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Therapeutic approaches using nitric oxide in infants and children

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Abstract

Pulmonary hypertension contributes significantly to the morbidity and mortality associated with many pediatric pulmonary and cardiac diseases. Nitric oxide, a gas molecule, is a unique pharmaceutical agent that can be inhaled and thus delivered directly to the lung. Inhaled nitric oxide was approved by the FDA in 1999 as a therapy for infants with persistent pulmonary hypertension. Since then, the use of inhaled nitric oxide has expanded to other neonatal and pediatric conditions, and our knowledge of its properties and mechanisms of action has increased tremendously. This review will discuss the physiology of nitric oxide signaling, the most common indications for its clinical use, and promising new investigations that may enhance endogenous production of nitric oxide and/or improve vascular response to it.

Introduction

The introduction of inhaled nitric oxide for the treatment of persistent pulmonary hypertension of the newborn represents a tremendous advance in neonatal intensive care. Since its initial approval in 1999, the use of inhaled NO has expanded to other conditions, and our knowledge of its properties and mechanisms of action has increased greatly. This potent and successful therapy continues to be studied intensively to better define its role in neonatal and pediatric lung and cardiac disease. In addition, there remains intense interest in possible new applications for newborns, as well as strategies that may enhance its efficacy.

Endogenous Nitric Oxide Physiology

Nitric oxide (NO) is synthesized from the terminal nitrogen of L-arginine by the enzyme nitric oxide synthase (NOS). All three NOS isoforms are expressed in the lung, and are distinguished by the regulation of their activities, as well as by specific sites and developmental patterns of expression (1). Endothelial NOS (NOS3 or eNOS) is expressed in vascular endothelial cells, and is believed to be the predominant source of NO production in the pulmonary circulation.

NOS is a complex oxidoreductase enzyme that functions physiologically as a dimer. A particularly important requirement for normal NOS function is sufficient availability of substrate (L-arginine), as well as co-factors such as heat shock protein 90, an intracellular molecular chaperone, and tetrahydrobiopterin, a bioactive form of folic acid (2). Depletion

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of these cofactors or inhibition of hsp90-eNOS interactions leads to “uncoupled” NOS activity, which promotes release of the oxygen radical superoxide instead of NO. In addition to substrate and cofactors, NOS expression and activity is regulated by multiple other factors, including oxygen tension, hemodynamic forces, hormonal stimuli (eg, estrogen), paracrine factors (eg, VEGF), and reactive oxygen species.

NO is a gas molecule that diffuses freely from the endothelium to the vascular smooth muscle cell. The biologic effects of NO in vascular smooth muscle are mediated primarily through activation of soluble guanylate cyclase, which promotes conversion of GTP to cGMP (Figure 1). cGMP is a critical second messenger that relaxes vascular smooth muscle through activation of cGMP-gated ion channels and activation of cGMP-dependent protein kinase (PKG). However, recent studies indicate that alternative NO signaling pathways likely also exist through reaction of NO with protein thiols to form S-nitrosothiols (SNO), which may induce vasodilation or protein modification (3).

Phosphodiesterases serve to catalyze the breakdown of cGMP, and are therefore able to regulate the magnitude and duration of vasodilation observed in response to NO. Each family of PDEs has a distinct pattern of specificity for cAMP and/or cGMP, and each PDE isoform has specific tissue and cellular distributions (4). The prevalent PDE within the lung is the cGMP-specific PDE5, although significant amounts of PDE1, PDE3, and PDE4 are also present (5, 6). As the primary enzyme responsible for regulating cGMP, PDE5 potentially represents the most important regulator of NO-mediated vascular relaxation in the normal pulmonary vascular transition after birth.

Developmental regulation of NO physiology

Pulmonary hypertension is a normal state for the fetus that is essential to fetal survival. Because the placenta, not the lung, serves as the organ of gas exchange, most of the right ventricular output crosses the ductus arteriosus to the aorta, and only 5-10% of the combined ventricular output is directed to the pulmonary vascular bed. Pulmonary vascular constriction plays a key role in maintaining high pulmonary vascular tone during fetal life. At the same time, the fetal lung and pulmonary vasculature must prepare for the dramatic adaptation to air breathing at the time of birth.

This complex orchestration of fetal and postnatal lung development is regulated in part by important maturational changes in expression and activity of nitric oxide synthase (NOS) isoforms. Lung eNOS mRNA and protein is present in the early fetus and increases with advancing gestation and during the early postnatal period in multiple species (7, 8). This increase in lung endothelial NOS content occurs at the same time the fetal pulmonary vasculature develops the capacity to respond to endothelium-dependent vasodilator stimuli, such as oxygen and acetylcholine (9). Recent data indicate that low fetal NOS activity may be maintained during fetal life in part through increased levels of asymmetric dimethyl arginine (ADMA), an endogenously produced arginine analogue that acts as a competitive NOS inhibitor (10, 11).

The neuronal and inducible NOS isoforms have been also been identified in the fetal lung of multiple species, and appear to be primarily localized to the airway epithelium. Similar to eNOS, their expression and activity appear to be regulated in specific developmentally-driven patterns. For instance, epithelial NO production increases during the third trimester, and occurs in parallel with rises in nNOS and eNOS expression in the proximal lung (12). NO is now recognized as important to epithelial development and function during lung development, and appears to regulate critical functions such as lung liquid clearance by the respiratory epithelium (13).

