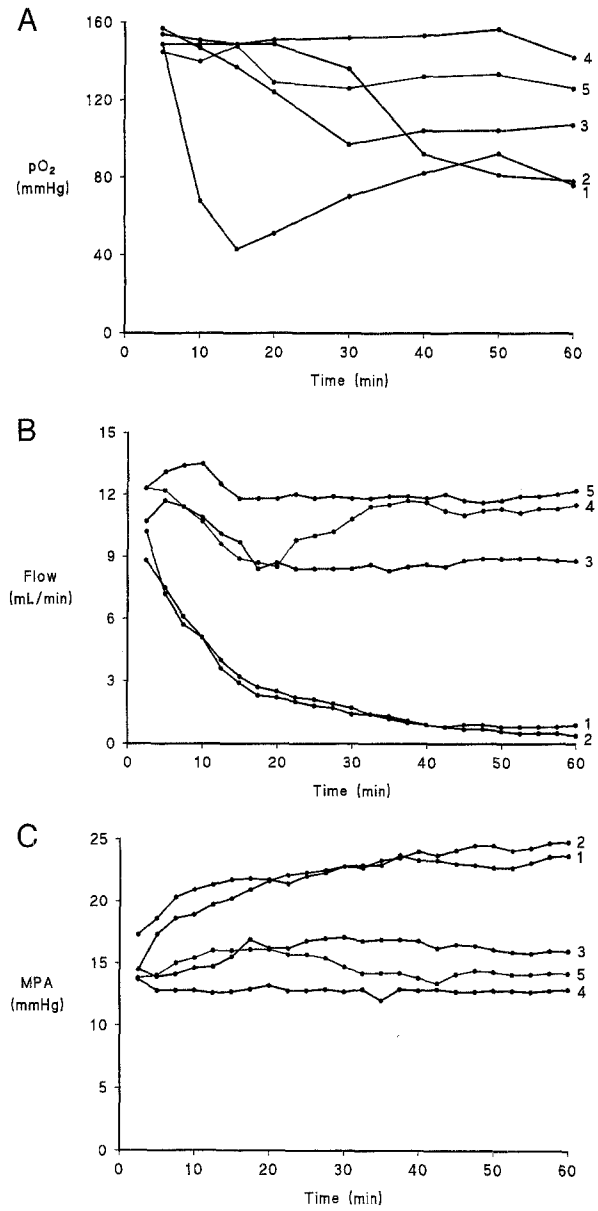

Fig. 6. Graft effluent Po_2 **(A),** blood flow **(B),** and mean pulmonary artery pressure **(C)** *(MPA)* during 1 hour of reperfusion of grafts stored for 24 hours in UW solution and receiving hydralazine, 200 μg/min, for the first 10 minutes of reperfusion. Data are for individual experiments. Grafts 4 and 5 performed at control levels. In graft 3, oxygenation and flow were moderately impaired, and grafts 1 and 2 functioned poorly.

It is now becoming clear that the NO/cGMP pathway can also be beneficially modulated in pulmonary preservation. Addition of GTN to storage

solutions improved rat lung graft function and reduced neutrophil and platelet accumulation after 6-hour storage in Euro-Collins solution[9] and 4-hour storage in Ringer's lactate.[10] In the same model, addition of a cGMP analog to the preservation solution also improved graft function.[4] We have previously demonstrated with our isolated rat lung reperfusion model that supplementation of Euro-Collins preservation solution with GTN yields significantly better graft function than supplementation with prostacyclin.[11] Prostacyclin is also a vasodilator with antineutrophil and antiplatelet activity (mediated through cyclic adenosine monophosphate) and is widely used because of these properties in clinical lung transplantation.

During lung reperfusion, it is possible to supplement NO levels by inhaled delivery of the gas. This produces vasodilation in well-ventilated parts of the grafts, thus improving ventilation/perfusion matching, while rapid inactivation of NO by hemoglobin prevents systemic hypotension. However, to date, mixed results have been achieved in attenuation of ischemia-reperfusion injury. In a rat lung transplantation model, inhaled NO treatment during reperfusion improved function in only 4 of 12 grafts whereas addition of a cGMP analog to the storage solution was uniformly beneficial.[25] Injury in rat lungs undergoing 90 minutes of warm in situ ischemia was significantly worsened when inhaled NO was administered from the onset of reperfusion.[26] This effect was reversed by addition of superoxide dismutase or by delaying inhaled NO therapy for 10 minutes. The authors hypothesized that interaction of NO with the burst of superoxide that is generated at reperfusion, with production of toxic peroxynitrite and hydroxyl radicals, may be responsible for these observations.[26]

In contrast, our current study has shown that administration of GTN during the first 10 minutes of reperfusion, which is when ischemia-reperfusion injury is triggered, has a sustained beneficial effect on lung graft function. GTN is an organic nitrate that is thought to exert its effects through liberation of NO intracellularly, as well as extracellularly, and also through production of S-nitrosothiol intermediates that directly stimulate cGMP production.[27] These mechanisms may be accompanied by generation of free NO in smaller quantities or in a different microenvironment compared with results with gas delivery by inhalation, thereby limiting interaction with superoxide. Another important factor may be that in our study grafts were ventilated with room air whereas in the inhaled NO studies, 70%[25] and 60%[26] oxygen were used. Oxidant injury in ischemic-reperfused rat lungs has been shown to increase when high oxygen concentrations are used during reperfusion.[28] Generation of reactive oxygen species and subsequent deleterious reaction with NO may therefore have been less marked in our model.

