The Therapeutic Potential of Nitric Oxide in Lung Transplantation*

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Endogenously produced oxides of nitrogen appear to play important roles in tissue and organ homeostasis. Endogenous production of nitric oxide, which can be altered in response to various stimuli, can modulate vascular tone, oxyradical cascades, cell adhesion, and other aspects of inflammation. Because exogenously administered (inhaled) nitric oxide can mediate pulmonary vasodilatation and improve pulmonary function in some patients with lung injury, treatment of lung allograft recipients with inhaled nitric oxide may ameliorate ischemia-reperfusion injury, thereby improving perioperative pulmonary function and diminishing ventilatory support requirements. This review examines the biology of nitric oxide and presents data that support its potential therapeutic effects for lung transplant recipients. (CHEST 1998; 113:1360-71)

Key words: lung transplantation; nitric oxide; peroxynitrites

Abbreviations: cGMP=guanosine 3',5'-cyclic monophosphate; iNOS=inducible calcium-independent nitric oxide synthase; L-NAME=N^6-nitro-L-arginine methyl ester; L-NMMA=N^5-monomethyl-L-arginine; NADPH=nicotinamide adenine dinucleotide phosphate; NANC=nonadrenergic noncholinergic; NO=nitric oxide; NO_2^-=nitrogen dioxide; NO_3^-=nitrite; NO_2^-=nitrate; NOS=constitutive, calcium-dependent nitric oxide synthase; ONOO^-=peroxynitrite; PMN=polymorphonuclear leukocyte

Allotransplantation of the human lung was first performed in 1963.1 However, lung transplantation was not successful enough to be accepted as a treatment for end-stage lung disease until the mid-1980s when the implementation of improved surgical techniques, development of improved organ preservation, and use of novel immunosuppressive drugs allowed long-term survival of allograft recipients.2 Over the past decade, a predominance of heart-lung transplants has been supplanted by single lung transplants or bilateral sequential lung transplants. Concurrently, operative mortality has declined and 1-, 2-, and 5-year survival has improved gradually. Two-year survival following transplantation for various causes of end-stage lung disease ranges from 55 to 70% according to data accrued from the St. Louis International Lung Transplant Registry.3 However, donor organ availability is a major problem, with many potential recipients achieving listing for lung transplantation but dying while awaiting transplantation.

Despite advances in lung transplantation and the acceptance of lung allotransplantation as a treatment for end-stage lung disease, graft and recipient survival are not optimal. In some instances, actuarial survival for lung transplant recipients may not be much better than the projected survival for the lung disorder for which allotransplantation is performed. Surgical techniques and operative mortality have improved gradually. However, infection and obliterator bronchiolitis4 are the major causes of morbidity and mortality after lung transplantation. Major infection risks include cytomegalovirus, aspergillosis, and bacterial bronchopneumonia. Unfortunately, retransplantation is seldom an option for recipients with graft failure, and outcome with retransplantation is significantly poorer than that following initial transplantation.5 Additionally, the dire shortage of
donor organs combined with the generally poor outcome of retransplantation severely limit the feasibility of retransplantation and underscore the need to optimize the outcome of initial primary lung transplantation by devising strategies to minimize early graft injury and subsequent rejection episodes.

Significant events immediately following lung transplantation that may adversely affect graft function and survival include oxyradical-mediated injury occurring secondary to ischemia-reperfusion, pulmonary leukocyte sequestration, and hyperoxia. Xanthine oxidase and granulocytes likely interact synergistically in mediating ischemia-reperfusion injury. Moreover, a subsequent need for prolonged ventilatory support with high oxygen concentration in inspired gas may further exacerbate oxyradical injury in this setting. Nitric oxide (NO) is known to inhibit xanthine oxidase generation of superoxide anion, possibly by alteration of key protein sulfhydryl, flavin, or iron-sulfur moieties. Because NO can inhibit xanthine oxidase as well as inhibit neutrophil chemotaxis and activation (discussed below), NO could be expected to be of therapeutic benefit in the immediate posttransplant period on the basis of its biochemical effects.

The Biology of NO

Identification of endothelium-derived relaxation factor in 1980, and demonstration in 1987 that NO produced by a vascular endothelial enzyme accounted for the biological properties of endothelium-derived relaxation factor, have opened exciting avenues of research that may lead to therapeutic benefit for a variety of disorders. NO has since been shown to have potent vasoregulatory and immunomodulatory properties. It is synthesized by various cell types via a family of enzyme NO synthases (NOS) that catalyze the production of NO via the five-electron oxidation of the guanidino nitrogen moiety of L-arginine to citrulline via N-hydroxy-L-arginine. At least two forms of cytosolic, Ca²⁺/calmodulin-dependent NOS are constitutively expressed. One is restricted to endothelium, but the other is found in numerous cell types, including nerve and bronchial epithelium. An inducible form of NOS is found in a wide variety of cell types, including macrophages, endothelial cells, and epithelial cells.

