The Role of Nitric Oxide in Solid-Organ Transplantation

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Abstract Ischemic reperfusion injury (IRI) is a complex cellular and molecular inflammatory response that occurs upon reperfusion of a transplanted organ. This injury is related directly to the ischemia time and reduces the graft’s regenerative capabilities. Surgeons strive to keep the ischemia time to a minimum, but IRI is an inevitable occurrence that particularly impacts marginal grafts, such as those from older donors or those donated after cardiac death. To maximize the use of these organs and achieve better outcomes, clinical researchers have studied the use of nitric oxide to minimize IRI. This antioxidant and anti-inflammatory agent affects cell signaling, inhibits nuclear proteins, and limits different levels of IRI. Inhaled nitric oxide has produced good outcomes in terms of primary graft dysfunction in lung-transplant recipients. More randomized trials are needed to further define the utility and safety of nitric oxide in patients who receive lung and other organ transplants.

I N F O R M A T I O N V I N T A G E

Ischemic injury is an inevitable event associated with organ transplantation. It begins with warm ischemic injury, particularly in organ donations resulting from cardiac death, starting with cardiac arrest and continuing with perfusion of the organ with preservative solution. Subsequent organ preservation in cold solution leads to cold ischemic injury, followed by warm ischemia upon implantation of the organ in the recipient. Reperfusion of the ischemic vasculature with blood is associated with the release of free radicals, which eventually produce ischemic injury and trigger an intense immune response in the organ.

At an Ikaria-sponsored satellite symposium held during the 2014 World Transplant Congress, three experts discussed the role and potential applications of nitric oxide in transplant medicine. The panelists included Rakesh P. Patel, PhD, Professor of Pathology at the University of Alabama at Birmingham; Arlin B. Blood, PhD, Associate Professor of Pediatrics in the Division of Neonatology at the Loma Linda University School of Medicine; and Ronald A. Bronicki, MD, Associate Professor of Pediatrics, Critical Care, and Cardiology at Baylor College of Medicine and the Texas Children’s Hospital in Houston.

ISCHEMIC REPERFUSION INJURY (IRI)

IRI is a combination of complex cellular and molecular inflammatory responses. The microcirculation endures endothelial dysfunction within minutes following reperfusion of the ischemic vasculature. Endothelial dysfunction is characterized by a loss in basal and agonist-mediated nitric oxide produced by the vascular endothelium. The loss of nitric oxide results in upregulation of cell adhesion molecules (CAMs), particularly P-selectin, following reperfusion. Thus, CAM upregulation renders the endothelium “sticky.” A marked degree of leukocyte adherence (particularly involving neutrophils) occurs following reperfusion (Figure 1), leading to neutrophil infiltration of the underlying tissue. The infiltration of neutrophils leads to reperfusion injury (ie, necrosis), which is significant after 3 hours and becomes profound after 4½ hours.

The degree of IRI is directly associated with the ischemia time. Some degree of such injury is unavoidable considering the nature of the procedure, but the ischemia time must be minimized, and other methods of decreasing IRI must be accomplished. Further, IRI is inversely proportional to the size of the allograft. IRI reduces the regenerative capabilities of the allograft, which directly impacts morbidity, mortality, and long-term patient outcomes. IRI limits the use of marginal grafts, particularly when tissues from extended-criteria donors (eg, older donors) are used.

Overall, the impact of IRI that affects the allograft so badly during the first few hours after reperfusion must be minimized to prevent early graft dysfunction or nonfunction. To achieve this goal, researchers must learn more about the best use of marginal grafts (eg, those from steatotic livers) or organs that have had a relatively long ischemia time. Transplant teams also need to know more about maximizing the use of allografts following cardiac death and using split-liver grafts.

HOW NITRIC OXIDE WORKS

In 1992, nitric oxide was considered to be “the molecule of the year.” This important cellular molecule is produced by nitric oxide synthases. These enzymes convert arginine into citrulline, produc-
ing nitric oxide as a byproduct. Oxygen and nicotinamide adenine dinucleotide phosphate are necessary cofactors. The three isoforms of nitric oxide synthase—neuronal nitric oxide, endothelial nitric oxide, and inducible nitric oxide—are named according to the activity or tissue in which they are found.

Nitric oxide, a signaling molecule that participates in different physiologic and pathologic processes, is a powerful vasodilator with a half-life of just a few seconds in the blood. Nitric oxide synthetase inhibits cell death, neutrophil migration and activation, platelet aggregation and adhesion, CAMs, and release of vasoconstrictors and growth factors.

