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Inhaled NO in the Experimental Setting

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Abstract

Nitric oxide, a gas molecule, is a unique pharmaceutical agent that can be inhaled and thus delivered directly to the lung. More than a decade of intensive laboratory and clinical investigation has culminated in the current role for inhaled NO as the only selective pulmonary vasodilator for the treatment of persistent pulmonary hypertension of the newborn (PPHN). Not surprisingly, this potent and successful therapy continues to be studied intensively to better define its mechanism of action and role in PPHN treatment. In addition, there remains intense interest in possible new applications for newborns, as well as strategies that may enhance its efficacy. This review describes several areas of current research on amplification of NO signaling in the neonatal pulmonary vasculature, and reviews our current knowledge about the role of iNO in other conditions such as congenital diaphragmatic hernia and congenital heart disease. In addition, laboratory and clinical studies addressing a potential role for iNO as a therapeutic modality for the preterm infant are reviewed.

Keywords

Pulmonary hypertension; Newborn; Phosphodiesterase; Congenital heart disease; Congenital diaphragmatic hernia; Bronchopulmonary dysplasia

A. Introduction

The introduction of inhaled nitric oxide (NO) for the treatment of persistent pulmonary hypertension of the newborn (PPHN) has without doubt been one of the most significant advances in neonatal intensive care. More than a decade of intensive laboratory and clinical investigation culminated in the current role for NO as the only selective pulmonary vasodilator approved by the Food and Drug Administration (FDA) for the treatment of PPHN. Our understanding of the role of NO in the normal and abnormal perinatal vasculature did not develop sequentially; rather the potent vascular effects of exogenous NO were appreciated before the mechanism of action was fully understood, and even before the role of endogenous NO production was characterized in the normal and abnormal lung. Therefore this potent and successful therapy continues to be studied intensively to better define its role in PPHN and mechanism of action. In addition, there remains intense interest in possible new applications for newborns, as well as strategies that may enhance its efficacy.

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B. Treatment of Vascular Disease

1. Persistent Pulmonary Hypertension of the Newborn

A family of three NO synthases (NOS) are expressed in the lung, including the calcium dependent endothelial and neuronal isoforms, and the calcium-independent inducible isoform. All three are associated with specific developmental patterns of expression in the lung airway, parenchyma, and vasculature through gestation and early infancy (1). Pulmonary expression of both endothelial NOS (eNOS), and its downstream target, soluble guanylate cyclase (sGC), increase during late gestation (Figure 1). At birth, increased NO production and sGC activity lead to increased cGMP concentrations in vascular smooth muscle cells, which produce vasorelaxation through a decrease in intracellular calcium. Acute or chronic inhibition of NOS in fetal lambs produces pulmonary hypertension following delivery, indicating the fundamental importance of endogenous NO in the normal pulmonary vascular transition. Further study has shown that newborn lung disease is associated with endothelial cell injury and decreased NO-cGMP signaling in both experimental animal models and clinical studies of human infants. These findings laid the groundwork for the translational and clinical studies that demonstrated the pulmonary vascular effects of inhaled NO (iNO).

NO exists as a gas molecule, and therefore is a unique pharmaceutical agent that can be inhaled and thus delivered directly to the lung. Early studies showed that inhalation of NO decreases pulmonary vascular resistance in neonatal lambs with acute pulmonary hypertension induced by pharmacologic agents. Similar benefits were subsequently reported in lambs born with chronic pulmonary hypertension after antenatal ductus ligation, which is associated with pulmonary vascular remodeling similar to that observed in the human condition. These studies were quickly followed by well-designed, multicenter, placebo controlled trials that led to the approval of iNO for treatment of PPHN. While iNO decreased the need for Extra Corporeal Membrane Oxygenation (ECMO) support, thus avoiding the accompanying risks of systemic heparinization and carotid ligation, it is important to note that iNO has never been shown to reduce the mortality associated with PPHN. Some have speculated that new treatment modalities such as iNO may delay ECMO cannulation, allowing progression of ventilator-related lung damage, thus increasing the morbidity and mortality for infants that continue to require ECMO. Therefore, we recently examined data from the Extracorporeal Life Support Organization's registry between 1996 and 2003, a time period capturing the 4 years before and the 4 years after FDA approval of iNO (2). We found that use of iNO was not associated with any adverse outcomes during ECMO, including increased hours on bypass support, or increased time to extubation. Further, iNO use was associated with a decrease in cardiopulmonary arrests prior to cannulation. As ECMO is proven therapy for severe respiratory failure, it is reassuring to learn that iNO has not had a negative impact on the most severely affected infants.