Endogenous NOs appear to play important roles in diverse biological processes for eukaryotic organisms ranging from Limulus polyphemus to vertebrates. Such biological processes include development, smooth muscle relaxation, neurotransmission, platelet adhesion, killing of malignant cells and bacteria, endocrine gland function, modulation of immune function, and wound healing. In many tissues, NO activates soluble guanylate cyclase, and its biological actions are mediated via guanosine 3′,5′-cyclic monophosphate (cGMP). An increase in cGMP levels reduces intracellular calcium concentration that can lead to smooth muscle relaxation, inhibition of platelet aggregation, and adherence or inhibition of leukocyte chemotaxis. However, not all actions of NO are cGMP dependent. The half-life of NO is very short (0.1 to 5 s in physiologic systems) due to rapid binding by deoxyhemoglobin with formation of methemoglobin as well as association with oxyhemoglobin to form S-nitrosohemoglobin. NO can also associate with other heme-containing proteins and can be sequestered as nitrosothiols by various nonhemoglobin proteins containing sulfhydryl groups, which can preserve its bioactivity but limit potential radical-dependent toxicity. Most NO is metabolized by erythrocytes where it is reduced to nitrite and nitrate with resultant conversion of hemoglobin iron from ferrous to ferric (methemoglobin). Subsequently, nitrate and nitrite enter plasma and are eliminated via the kidneys.

Several inhibitors of NOS have been discovered and include L-NAME, an Larginine analog, and L-NMMA, an L-dimethylarginine. These molecules competitively inhibit NOS both in vitro and in vivo, with constitutively expressed forms of NOS being more sensitive to L-NAME and inducible forms more sensitive to L-NMMA. Such inhibitors have been used to better understand the role of NOS and NO generation in inflammatory responses. For example, L-NMMA increases pulmonary vascular resistance when injected into the pulmonary artery of humans, suggesting that NOS regulates basal pulmonary vascular tone and blood flow. However, acute lung injury in rats, adjuvant-induced arthritis in rats, chronic ileitis in guinea pigs, progression of inflammatory arthropathy and immune complex glomerulonephritis in MRL-lpr/lpr mice, or systemic hypotension in sepsis have all been suppressed by administration of L-NMMA. These and other experiments demonstrate the complexity of the NOS system and potential for benefit or harm when NOS function is inhibited or enhanced.

NO is quite stable under anaerobic conditions for prolonged periods, but will form the more potent oxidizing agent, nitrogen dioxide (NO₂), another radical species, when exposed to oxygen. However, NO has a very short half-life in biological systems, and the formation of NO₂ is insignificant due to the relatively low concentrations of NO under physiologic or most pathologic conditions in vivo, in part due to reactions of NO with transition metals.
reacts even more efficiently with superoxide anion to form the potent oxidant, peroxynitrite (ONOO\(^-\)), than superoxide dismutase reacts with superoxide anion\(^27\) as shown in Figure 1. Under normal conditions, the concentration of superoxide dismutase is much greater than that of NO and superoxide anion, and little ONOO\(^-\) is formed. However, under pathologic conditions (eg, acute lung injury), formation of ONOO\(^-\) is increased and may contribute to tissue injury.\(^{28}\) Alternatively, NO may protect against cellular injury by shunting superoxide anion away from pathways that generate more damaging oxidants.