In addition, nitric oxide works as an antioxidant and anti-inflammatory agent, inactivates superoxides, and imparts beneficial effects on cell signaling and inhibition of nuclear proteins. By doing so, nitric oxide has important stopping power on different levels of the IRI cascade. Exogenous and endogenous nitric oxide protect against IRI.

#### INHALED NITRIC OXIDE (INO)

INO is a selective pulmonary vasodilator. Its role is well established in ameliorating the effects of pulmonary hypertension in infants. More recently, iNO is being tested for benefits in patients with such pulmonary pathologies as acute respiratory distress syndrome, where it acutely improves hypoxemia. Administration of iNO reduces the need for extracorporeal membrane oxygenation in term and near-term infants who have persistent pulmonary hypertension.

However, these short-term benefits of iNO have not been shown conclusively to outweigh its potential toxicities. For example, high-dose iNO use reduces surfactant function in the lungs. It also acts as a pulmonary irritant, causing activation of pulmonary macrophages and oxidative injury to the pulmonary epithelium. At high concentrations—greater than 8–100 ppm—iNO has pro-inflammatory and pro-oxidant effects, increasing macrophage production of tumor necrosis factor-α, interleukin (IL)-1, and reactive oxygen species.

Extrapulmonary effects of iNO have been described in the literature. Cardillo and others reported that iNO increases blood flow in the forearm during nitric oxide deficiency. Hataishi et al described the utility of iNO in protecting myocardial reperfusion injury in mice. Fox-Robichaud and colleagues showed that iNO inhibited inflammation and reduced mesenteric ischemic reperfusion injury in cats.

In clinical research on the effect of iNO on human ischemic tissue, Mathru et al reported that use of 80 ppm of iNO significantly reduced inflammation in tourniquet-induced lower-extremity ischemia.

iNO has been shown to accelerate restoration of liver function in adults following orthotopic liver transplantation. Lang and colleagues reported that alanine transaminase (ALT) and aspartate transaminase (AST) levels trended downward with administration of 80 ppm of iNO.

Lang et al performed another study at the University of Alabama and the University of Washington. They documented iNO-enhanced allograft function by down-trending liver function tests ($P < 0.05$ at the University of Washington; $P < 0.03$ for ALT with data from both centers combined) and reduced complications at 9 months (data from both centers combined: odds ratio, 0.15; 95% confidence interval, 0.04–0.59; $P = 0.0062$). Use of iNO increased concentrations of nitrate ($P < 0.001$), nitrite ($P < 0.001$), and nitrosyl hemoglobin ($P < 0.001$), with nitrite possibly having a protective mechanism. The mean cost of iNO was $1,020 per transplant. The study showed use of iNO to be safe; further, it improved liver graft function at one center and trended toward improving liver graft function at the other.

Other pulmonary vasodilators (eg, prostacyclin) also work like nitric oxide in terms of IRI.

#### DURATION AND DOSING OF INO: LUNG TRANSPLANT EXPERIENCE

Primary graft dysfunction (PGD) after lung transplant is associated with severe hypoxemia, lung edema, and the radiographic appearance of diffuse pulmonary opacities without another identifiable cause. The typical pathologic pattern of PGD is diffuse alveolar damage. Despite significant advances in organ preservation, surgical technique, and perioperative

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**FIGURE 1** Cellular response in ischemic reperfusion injury. CD11b/CD18 = sargosomal neutrophil integrin; $O_2 =$ carbon dioxide; ICAM-1 = intercellular adhesion molecule-1; IL = interleukin; LT = lymphotixin; NADPH = nicotinamide adenine dinucleotide phosphate; NO = nitric oxide; $O_2 = $ oxygen; PAF = platelet-activating factor; PECAM = platelet-endothelial cell-adhesion molecule; PSGL-1 = P-selectin glycoprotein ligand-1; Rec IL-8 = neutrophil interleukin-8 receptor; ROS = reactive oxygen species; TNF-α = tumor necrosis factor-alpha; VasoC = vasoconstrictor. Reproduced, with permission, from Gourdin and Dubois.3
care, PGD is still responsible for significant morbidity and mortality after lung transplantation.17-19

IRI, the major cause of PGD after lung transplant, is an important factor for long- and short-term morbidity. Mild IRI is seen in more than 95% of lung-transplant recipients. In about 30% of lung-transplant recipients, severe IRI is noted.20

Pasero et al21 and Tavare et al22 reviewed multiple studies and reported that administration of iNO improves gas-exchange properties and selective pulmonary vasodilation, decreases pulmonary vascular resistance, and improves ventilation/perfusion mismatch. Although improved graft function was not always seen in these studies, improved hemodynamics were observed. Only a few clinical studies assessed primary graft failure; most were limited by small sample size and, unfortunately, were not randomized and/or controlled studies.