An important and consistent finding in the large clinical trials is that 30% or more of infants do not improve oxygenation or maintain a response to iNO. This has led to continued intense investigation into the downstream mechanisms of NO signaling in the remodeled or diseased pulmonary vasculature. As the response to iNO is believed to be mediated primarily by activation of soluble guanylate cyclase and cGMP-dependent protein kinase, it is logical to pursue other mechanisms that might enhance cGMP accumulation. Inhibition of cGMP-metabolizing phosphodiesterase type 5 (PDE5) activity may increase cGMP concentrations and result in pulmonary vasodilation and/or increase the efficacy of iNO. For example, rebound pulmonary hypertension is a concern during use of iNO because of its short half-life, as well as potential NO-mediated alterations of downstream signaling mechanisms. PDE5 inhibition with sildenafil may attenuate rebound pulmonary hypertension after withdrawal of iNO in newborn and pediatric patients (3). In lambs with experimental pulmonary hypertension, both enteric and aerosolized sildenafil have been shown to dilate the pulmonary vasculature and

augment the pulmonary vascular response to iNO. Intravenous sildenafil was found to be a selective pulmonary vasodilator with efficacy equivalent to inhaled nitric oxide in a piglet model of meconium aspiration. When combined with iNO, sildenafil enhanced the reduction in pulmonary vascular resistance, although systemic hypotension and worsening oxygenation also resulted (4,5). Data have only recently begun to emerge on the use of sildenafil in combination with iNO in clinical populations. Preliminary findings from a pilot study of intravenous sildenafil in newborns with pulmonary hypertension indicate that it was generally well tolerated, with improvements in oxygenation noted in the cohorts that received higher infusion doses (6).

Similar to cGMP, cyclic adenosine monophosphate (cAMP) also stimulates vasodilatation. Emerging data indicate that prostacyclin (PGI₂) stimulates membrane bound adenylate cyclase, increases cAMP, and inhibits pulmonary artery smooth muscle cell proliferation *in vitro*. Although the use of systemic infusions of PGI₂ may be limited by systemic hypotension, inhaled PGI₂ has been shown to have vasodilator effects limited to the pulmonary circulation. The actions of inhaled PGI₂ and iNO appear to be additive in humans and even synergistic in some studies. Reports of inhaled PGI₂ use in neonates with PPHN are limited, but case reports indicate it may enhance oxygenation in infants that are poorly responsive to iNO (7). Rebound pulmonary hypertension following withdrawal of iNO has been mitigated by intravenous PGI₂ in children with pulmonary hypertension following congenital heart disease. Another potential approach taking advantage of cAMP signaling is inhibition of phosphodiesterase type 3 (PDE3), that metabolizes cAMP. Milrinone, a PDE3 inhibitor, has been shown to decrease pulmonary artery pressure and resistance, and to act additively with iNO in animal studies. A recent report indicates that the addition of intravenous milrinone to neonates with severe PPHN and poor iNO responsiveness was associated with improvements in oxygenation without compromising hemodynamic status (8).

Hyperoxic ventilation is standard therapy for PPHN, but may be toxic to the developing lung through the formation of reactive oxygen species (ROS), such as superoxide anions (9). Superoxide anions also inactivate NO and in the process form peroxynitrite, a potent oxidant with the potential to produce vasoconstriction and cytotoxicity. Since iNO is usually delivered with high concentrations of oxygen, there is the potential for enhanced production of these ROS (Figure 2). In addition, increased production of ROS has been reported in experimental models of PPHN. For instance, an increase in superoxide anions in the smooth muscle and adventitia of pulmonary arteries and a reduction in superoxide dismutase (SOD) activity have been demonstrated in the lamb ductal ligation model of PPHN (10). It has been suggested that ROS may have a role in vascular smooth muscle cell proliferation in PPHN, and may contribute to inadequate responses to iNO. Superoxide dismutase scavenges and converts superoxide radical to hydrogen peroxide, which is subsequently converted to water by the enzyme catalase. New studies indicate that scavengers of ROS may augment responsiveness to iNO. We studied the effects of recombinant human SOD (rhSOD) administered intratracheally, in a dose whose safety profile has been established in clinical trials (11). A single dose of rhSOD (administered either at birth or at 4 hours of age) improved oxygenation to a degree similar to that observed after 20 ppm of iNO. These physiologic improvements were associated with reduction in formation of isoprostanes and peroxynitrite. We speculate that scavenging superoxide may make both endogenous and inhaled NO more available to stimulate vasodilatation, and may also reduce oxidative stress and limit lung injury. It is hoped that human trials will begin soon.