The role of NO in oxidant-mediated tissue injury (eg, pulmonary dysfunction related to hypoxia, inflammation, or ischemia-reperfusion) is unclear. Activated inflammatory cells can be stimulated to produce both superoxide anion and NO. Hydroxyl radical (\(\text{HO}^*\)), formed secondarily via the Haber-Weiss reaction, has been frequently proposed as the mediator of tissue injury. However, superoxide anion, hydrogen peroxide, and free, appropriately chelated iron (all of which are required to drive hydroxyl radical \([\text{HO}^*]\) production) are maintained in very low \textit{in vivo} concentration by very efficient scavenging mechanisms, and rates of individual reactions comprising the Haber-Weiss pathway are relatively slow.\(^{29}\) In contrast, ONOO\(^-\), which is very rapidly formed from NO and superoxide anion as mentioned above, has potent oxidant properties and can mediate lipid peroxidation.\(^{30}\) Peroxynitrous acid (ONOOH, \(\text{pK}_a=6.8\)) is known to decompose to \(\text{NO}_2\) and a species with hydroxyl anion-like activity. NO may participate in many toxic reactions with biological molecules, including lipid peroxidation,\(^{30}\) sulfhydryl oxidation,\(^{31,32}\) inhibition of mitochondrial function,\(^{33}\) or DNA cleavage.\(^{34}\) However, antioxidant chain-breaking termination reactions with lipid-based radicals,\(^{35}\) inhibition of superoxide anion production via nicotinamide adenine dinucleotide phosphate (NADPH) oxidoreductase,\(^{36}\) and various anti-inflammatory properties of NO (eg, diminished neutrophil recruitment\(^{37}\)) may actually diminish tissue injury, despite formation of ONOO\(^-\). Potential anti-inflammatory effects of NO are summarized in Table 1.

\[
\begin{align*}
\text{O}_2^- + \text{NO}^+ &\quad k = 2.3 \times 10^9 \text{ m}^{-1} \cdot \text{sec}^{-1} \quad \text{SOD} \quad \text{H}_2\text{O}_2 \\
\text{O}_2^- + \text{NO}^+ &\quad k = 6.7 \times 10^9 \text{ m}^{-1} \cdot \text{sec}^{-1} \quad \text{ONOO}^- \\
\end{align*}
\]

\textbf{Figure 1.} Dual metabolic fates of superoxide anion.

\begin{table}[h]
\centering
\caption{Potential Anti-inflammatory Effects of NO*}
\begin{tabular}{|l|}
\hline
1. Alteration of iron to inhibit Fenton and Haber-Weiss chemistry & \\
2. Reduction in the production of hydrogen peroxide and related downstream oxidants & \\
3. Augmentation of cGMP-mediated anti-inflammatory pathways & \\
4. Termination of autocatalytic lipid peroxidation & \\
5. Induction of anti-inflammatory and inhibition of proinflammatory gene expression & \\
\hline
\end{tabular}
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\textbf{NO in Immune Responses and Wound Healing}

Endogenous NO generated during an immune response has important antimicrobial and immunomodulatory effects. Macrophages and neutrophils utilize NO to kill various microorganisms, and administration of NOS inhibitors such as NMMA can impede antimicrobial actions of macrophages.\(^{35-40}\) Reactive oxygen and nitrogen intermediates formed simultaneously in phagocytes via NADPH oxidoreductase and inducible NOS, respectively, may have synergistic antimicrobial effects. For example, decomposition of ONOO\(^-\) to the hydroxyl-like species is promoted in an acidic environment such as that found in phagocyte vacuoles,\(^{41}\) and could enhance the antimicrobial actions of NO and superoxide anion.\(^{42}\) In addition to its potent antimicrobial properties, NO can cause injury or death of tumor cells,\(^{43,44}\) and may induce apoptosis following metabolism to ONOO\(^-\).\(^{45}\) In addition, NO via ONOO\(^-\) is known to alter cell signaling by generating nitrosine; such ONOO\(^-\)-promoted nitration of tyrosine residues is irreversible and can compromise the regulatory function of proteins that serve as substrates for protein kinases.\(^{46}\)

NO production is not only important for host defense against infectious agents, but is also an important immune response modulator. Enhanced NO synthesis by immune cells with mitogen stimulation or induction of alloimmune responses has been demonstrated both \textit{in vitro} and \textit{in vivo}.\(^{47,48}\) Low-level (constitutive) NO production appears to be necessary for maximal cell proliferation,\(^{49}\) but activation of inducible NOS, likely by macrophages, is associated with a reduced lymphocyte proliferative capacity.\(^{49}\) Interferon produced by primed T cells may inhibit lymphocyte proliferation by upregulating macrophage NO production,\(^{50}\) and NO produced by interferon-stimulated macrophages can inhibit expression of class II major histocompatibility complex molecules, thereby limiting antigen presentation to T cells with subsequent limitation of immune activation via diminished T-cell interferon production and
macrophage NO production.51 If large amounts of NO are produced rapidly in alloantigen-stimulated in vitro cultures of T cells and antigen-presenting cells, the T-lymphocyte proliferative response can be completely inhibited, and such inhibition is reversed if NOS inhibitors are present.52 Various in vitro experiments have shown elevated NO2− and NO3− in serum reflecting increased NO synthesis coincident with organ rejection or graft-vs-host reactions.53 Increased serum levels of NO2− and NO3− can be abrogated by administering the potent immunosuppressive agents, cyclosporine A or tacrolimus (FK 506). These data and the fact that NOS inhibitors can diminish organ inflammation in animal models20,22,54 underscore the fact that the relationship of endogenous NO synthesis to immune responses and tissue inflammation is complex and needs to be better understood.