In a previous study, we showed that if rat lung grafts were reperfused at physiologic pressure after 24 hours of storage, marked deterioration of function occurred, whereas if for the first 10 minutes reperfusion pressure was reduced by 50%, subsequent function was similar to that in control lungs.[12] In the current study, too, the protective effect of intervention during the first 10 minutes was sustained subsequently throughout the period of reperfusion, which underlines the critical importance of this initial phase of reperfusion. Although it is not possible from our results to identify which of the actions of GTN and NO discussed were responsible for the observed protection, vasodilation must play a major role in view of the partial benefit seen with hydralazine.

Part of the vascular endothelial injury associated with lung graft preservation may derive from the flush/storage solution. There is concern that the high potassium concentration of the widely used Euro-Collins and UW solutions may be detrimental to endothelial function, and studies in which standard UW solution has been compared with modified low-potassium UW solution have shown the latter to yield better graft function.[29] However, isolated canine lobe studies have demonstrated that the increase in vascular tone caused by UW solution is slight and is less than that observed with Euro-Collins solution.[30] This may reflect the complex composition of UW solution, which includes the vasodilator adenosine. We elected to use UW solution because it has repeatedly been shown to be superior to Euro-Collins solution for lung graft preservation in experimental studies and is increasingly being used in clinical lung transplantation. The mechanism of the effect of GTN administration in our study may have included alteration of the endothelial response to exposure to UW solution.

The dose of GTN used in this study was relatively high, and although the GTN did not adversely affect the support animal, it would probably cause unacceptable systemic hypotension if given to a trans-

plant recipient. However, the results highlight the potential benefits of supplementing the NO/cGMP pathway during lung graft reperfusion, and further studies are warranted.

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Discussion

Dr. David A. Fullerton *(Chicago, Ill.).* Dr. Bhabra, I would like to congratulate you and your colleagues on a beautiful study. I wonder how many appreciate just how elegant the authors' model is. Any of us who are interested in experimental studies dealing with lung transplantation have struggled with setting up a model. This really is a sophisticated yet simple and elegant model of lung transplantation. It offers the opportunity to study just about any aspect of the transplanted lung. I think that the authors have effectively demonstrated that by controlling

the initial reperfusate, one can clearly improve lung function.

In this particular study, they elected to provide NO moiety through the reperfusion route, in other words, through the flow point of the ventilation-perfusion ratio. I was curious if Dr. Bhabra might first discuss the pros and cons of offering inhaled NO as another route of delivering the NO moiety.

Dr. Bhabra. As you say, with lung grafts it is possible to deliver NO by inhalation. There have been a couple of papers published recently that both showed that inhaled NO was not beneficial. The reasons offered for this lack of benefit were that NO can react with superoxide and generate peroxynitrite and hydroxyl radicals, which can cause further injury. However, our model is amenable to all sorts of studies, and since the current study we have used low-dose inhaled NO and under specific conditions it produces a similar benefit to that obtained with GTN. We have also done other studies in which we have been able to demonstrate that the detrimental effect of NO can be overcome if you take certain precautions.

Dr. Fullerton. Although you used the hydralazine as a cohort to examine simply the vasoactive component of the nitroglycerin, I must say that I was impressed that three out of the five lungs demonstrated very good function after hydralazine administration, and I acknowledge that the authors appropriately analyzed the data in a different fashion. I found it intriguing that three of those five lungs had near normal function, and so I was curious as to whether the authors have, for instance, had the opportunity to sample von Willebrand's factor or to isolate the neutrophils that might be coming through this lung to study them in vitro to see whether they might behave differently in the various treatment groups.

Dr. Bhabra. I believe that the vasodilation aspect is very important. We had a study published in the *Annals of Thoracic Surgery* this month in which we had found that simply by reducing the reperfusion pressure for the first 10 minutes we obtained sustained benefits. I think that an important component of this finding is that during initial reperfusion there is an increase in endothelial permeability, and if the capillary or the PA pressure can be reduced, that in itself can be beneficial in preventing edema formation. In studies in which we have measured the endothelial permeability in the initial stage of reperfusion, we have found that permeability is quite high in the first 5 minutes or so but then comes down. Hence if you protect the graft by either reducing reperfusion pressure or using dilators during this time, that in itself has a benefit.

As for looking at other components, for example, neutrophils, we are working on that and are developing other assays too.

Dr. Joseph Bavaria *(Philadelphia, Pa.).* We have done about 150 lung transplantations now clinically, and I think this is an interesting situation. The first question I have is, exactly when is the administration of nitroglycerin started? Are full dose and full levels achieved before opening up the reperfusion of the lung?

Dr. Bhabra. The way the model works is that the reperfusion limb of the circuit has to be clamped while we put the graft onto the circuit. Immediately before releasing the clamp, we started the GTN infusion, so there is not any preceding period of administration into the circuit.

Dr. Bavaria. Second, in clinical double-lung transplantation, in our bilateral sequential lung transplantation technique, the first lung is implanted, the clamps are taken off, and while both the native lung and the newly implanted lung are being perfused there are reasonably good, that is, low, pulmonary artery pressures. Then, as soon as explantation of the second lung begins the first lung is relied on to do all the work, and the PA pressures generally rise by about 10 to 15 mm Hg for the 90 minutes or so that it takes to implant the second lung. Do the authors have any ideas for us that we might want to use during that period to help us with the reperfusion injury and the injury that occurs in the first implanted lung, which generally, even though it has a short ischemic time, generally has a worse appearance on the chest x-ray film afterward?

Dr. Bhabra. I believe that this study is just setting out the concept that if NO is supplied, the outcome can be improved, but I do not really believe that the doses of GTN that we were using in this study would be appropriate clinically, because they would almost certainly cause systemic hypotension. We have to look at other ways of delivering NO, and I do believe that inhaled NO is the future. It would be possible to use this in the first lung while procedures are being done in the second lung.