2. Congenital Diaphragmatic Hernia

Congenital diaphragmatic hernia (CDH) occurs in approximately 1 in 3000 births, and represents approximately 8% of all major congenital anomalies. Infants with CDH have severe cardiopulmonary disease characterized by decreased lung volume and weight, fewer alveoli,

structural and functional abnormalities of the pulmonary vasculature, and abnormal cardiac function. Due to the complexity of their disease, mortality for infants with CDH remains very high (30–40%), and has not changed significantly over the past few decades despite medical advances that have improved outcomes for other neonatal respiratory diseases.

While initial case reports suggested that iNO improved oxygenation in some infants, large randomized, controlled trials demonstrated that early administration of iNO did not reduce death or ECMO utilization in CDH (12). eNOS mRNA and protein have been found to be decreased in the lungs of infants who died from CDH (13). However, the inadequate clinical responses to iNO suggest that the complex anatomic and functional pulmonary vascular abnormalities in CDH are further complicated by alterations in downstream vasoactive signaling pathways.

Animal models have been utilized to further characterize impaired NO signaling in CDH. The predominant model is the rat CDH model, created by fetal exposure to the herbicide nitrofen. Nitrofen interferes with branching morphogenesis, creating a diaphragmatic defect and uniformly lethal pulmonary hypoplasia in roughly half of the litter. Other investigators have surgically produced a diaphragmatic defect in the second trimester of fetal lambs, which results in a decrease in the total size of the pulmonary vascular bed, a decrease in the number of vessels per unit lung, and an increase in the muscularization of the pulmonary arterial tree compared to controls. Endothelial NOS expression and activity are diminished in the nitrofen rat model, but not in the lamb model of CDH, a finding that emphasizes the differences between the two experimental conditions. However, both demonstrate abnormalities in downstream signaling mechanisms, as evidenced by decreased pulmonary artery relaxations to the NO donor sodium nitroprusside or 8-bromo cGMP, a stable cGMP analogue (14,15). NO-cGMP signaling in CDH has been further explored through examining the effects of PDE5 inhibition. Concurrent treatment with sildenafil restores normal vasodilation in response to SNP and 8-bromo cGMP in rats with nitrofen induced CDH. Pulmonary arteries from lambs with CDH show normal dilation in response to zaprinast (a PDE5 inhibitor), which has been suggested to support a role for decreased activity of soluble guanylate cyclase in CDH.

Inhaled NO does not appear to reduce early mortality or ECMO utilization, although it still likely has a role in supporting the hypoplastic lung. For instance, data from the ELSO registry suggest that iNO decreases cardiopulmonary arrest before ECMO (2), which may improve later morbidity.

While NO signaling is commonly considered in the context of acute changes in pulmonary vascular tone, NO-cGMP signaling mechanisms may be even more important in supporting antenatal and postnatal vascular growth in CDH. Growth of lung explants taken from rat fetuses with CDH was enhanced by treatment with NO, but not vascular endothelial growth factor (VEGF) (16). In intact fetal rats with nitrofen-induced CDH, preliminary data suggest that chronic fetal treatment with sildenafil may lead to improved structural maturation and decreased medial wall thickness of pulmonary arterioles (17). This strategy may also restore vascular reactivity: in the same study, sildenafil also increased fetal lung cGMP and pulmonary artery responsiveness to NO donors. In infants with severe CDH, sustained elevations of PVR may persist despite repair of the diaphragmatic defect and improvement in parenchymal lung disease. In this setting, chronic treatment with iNO or sildenafil may provide sustained improvement in oxygenation by lowering pulmonary vascular resistance, and stimulating lung growth (18,19). A study designed to address the utility of chronic sildenafil in infants with severe CDH is currently underway.