In addition to altering lymphocyte and macrophage activity during alloimmune responses and chronic tissue inflammation, NO can modulate acute tissue inflammation and wound healing. NO appears to be generated by infiltrating polymorphonuclear leukocytes (PMNs) during early phases of wound healing,55 which is supplanted by arginase-dependent NO production by macrophages (increased arginase activity sequesters arginine required by macrophage NOS) as wound healing progresses.56 Although NO production by endothelial cells and PMNs may reduce adherence of PMNs to vascular endothelium,57 superoxide anion production by endothelium and/or PMNs could inactivate NO and increase leukocyte adherence and migration through vessel walls. Inhibition of NO production causes leukocyte rolling via induction of P-selectin58 and leukocyte adhesion due, in part, to the CD11/CD18 complex.57 Experiments with inflamed cat mesentery in vivo have shown that NOS inhibition by NMMA was associated with increased effectiveness of adhesion molecules (CD11/CD18) accompanied by increased PMN adherence and migration through the vessel wall.57 Local infusion of the NOS inhibitor, L-NAME, into the cat intestine caused a very rapid and persistent increase in microvascular protein and fluid leakage from the vessels that was independent of capillary hydrostatic pressure.59 Inhibition of adhesion prevented the later phase of vascular protein leakage caused by L-NAME infusion but not the early phase. In addition to causing leukocyte infiltration and increased microvascular permeability, inhibition of NO synthesis causes mast cell degranulation.60 In contrast to the proinflammatory effects of NOS inhibitors, NO donors attenuate organ permeability and injury in various models of ischemia-reperfusion.61-64 Attenuation of capillary leak and tissue injury by NO may not be due only to diminished PMN adhesion and suppression of mast cell degranulation but to inhibition of superoxide anion production by PMN NADPH oxidase.65 Inhaled NO has been demonstrated to prevent neutrophil-mediated, oxygen radical-dependent capillary leak in isolated, perfused rat lungs.66 Similarly, inhalation of NO at 5 to 10 ppm significantly attenuates mortality in hyperoxia-exposed adult rats, and NO inhibits increased epithelial permeability induced in vitro by exposure to reactive oxygen intermediates.37

NOS activity is absent in late phases of wound healing due to high local arginine activity, depriving macrophage NOS of arginine substrate.66 Increased ornithine concentrations due to increased arginine activity may promote polyamine synthesis and/or promote collagen synthesis by fibroblasts due to metabolism or ornithine,67 both of which would facilitate wound healing. Expression of NOS in later phases of wound healing could promote dysfunction and premature death of fibroblasts and macrophages,68,69 thereby adversely affecting successful wound healing.

**NO AND THE LUNG**

NOs can affect airway and vascular smooth muscle, neurotransmission, and host defense. Inhaled NO donors have been used to treat bronchospasm with variable results.70 Nebulized isosorbide was demonstrated to prevent exercise-induced bronchospasm in asthmatic subjects,71 although instillation into distal airways can increase peripheral airways resistance, possibly due to vascular engorgement.72 When delivered via the bronchial circulation, nitrogen oxide donors appear to relax proximal airways in both dog73 and man.74 In vivo investigations have demonstrated that NO or its reaction products can relax airway smooth muscle,75-77 although ONOO− has been implicated as causing airway or alveolar inflammation and edema.74,78

The effects of NO on pulmonary vascular tone and matching of ventilation with perfusion have been studied extensively. Endothelial cells from the pulmonary arterial and venous circulation can release NO when stimulated by agonists that relax vascular smooth muscle (eg, acetylcholine, bradykinin).79 NO modulates pulmonary arterial contractile responses to catecholamines80 or prostaglandin F2α,81 reverses hypoxic pulmonary vasoconstriction,82 and decreases pulmonary vascular resistance.83 Inhibitors of NOS prevent histamine- and endothelin-mediated pulmonary vasodilation,80,81 and L-NMMA increases pulmonary vascular resistance when injected into the pulmonary artery of human subjects.18 Nebulization of a NO donor in dogs has been shown to reverse
pulmonary embolism-related bronchoconstriction, and endothelium-dependent relaxation of pulmonary artery smooth muscle is impaired in patients with chronic obstructive lung disease accompanied by hypoxemia and hypercapnia. These findings suggest that hypoxic pulmonary vasoconstriction is mediated by inhibition of NO release, because inhaled NO is delivered only to ventilated areas of the lung.