Use of iNO decreases primary graft dysfunction in animal models by limiting the inflammatory cascade and neutrophil adhesion. Different cytokines (eg, IL-6, IL-8, and IL-10) are markers for PGD. In a study conducted in pigs,23 nitric oxide pretreatment at the time of harvesting reduced allograft reperfusion injury, in part because of its effects on IL-8 release. iNO was studied at various doses and timings. Extrapulmonary protective effects were observed only when 80 ppm of iNO was given.

Moreno and others24 studied the effects of iNO administration on primary graft dysfunction in human lung transplantation. Of 49 patients, 29 were treated with 10 ppm of iNO for up to 48 hours, and 20 were controls. The iNO group had a significantly lower incidence of PGD than did the control group (17% vs 45%; P < 0.035). A significant difference (P < 0.05) also was observed between the iNO-treated and control groups when levels of IL-6, IL-8, and IL-10 were assessed in blood and bronchoalveolar lavage fluid at 12 and 24 hours.

Outcomes of studies of iNO in human lung transplantation are limited because of small sample size, duration and time of initiation of iNO therapy, and iNO dosing.

Inhaled prostacyclin also may have a role in heart and lung transplantation. Both inhaled prostacyclin and iNO reduce pulmonary artery pressures and central venous pressure, as well as improve cardiac index and mixed venous oxygen saturation, in heart- and lung-transplant recipients.25 Although administration of iNO was superior to other inhaled vasodilators in terms of hemodynamics for lung-transplant recipients,26 the prophylactic role of iNO in patients undergoing lung transplantation has not been definitively established.21

PHARMACOLOGY OF THE NITRIC OXIDE PATHWAY

Nitric oxide is a free radical with a short half-life in biologic fluids. The transit time for nitric oxide in blood from lung to periphery is about 5–15 seconds; the half-life of free nitric oxide in whole blood is less than 10 ms (Table 1). Nitric oxide acts on soluble guanylyl cyclase, resulting in increased levels of cyclic guanine monophosphate in smooth muscle cells of the lungs. It also may regulate pulmonary vasodilation by direct activation of potassium channels or by modulation of the expression and activity of angiotensin II receptors. After inhalation, nitric oxide diffuses into the bloodstream and rapidly reacts with oxyhemoglobin to form methemoglobin and nitrate and with deoxyhemoglobin to form iron-nitrosylhemoglobin (Figure 2).27

The two reaction mechanisms are S-nitrosation of thiols (including cysteine residues in proteins) and the nitrosylation of transition metal ions. Inhalation of nitric oxide also increases the level of nitrite and nitrate in blood. These compounds work as a nitric oxide donor, increase nitric oxide bioactivity throughout the

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<td><strong>Half-Life of Nitric Oxide in Different Media</strong></td>
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<tr>
<td>Matrix</td>
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<tr>
<td>Deoxygenated buffer</td>
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<td>21% Oxygen buffer</td>
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**FIGURE 2** Nitric oxide in blood cells. AE1 = anion-exchange protein 1; NO = nitric oxide; O2 = oxygen; SH = thiol; SNO = S-nitrosothiol; X-NO = exported nitric oxide. Reproduced, with permission, from Gross.27
body, and account for the peripheral effects of iNO on distant organs. As shown by Kubes and McCafferty, iNO increases the intestinal blood flow in cats and decreases leukocyte adhesion.

In the blood, nitric oxide is converted to nitrite, a relatively stable compound that generates nitric oxide in the setting of hypoxia. Ibrahim et al showed that the levels of nitrite significantly rise in serum and cerebrospinal fluid after inhalation of nitric oxide. Nitrite is a potent vasodilator in the human forearm.

Nitrite has a protective role in ischemic/reperfusion studied in animal models. In humans, nitrite has proven beneficial in setting of crush injury/ischemia reperfusion. Duranski et al described how nitrite decreases in vivo liver injury in a U-shaped, dose-dependent manner (Figure 3).

Almost 70% of iNO is excreted within 48 hours as nitrate in the urine. Blood levels of nitrate have been reported to increase fourfold during inhalation of 80 ppm of iNO.

**CONCLUSION**

In the treatment of premature infants and patients with acute lung injury and acute respiratory distress syndrome, iNO is a well-established treatment modality. However, in the setting of IRI associated with organ transplantation, its promise is still largely untested. More studies are needed to explore its multifactorial and complex mechanisms of action in lung and other solid-organ transplant recipients and its potential value in these patients.

REFERENCES