3. Congenital Heart Disease

While an increase in pulmonary blood flow is necessary for adequate cardiopulmonary transition from fetus to newborn, infants with congenital heart lesions associated with increased pulmonary blood flow, such as truncus arteriosus or atrioventricular canal, will develop vascular remodeling and secondary pulmonary hypertension. The incidence of these types of congenital heart disease (CHD) is difficult to determine, but can reasonably be estimated as ~3 in 1000 births. Pulmonary hypertension following cardiopulmonary bypass is a serious clinical problem even in very young infants, and if the heart lesion is not corrected early, vascular changes of medial and intimal thickening occur, which ultimately lead to luminal obliteration.

The obvious differences in pulmonary hypertension induced by a chronic increase in pulmonary blood flow from that induced by antenatal remodeling has led to the development of specific animal models. Lambs with aorto-pulmonary graft shunts placed prior to birth have been very useful models for the study of the pulmonary vascular effects of excessive postnatal pulmonary blood flow. Shunted lambs have anatomic and physiologic features consistent with children with CHD, manifesting as initial increased pulmonary blood flow and heart failure, followed by secondary pulmonary hypertension due to pulmonary vascular disease. Pulmonary vascular signaling is altered as early as 1 week after birth and progresses over time.

At one week of age, NO signaling is similar to control lambs, and the manifestations of pulmonary vascular alterations are limited to increased pathways for vasoconstriction by endothelin-1(20). These alterations in endothelin signaling persist or expand through at least 8 weeks of age. Over the subsequent weeks, NO-cGMP signaling progressively changes. By 4 weeks of age, eNOS mRNA and protein are increased, but NO mediated dilation is diminished. This seems to be due to uncoupling of NOS so that superoxide rather than NO is produced (21), and it is interesting to note that SOD enhances pulmonary artery dilation in response to NOS stimulation (22). By 4 weeks, PDE5 expression is also increased, representing another mechanism that may inhibit NO-cGMP mediated vasodilation (23).

By 8 weeks, shunted lambs have progressive failure to thrive and pulmonary vascular remodeling. Their eNOS protein and basal NO activity is normal, but endothelium dependent vasodilation is decreased (24). Increased pulmonary vascular flow over time leads to sustained endothelial injury, progressive changes in NO and endothelin signaling, and shifts the vasoactive balance toward vasoconstrictive pathways. After a certain point, the changes become permanently imbalanced. These findings suggest that in the chronic setting of CHD, after pulmonary vascular disease is established, therapies aimed at improving the balance between vasodilation and vasoconstriction such as iNO, sildenafil, or endothelin antagonists may help improve pulmonary hemodynamics and alleviate symptoms.

Given the pulmonary vascular effects of iNO, it should probably be avoided in clinical conditions associated with a significant left to right shunting, as it may aggravate the hemodynamic mismatch. A setting in which this has not been seen to be a problem, however, is in premature infants treated with iNO to prevent BPD (see separate section below). The large randomized studies suggest that iNO given to premature infants does not precipitate or aggravate heart failure despite a high incidence of PDA before or during treatment. This suggests that chronic administration of iNO may not lead to significant enhancement of pulmonary blood flow, even when a potential left to right shunt is present. The same cannot be said for term neonates or older infants.

Acute changes in pulmonary vascular signaling can arise following surgical correction of CHD, especially if functional or structural vascular responses to excessive pulmonary blood flow have occurred. A recent meta-analysis examined the efficacy of iNO in the post-operative

management of infants and children with congenital heart disease (25). The objectives were to compare the effects of post-operative iNO versus placebo and/or conventional management on infants and children with CHD. The primary outcome was mortality, and the secondary outcomes were length of hospital stay, number of pulmonary hypertensive crises, as well as changes in hemodynamics and oxygenation. No differences were observed with the use of iNO versus control groups in the majority of outcomes reviewed, and the authors noted many concerns regarding methodological quality of the studies included in the meta-analysis. No data were available for analysis with respect to several important clinical outcomes including long-term mortality and neurodevelopmental outcome. This is clearly an area that needs further study in both the laboratory and clinical settings.