NO also appears to play an important role in airway neurotransmission. For example, in the guinea pig, NO appears to be involved in vasoactive intestinal polypeptide-mediated and nonvasoactive intestinal polypeptide-mediated nonadrenergic, noncholinergic (NANC) relaxation of airway smooth muscle. NO-dependent NANC responses in human bronchi are significantly diminished in denervated (transplanted) lung and specimens from patients with cystic fibrosis, suggesting that NO-mediated airway relaxation is of central importance to the NANC neural response in humans.

NO likely plays an important role in pulmonary antimicrobial defenses and regulation of airway inflammation. NO production by "juxtaposed" alveolar macrophages appears to depress antigen-presentation abilities of dendritic cells, thereby preventing inappropriately exuberant responses to inhaled alveolar antigens that are benign and can be cleared via phagocytosis and/or the mucociliary escalator. Additionally, nitrosothiols are bacteriostatic, and NO and superoxide anion can form the potent bactericidal agent, ONOO-. However, ONOO- and other nitrogen oxides can damage the lung. L-NMMA can block such lung injury. ONOO- is known to inhibit the ability of surfactant protein A to aggregate phospholipids. Hence, while NO pathways are important for lung homeostasis and protection against pulmonary pathogens, reactive nitrogen intermediates formed in response to such pathogens may damage normal lung tissue.

Therapeutic Applications of NO: Rationale and Clinical Use

NO is involved in local regulation of blood flow not only in the lung, but also in various other organs, including heart, brain, liver, and kidney. Inhibition of NOS in experimental animals, either short term or long term, caused an increase in systemic arterial pressure that could be reversed by L-arginine administration. Administration of L-arginine alone in various animals, however, generally does not alter BP. In contrast, L-arginine infusion in humans who are normal volunteers or have essential hypertension can decrease systemic pressure, although this was not observed in another study of patients with scleroderma and pulmonary hypertension or normal control subjects.

Agonists such as bradykinin or acetylcholine can cause NO release by pulmonary arterial and venous endothelial cells. Administration of inhaled NO to various animals including lambs given a thromboxane endoperoxide analog, sheep infused with heparin-protamine or subjected to hypoxia, or dogs given a thromboxane analog to induce pulmonary hypertension either decreased or reversed the pulmonary hypertension. Similarly, in dogs given oleic acid, pigs given oleic acid, or pigs administered an endotoxin infusion to induce acute lung injury (ie, ARDS models), inhaled NO partially or completely prevented increased pulmonary artery pressure; ventilation-perfusion maldistribution, oxygenation, and lung edema all improved in the pig models following NO administration.

In a sheep model of acute lung injury, inhaled NO at 60 ppm improved arterial oxygenation and decreased venous admixture but did not alter systemic hemodynamics. On the basis of these and other animal investigations, the finding that patients with primary pulmonary hypertension may have basic defects in the endothelial NOS/L-arginine pathway and the observations that neonatal disorders (bronchopulmonary dysplasia, surfactant deficiency, persistent pulmonary hypertension of the newborn, and congenital diaphragmatic hernia) are often characterized by pulmonary hypertension, various investigators have suggested that inhaled NO may have a significant beneficial impact on patients with various acute or chronic disorders characterized by pulmonary hypertension and impaired gas exchange.