B. Prevention of Bronchopulmonary Dysplasia

In the United States, more than 10,000 babies develop bronchopulmonary dysplasia (BPD) each year, a disease characterized by persistent abnormalities of lung structure, including abnormal vascular growth and impaired alveolarization. A surge of interest in a potential role for iNO in respiratory failure of preterm infants has now evolved to studies designed to characterize a potential role for NO in supporting growth of the preterm lung, thus preventing BPD and its sequelae.

1. Laboratory Studies

Laboratory studies have demonstrated that iNO improves ventilation/perfusion matching, decreases lung inflammation and oxidant stress, and restores more normal patterns of angiogenesis and parenchymal growth in the immature lung. In animal models, there appear to be multiple mechanisms by which iNO might improve respiratory function in the preterm lung, thus creating a strong rationale for its potential role in premature infants with respiratory failure and at risk for BPD. Early studies confirmed that endogenous NO modulates pulmonary vascular tone and contributes to pulmonary vasodilation at birth in extremely preterm sheep. Further, treatment with low-dose iNO (5 ppm) improved gas exchange and reduces pulmonary vascular resistance during mechanical ventilation of premature lambs with severe respiratory distress syndrome (RDS).

Even more importantly, evidence has emerged indicating that impaired production of endogenous NO contributes to the pathogenesis of BPD. Multiple studies have demonstrated that lung eNOS expression is decreased in premature lamb and baboon models of BPD. Recent investigations have focused on the role of exogenous NO in reversing these changes. In the premature baboon model of chronic lung disease (CLD), 14 days of NO inhalation (5 ppm) improved lung compliance and lung volume, reversed lung growth inhibition, and improved lung morphology (26). Additional studies in this model and others have demonstrated that iNO protects against surfactant protein dysfunction associated with CLD, and preserves structure and function of airway smooth muscle.

The role of NO in abnormal alveolarization has also been explored in rat and mouse models. Mice deficient in eNOS have abnormal lung architecture at birth, supporting the critical role for NO in lung development. Exposure of neonatal rats to hyperoxia produces lung alterations similar to BPD; iNO given during the recovery period was found to improve growth of lung parenchyma and blood vessels in this model (27). In another neonatal rat model created by inhibition of vascular endothelial growth factor (VEGF), prolonged iNO treatment reduced vascular remodeling, improved lung vascular growth, and normalized lung development (28). These studies and others indicate that iNO may normalize the abnormal lung development associated with BPD.

2. Clinical Trials

Over the last several years, the use of iNO in the treatment of premature infants has been widely investigated. Early case reports of NO inhalation in premature newborns with severe respiratory failure demonstrated improvement in pulmonary hypertension, accompanied by marked improvement in oxygenation. These early reports also raised speculation about potential adverse effects, especially intracranial hemorrhage (ICH). These concerns were based, in part, on laboratory and clinical studies in adults suggesting that high doses of iNO (30–40 ppm) prolong bleeding times.

After these early case reports, several unmasked, randomized trials were subsequently published, and have been reviewed in detail elsewhere (29,30). More importantly, four large randomized, masked clinical trials focusing on iNO administration in preterm infants have published their short-term outcomes, and a fifth large-scale randomized trial in Europe is just now completing patient enrollment. It is important to note that all five of these studies focused on preterm infants requiring mechanical ventilation, and further study will be needed to address the potential role for iNO in preterm infants managed with CPAP, etc. However, the data thus far represent more than 2000 babies, and the individual trials report on a wide range of patient populations, disease severity, and potential treatment approaches (Table). Two studies have published long-term neurodevelopmental data, and preliminary data are available from a third.

Schreiber et al completed a single-center trial that provided for early randomization of infants to receive placebo gas or iNO at 10 ppm for 24 hours, followed by 5 ppm for up to 6 days (31). iNO reduced the composite outcome of death or chronic disease (CLD) by 24%, and post hoc analysis indicated that iNO predominately benefited infants with relatively mild respiratory failure (oxygenation index less than 6.94, the median for the study). These positive findings were reported at the same time as the multi-center study by Van Meurs et al (32), which also focused on an early enrollment strategy, but only included infants with much more severe respiratory failure (mean oxygenation index of 22–23). The mean duration of exposure for the iNO group was 76 hours, the shortest of all the clinical trials. Further, by protocol design, infants without improved oxygenation had the study gas discontinued almost immediately. In this population, iNO did not reduce death or BPD. Further, post hoc analysis of the 316 infants with birth weights of <1000 grams suggested the worrisome finding that iNO was associated with an increased risk of death and brain injury (Grade 3 or 4 IVH or PVL). Interpretation of these data has been somewhat difficult as this study did not require a screening head ultrasound, enrolled only infants with severe respiratory failure, and provided the shortest duration of iNO treatment. However, these findings suggest it is prudent to avoid iNO use in infants < 750 gm with severe hypoxemic respiratory failure due to parenchymal lung disease until more is known.

Kinsella et al also used an early enrollment strategy to determine the effects of low dose iNO in a large population of preterm infants (33). The population had a mean OI of 5.6, indicating a milder degree of respiratory failure, similar to the Schreiber trial. Median duration of treatment was 14 days for the iNO group. iNO did not significantly decrease the risk of death or BPD. However, as randomization was stratified by weight, this study also revealed that iNO reduced the risk of BPD by 50% in the 1000–1250 gram group (relative risk reduction 40%, $p = 0.004$). It is interesting to note that the Van Meurs report included a post hoc analysis of a similar group of 1000–1250 gram babies, and also observed a benefit (relative risk reduction 28%, $p = 0.03$).

Ballard et al studied the effects of iNO on in-hospital mortality and respiratory morbidity at a later time point, after the risk of BPD was established (34). In this trial, infants receiving mechanical ventilation or CPAP between 7 and 21 days of age were randomized to receive placebo gas or iNO at 20 ppm, followed by stepwise weaning, with a total length of treatment

of at least 24 days. Infants treated with iNO had improved pulmonary outcomes: a 23% increase in survival without CLD was observed at 36 weeks postmenstrual age, and improved outcome (survival without need for supplemental oxygen) was also observed at 40 and 44 weeks postmenstrual age. There were no differences in any complications of prematurity, including brain injury. Post hoc analysis showed a significant effect of age at study entry. Inhaled NO started between 7 and 14 days resulted in an 77% increase in survival without CLD, while there was no benefit if iNO was started after 15 days. Ballard's findings raise important questions about the timing and potential reversibility of lung injury, as well as whether higher doses or longer exposures to iNO are significant factors.

C. Is NO Neuroprotective?

Many clinicians were initially concerned that inhaled nitric oxide would increase the rate of intracranial hemorrhage in preterm infants, and debate continues on this critically important topic. Unfortunately, the results of the Van Meurs trial indicate that further study is needed to determine if there is a role for iNO in the management of the smallest infants with severe respiratory failure.

In contrast to the Van Meurs trial, the Schreiber and Kinsella showed a short-term neurologic benefit. Schreiber et al reported a 47% reduction in severe intraventricular hemorrhage (IVH) or periventricular leukomalacia (PVL) in the group that received iNO. Kinsella found that iNO reduced neurological morbidity (IVH, PVL, or ventriculomegaly) by 27%, an effect that was particularly striking in the 750–999 gram group. While follow-up data are still needed for the Kinsella study, 2-year developmental follow-up of the Schreiber cohort demonstrated that the early benefits were sustained (35). Infants treated with iNO had approximately half the risk of having an abnormal neurodevelopmental outcome as those in the placebo group, a reduction that persisted even after adjustment for birth weight, male gender, CLD, or severe IVH or PVL. In post hoc analysis, iNO was specifically protective against Mental Developmental Illness score less than 70.

These findings will likely lead to a better understanding of the pathogenesis of early brain injury in critically ill premature babies, and its interaction with NO signaling. Neuroprotection could be an indirect pulmonary benefit, perhaps through decreased neutrophil activation and cytokine release in the lung. However, extra-pulmonary effects of iNO are also highly plausible and have been demonstrated in other model systems.