Many clinical investigations have now been performed in humans to evaluate the effect of inhaled NO on pulmonary vascular tone and ventilation-perfusion matching. Inhaled NO reversed hypoxic pulmonary vasoconstriction in healthy subjects at 40 ppm without altering hemodynamics or gas exchange, caused selective pulmonary vasodilation at 40 ppm with improved gas exchange in patients with COPD, and decreased pulmonary artery pressure and vascular resistance at 15 ppm in patients with COPD. Administration of NO at 80 ppm to six newborns with persistent pulmonary hypertension improved predeltal and postdeltal oxygen saturation, and 10 to 20 ppm inhaled NO improved oxygenation in infants with severe persistent pulmonary hypertension being considered for extracorporeal membrane oxygenation. Ten to 20 ppm NO effectively controlled pulmonary hypertension following surgical repair of congenital diaphragmatic hernia in a neonate, 40 to 80 ppm selectively dilated pulmonary vessels in infants and children with congenital heart disease during diagnostic car-
cardiac catheterization,118 and inhaled NO at concentrations ranging from 5 to 50 ppm improved gas exchange in infants with severe hypoxemia referred to extracorporeal membrane oxygenation therapy.120 Inhaled NO at 80 ppm effectively lowered pulmonary artery pressure in patients with congenital heart disease associated with pulmonary hypertension before and after cardiopulmonary bypass,121 and inhaled NO (but not IV vasodilators) decreased pulmonary artery pressure and vascular resistance significantly but did not impair oxygenation during one-lung ventilation in patients with or without moderate pulmonary hypertension who were undergoing surgery.122 In addition to the foregoing reports, inhaled NO has controlled pulmonary hypertension postoperatively in infants, children, and young adults who underwent surgery for congenital heart disease,123,124 and intraoperatively for patients undergoing surgery for mitral valve replacement associated with pulmonary hypertension.125 Under various study protocols, most state-of-the-art pediatric and neonatal ICUs utilize NO to reduce pulmonary artery pressure and improve arterial oxygenation for diverse lung diseases. Inhaled NO has also been administered therapeutically to patients with ARDS. Rossaint et al126 observed that inhaled NO at 18 ppm acutely reduced pulmonary artery pressure and intrapulmonary shunting in patients with severe ARDS. Gerlach et al127 investigated short-term effects of inhaled NO in patients with ARDS. NO enhanced oxygenation within 1 to 2 min (which reached a plateau by 8 to 12 min) and decreased pulmonary artery pressure at very low concentrations of NO in inspired gas (<3 ppm). Long-term administration of NO at low concentrations (60 to 230 parts per billion) for 9 to 13 days to patients with ARDS improved oxygenation and was not necessarily associated with a reduction in pulmonary arterial pressure.128 A retrospective review of 87 patients with severe ARDS could not confirm improved survival in the NO-treated group.129 Nevertheless, inhaled NO at concentrations ranging from 5 to 80 ppm (and perhaps as low as 0.1 ppm) improves hemodynamics and, frequently, oxygenation status, in a variety of pulmonary pathologic states.

**NO in Transplantation**

NO production has been studied extensively in solid organ transplantation in various animal models. Increased systemic nitrite levels and induction of iNOS messenger RNA in areas of inflammatory cell infiltrates have been demonstrated by Kuo et al120 for acute rejection models of heterotopic heart and orthotopic liver transplantation in rats. Ohdan et al130 found a dramatic increase in NO synthesis in allogeneic liver transplantation as compared with syngeneic transplants in rats, and Kuo et al120 observed increased iNOS expression in hepatocytes as well as infiltrating inflammatory cells in acute rejection. Additionally, Worrall et al131 observed increased systemic nitrite levels in an in vivo model of cardiac transplantation in rats in association with increased iNOS messenger RNA levels, and the inhibitor of NOS, aminoguanidine, suppressed NO production and attenuated cardiac rejection. Similarly, Winlaw et al132 reported that L-NMMA could abolish the increase in urinary nitrite excretion in a rat model of cardiac allotransplantation and increase graft survival, although allograft rejection could proceed to complete graft loss despite complete inhibition of rejection-associated increased NO production. Worrall et al133 demonstrated that iNOS inhibition by aminoguanidine significantly reduced cardiac allograft serum nitrite/nitrate levels and attenuated graft and systemic vascular barrier dysfunction in a rat model. However, Paul et al134 reported that administration of L-NAME to block NOS in rats given cardiac allografts decreased graft survival time and caused ischemic necrosis, possibly as a consequence of unopposed vasoconstriction.

Investigations in humans have also suggested that NO pathways are activated following graft implantation and with episodes of rejection. In patients who underwent cardiac transplantation, rejection grade on endomyocardial biopsy specimens correlated closely with serum nitrate concentrations.135 These authors suggested that serum nitrate determinations could play an important role in monitoring heart allograft recipients. In orthotopic liver transplantation, serum nitrate levels significantly increased in patients with episodes of rejection.136 Nitrate levels were also increased in the first few days following liver transplantation but returned to baseline within a few days in the absence of rejection. Devlin et al137 also found elevated levels of acid-labile nitroso compounds in plasma during episodes of acute hepatic rejection in humans that correlated with rejection severity and declined with augmented immunosuppressive therapy.