D. Summary

Inhaled NO is now established as the only FDA-approved selective pulmonary vasodilator for newborn infants with hypoxemic respiratory failure and/or PPHN. In large part due to the ongoing laboratory and clinical experimental studies described in this review, our understanding of the role and mechanism of action of inhaled NO continues to expand. The major focus over the last several years has been on understanding pathways that may potentially amplify the pulmonary vascular response to NO, including inhibition of cGMP phosphodiesterases, amplification of cAMP-mediated signaling, and reduction of oxidant stress. It is not surprising that the use of iNO has extended to other neonatal conditions associated with pulmonary hypertension, including congenital diaphragmatic hernia and infants with pulmonary hypertension associated with congenital heart disease. In the latter group, while iNO is increasingly used in the post-operative management, the potential benefit is still not clear.

Inhaled NO may also play a role in the developing lung and pulmonary vasculature of the preterm infant. Five large multicenter randomized trials have now completed enrollment, and three trials have reported neurodevelopmental outcomes at 18–22 months. While early use of

iNO is not of clear benefit to all babies, specific protocols will likely emerge as reasonable strategies to reduce bronchopulmonary dysplasia. The potential neuroprotective effects are especially intriguing.

D. Future Directions

Future research will undoubtedly lead to new strategies that will enhance the vascular effects of both exogenous and endogenous NO. It is possible that amplification of endogenous nitric oxide synthase activity will become possible, or that newer NO-donor drugs, such as *S*-nitrosothiols, may emerge as superior vehicles to deliver NO to specific tissue beds. Many investigators are pursuing inhibition of cGMP phosphodiesterases using laboratory and clinical approaches. It is likely that future studies will determine a potential role for phosphodiesterase inhibitors to enhance the effects of NO, and these agents may even serve as selective pulmonary vasodilators without iNO.

- New data indicate that oxidant stress is an important mechanism in pulmonary hypertension, and may reduce vascular responses to NO.
- Future research will better define the enzymatic and cellular sources of oxidants such as superoxide anion, delineate the role of the endogenous antioxidant systems, and determine whether anti-oxidant therapies are beneficial.
- While the results of large-scale trials will provide initial answers about the role for iNO in protecting the preterm lung and brain, this is an area that will continue to be studied and debated intensively for the next several years.
- Additional studies are required to determine whether there are benefits that are specific to the timing of initiation, dose, or length of iNO therapy in the preterm lung, and whether noninvasive delivery of iNO via CPAP or nasal cannula will provide additional advantages.
- Extra-pulmonary effects of iNO, such as the potential neuroprotective effects seen in some populations of preterm infants represent a particularly exciting avenue for future research.

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Figure 1.

Nitric oxide (NO) and prostacyclin (PGI₂) signaling pathways in the regulation of pulmonary vascular tone. NO is synthesized by nitric oxide synthase (NOS) from the terminal nitrogen of L-arginine. NO stimulates soluble guanylate cyclase (sGC) to increase intracellular cGMP. PGI₂ is an arachidonic acid (AA) metabolite formed by cyclooxygenase (COX-1) and prostacyclin synthase (PGIS) in the vascular endothelium. PGI₂ stimulates adenylate cyclase in vascular smooth muscle cells, which increases intracellular cAMP. Both cGMP and cAMP indirectly decrease free cytosolic calcium, resulting in smooth muscle relaxation. Specific phosphodiesterases hydrolyze cGMP and cAMP, thus regulating the intensity and duration of their vascular effects. Inhibition of these phosphodiesterases with agents such as sildenafil and milrinone may enhance pulmonary vasodilation.

Figure 2.

Proposed role for reactive oxygen species and their metabolites in mediating increased pulmonary arterial contractility following exposure to 100% oxygen. Exposure to high oxygen concentrations results in formation of superoxide anions ($O_2^{\cdot-}$). Superoxide anions combine with nitric oxide (NO) to form peroxynitrite, a potent pulmonary vasoconstrictor. Superoxide dismutase (SOD) enzyme converts superoxide anions to hydrogen peroxide (H_2O_2), also a pulmonary vasoconstrictor. When membrane lipids (arachidonic acid and poly unsaturated fatty acids, PUFA) are exposed to reactive oxygen species such as superoxide anions and hydrogen peroxide or peroxynitrite, a variety of isoprostanes are formed. Isoprostanes are known constrictors of pulmonary arteries. *From Reference 8, with permission.*