Some investigators have also examined the role of NO in animal models of lung transplantation. Shiraishi et al138 reported that treatment of allotransplanted rats with the inducible NOS inhibitor, aminoguanidine, starting 6 h after transplantation significantly reduced serum nitrite/nitrate levels and suppressed lung rejection at day 7 and 14 following transplant. Naka et al139 found that increased cGMP decreased pulmonary vascular resistance, improved oxygen tension, reduced myeloperoxidase activity, and improved recipient survival in a rat model of
lung allotransplantation, but inhaled NO administered during reperfusion failed to significantly alter any of these parameters. In contrast, Pinsky et al.\(^{140}\) found that augmenting the NO pathway at the level of cGMP via use of a membrane-permeable cGMP analog in the preservation solution kept NO levels at the lung surface from plummeting upon reperfusion of the allograft in an orthotopic rat model. This was associated with lower pulmonary vascular resistance, increased blood flow to the graft, improved arterial oxygen tension, decreased neutrophil infiltration, and improved recipient survival. Katayama et al.\(^{141}\) administered inhaled NO to rats with monocrotaline-induced pulmonary hypertension for 24 h immediately following single lung transplantation and found that pulmonary artery pressure was significantly higher in animals not receiving NO. Blood flow to the orthotopic graft was increased in comparison to control animals, and edema of the grafted lungs was histologically less severe at 24 h following transplant in the animals that received NO. Lindberg et al.\(^{142}\) also demonstrated a reduction of pulmonary vascular resistance in pigs following single lung transplantation with contralateral pneumonectomy, but NO therapy was not begun until 24 h after transplantation. These investigators noted rebound pulmonary vasoconstriction in both control animals and, to a lesser degree, in animals with transplants following termination of NO and suggested that NO should be gradually weaned if used in clinical situations.

Relatively few investigators have reported the use of NO in human lung transplantation. Adatia et al.\(^{143}\) treated six lung allograft recipients with NO at 80 ppm and observed a lowered mean pulmonary artery pressure, reduced intrapulmonary shunt fraction, and diminished transpulmonary pressure gradient with only minor concomitant effects on the systemic circulation. Sustained improvement in oxygenation and pulmonary artery pressure was observed in two patients given extended NO inhalation at 10 ppm. Macdonald et al.\(^{144}\) recently reported the successful treatment of life-threatening acute reperfusion injury following lung allotransplantation with inhaled NO. A more recent, retrospective study by Date et al.\(^{145}\) reported that inhaled NO when administered postoperatively to 15 patients for severe allograft dysfunction was safe and significantly lowered mean pulmonary artery pressure and improved the arterial oxygen tension to inspired oxygen fraction ratio within 1 h of administration. Improvement in these parameters was sustained for the duration of therapy, and postoperative complications were less in the treated group as compared with a historical control group of 17 patients. Allograft recipients at the authors’ institution have similarly been treated with inhaled NO for severe postoperative allograft dysfunction and found a significant reduction of pulmonary vascular resistance and mean pulmonary artery pressure accompanied by a significant improvement in PaO\(_2\)/fraction of inspired oxygen ratio (unpublished data; Robert Love, MD; Madison, Wisc; 1997) within the first hour after initiation of inhaled NO.

**Potential Adverse Effects of Inhaled NO**

Major potential toxicities of inhaled NO appear to be related to the formation of NO\(_2\), ONOO\(^-\), and methemoglobin.\(^{146}\) Methemoglobinemia generally does not pose a problem during inhaled NO therapy if NO concentration is effectively monitored. Because NO\(_2\) can be generated from NO and can cause pulmonary edema, NO\(_2\) formation is a concern. The estimated drug dose resulting in 50% mortality (LD\(_{50}\)) for humans with 1-h exposure to NO\(_2\) is 174 ppm, and short-term exposure to >150 ppm NO\(_2\) is generally fatal.\(^{147}\) Two parts per million of NO\(_2\) is the high end of the acceptable range for NO\(_2\) exposure in humans. In a rat model in which the animals were exposed to 2 to 17 ppm of NO\(_2\), histologic changes (alveolar cell hyperplasia and terminal bronchial epithelial hypertrophy) developed within 2 to 3 days of exposure.\(^{40}\) While it is unlikely that such high concentrations of NO\(_2\) would be generated or go undetected with levels of NO used clinically, the assumption that expired NO\(_2\) concentrations are associated with the extent of NO\(_2\)-related lung injury may not be entirely accurate, because NO\(_2\) is avidly taken up by epithelial lining fluid in proportion to its rate of reaction with glutathione.\(^{147-149}\) The findings that NO\(_2\) concentrations >1.5 ppm increase granulocyte and macrophage recruitment in the lungs of hamsters, and that 0.4 to 5 ppm depresses surfactant hysteresis,\(^{148}\) alters epithelial ultrastructure,\(^{149}\) and increases the expression of proinflammatory cytokines\(^{150}\) raise concern about the possibility of significant tissue injury even with relatively low NO\(_2\) concentrations in inspired gas, especially if high concentrations of oxygen are concomitantly administered that can enhance the oxidation of NO to NO\(_2\). ONOO\(^-\) has numerous potential adverse pulmonary effects, including surfactant alteration, macromolecular structural changes (eg, DNA strand breaks), metabolic injury (eg, oxygen consumption, ion transport, and signal transduction interference), and induction of apoptosis.\(^{151,152}\) Evidence for in vitro pulmonary ONOO\(^-\) production (nitrotyrosine) in human acute lung injury has been ascertained.\(^{153}\)

Numerous studies in animals and humans have investigated potential adverse effects of inhaled NO. Dogs breathing high concentrations of NO (20,000 ppm) develop pulmonary edema and methemoglobin-
binemia.\textsuperscript{154} Exposing lambs to NO to 80 ppm for 3 h in 21% oxygen did not cause methemoglobinemia, pulmonary edema, or histologic changes.\textsuperscript{82} However, rabbits exposed to 5 ppm NO for 14 days developed fluid-containing vacuoles inside arteriolar endothelial cells, changes in intercellular junctions, and thickening of alveolar capillary membranes.\textsuperscript{135} Exposure of rats to 0.5 ppm NO for 9 weeks also has been shown to produce alveolar septal injury.\textsuperscript{126} In contrast, inhalation of 10 ppm NO for 2 weeks protected rats from muscularization of pulmonary vessels and right ventricular hypertrophy when subjected to hypoxia,\textsuperscript{157} and 5 to 10 ppm NO decreased mortality in rats exposed to 100% oxygen.\textsuperscript{37} In addition to potential adverse effects on the lung, inhalation of NO (50 ppm) by rats resulted in impaired performance of learned tasks and prolonged brainstem-evoked potential responses,\textsuperscript{139} and long-term inhalation of 10 ppm was associated with altered immune function in mice.\textsuperscript{130}

Cigarette smoke delivers a concentration of 600 to 1,000 ppm NO,\textsuperscript{160} but such intermittent exposure to NO does not appear to cause acute morbidity to humans. However, brief exposures to low concentrations of NO could have deleterious effects on human airways. Inhalation of as little as 1 ppm NO for 2 h caused a small decrease in specific airway conductance in normal subjects,\textsuperscript{161} and inhalation of 15 to 20 ppm NO for 15 min by normal subjects was associated with a modest decrease in PaO\textsubscript{2} and increased airway resistance.\textsuperscript{162} Although such potential adverse effects of inhaled NO are of concern, there is no evidence that they are clinically significant when relatively low concentrations of inhaled NO are administered (≤80 ppm) with careful monitoring for methemoglobinemia and NO\textsubscript{2} (eg, numerous ongoing pediatric studies examining inhaled NO for pulmonary hypertension).

CONCLUSIONS

NO can alter vascular tone via smooth muscle relaxation, inhibit platelet aggregation and adherence to endothelium, suppress leukocyte chemotaxis and superoxide anion generation, and diminish capillary leak. NO is capable of modulating pulmonary vascular tone, and it can improve ventilation/perfusion matching and oxygenation in injured or diseased lungs. These properties make NO an attractive therapeutic agent for treatment of acute lung injury, particularly ischemia-reperfusion injury to which the newly transplanted lung allograft is predisposed. Experimentation in animal models of lung transplantation is inconclusive but suggests that administration of inhaled NO or use of cGMP analogs that augment NO-dependent pathways can improve early severe graft dysfunction. Administration of inhaled NO may attenuate injury and graft dysfunction immediately following implantation when the graft is subject to ischemia-reperfusion injury. In contrast, inhibition of NO production by inducible NOS may suppress acute graft rejection. Additional animal research will better define the effect of altering NO pathways following lung transplantation. Preliminary, uncontrolled experience with inhaled NO in human lung transplantation suggests that such therapy may prove beneficial for severe postoperative allograft dysfunction. Perioperative administration of inhaled NO may be particularly beneficial in single lung transplantation, especially for patients undergoing transplantation for severe pulmonary hypertension. When monitored carefully, therapeutically administered inhaled NO appears to be well tolerated with little evidence of clinically relevant toxicity. Both animal studies and early clinical experience support the need for prospective, controlled clinical trials of inhaled NO for treatment of postimplantation injury in human lung transplantation. Although additional investigations with animal models and clinical applications of inhaled NO or ingested iNOS inhibitors are needed, the animal and clinical data currently available suggest that use of inhaled NO in the perioperative period may benefit lung allograft recipients.

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