At the time of birth, dramatic cardiopulmonary events facilitate the transition to gas exchange by the lung. These include a rapid fall in pulmonary vascular resistance and pulmonary artery pressure, and a 10-fold rise in pulmonary blood flow. Birth-related stimuli, such as ventilation, increasing oxygen tension, and increasing shear stress rapidly increase NOS activity and NO production. Acute or chronic inhibition of NOS in fetal lambs produces pulmonary hypertension following delivery, indicating the fundamental importance of endogenous NO-cGMP signaling in the normal pulmonary vascular transition (14, 15).

Not surprisingly, the enzymes responsible for the downstream vascular responses to NO are also developmentally regulated. Soluble guanylate cyclase, which produces cGMP in response to NO activation, is expressed and active by late gestation (16). Interestingly, the developmental patterns of PDE5 expression are not as clearly defined as for NOS and sGC. For instance, in rats, PDE5 expression and activity steadily increase through the end of gestation, peak shortly after birth, and then drop dramatically into adulthood (17). In contrast, in neonatal lambs, PDE5 activity and expression appear to acutely decrease at birth, and then rise again at 4 to 7 days of life (18).

Role of NO in Pediatric Pulmonary Disease

Persistent Pulmonary Hypertension (PPHN)

Neonatal respiratory failure affects 2% of all live births, and is responsible for over one third of all neonatal mortality. Pulmonary hypertension complicates the course of more than 10% of all neonates with respiratory failure, and the most severe PPHN has been estimated to occur in 1 of every 500 term infants (19). Even with appropriate therapy, the mortality for moderate-severe PPHN remains just under 10%, and up to 25% of surviving infants are at risk for long-term neurodevelopmental disability (20, 21).

PPHN occurs in association with a diverse group of illnesses, but generally occurs in the following conditions: 1) the abnormally constricted pulmonary vasculature due to lung parenchymal diseases associated with alveolar hypoxia, such as meconium aspiration syndrome, respiratory distress syndrome, or pneumonia; 2) the lung with normal parenchyma and remodeled pulmonary vasculature, also known as idiopathic PPHN; or 3) the hypoplastic vasculature as seen in congenital diaphragmatic hernia. While “pure” idiopathic pulmonary hypertension occurs in the minority (~20-25%) of infants with PPHN, severe cases are almost certainly affected by both parenchymal and vascular disease. Severe, early pulmonary vascular disease is believed to be the result of antenatal events that lead to significant pulmonary vascular remodeling *in utero* (22).

Because of the inherent difficulties in studying the pulmonary vasculature in the fetal and neonatal period, much of our knowledge about altered NO-cGMP signaling in PPHN has been derived from animal models. Ligation or constriction of the ductus arteriosus in the fetal lamb results in rapid antenatal remodeling of the pulmonary vasculature, which has been extensively studied in the neonatal lamb. At birth, ductal ligation lambs exhibit the increased fetal pulmonary artery pressure, severe hypoxemia, and high mortality characteristic of severe human PPHN. Decreased expression of eNOS mRNA and protein, as well as diminished eNOS activity have been documented in pulmonary vascular tissue from this model (23, 24). In addition, expression and activity of vascular soluble guanylate cyclase is diminished (25), and activity of PDE5 is increased (26-29), both of which promote decreased cGMP concentrations (30). Others have used small and large animal models of chronic alveolar hypoxia after birth to induce pulmonary vascular dysfunction and remodeling. For instance, piglets exposed to 10 days of hypoxia exhibit impaired nitric oxide production due to eNOS dysfunction (31).

Clinical treatment of PPHN with inhaled nitric oxide (iNO) represents one of the most significant advances in neonatal intensive care. Inhaled NO is well suited for this task because it is a rapid and potent vasodilator that can be delivered as inhalation therapy to airspaces approximating the pulmonary vascular bed. NO that reaches the blood stream binds avidly to hemoglobin, which further localizes its effect to the lung vasculature. Inhaled NO is believed to also have a 'micro-selective effect', and may improve ventilation/perfusion matching by redirecting blood from poorly aerated, diseased lung regions to better-aerated distal air spaces. In contrast, intravenous dilators such as prostacyclin, tolazoline, and sodium nitroprusside often produce non-selective effects, leading to systemic hypotension, worsening ventilation/perfusion matching, and impaired oxygenation. There are relatively few contraindications to iNO, which mainly consist of congenital heart disease with left ventricular outflow tract obstruction, such as interrupted aortic arch, critical aortic stenosis, hypoplastic left heart syndrome, or severe left ventricular dysfunction. These children are likely to have pulmonary venous hypertension due to high left atrial pressures, and without correction of the underlying abnormality, use of iNO may aggravate pulmonary edema.

While large well-designed studies paved the way to FDA approval of iNO (summarized in Table 1), it is equally important to note that iNO did not reduce the mortality, length of hospitalization, or the risk of significant neurodevelopmental impairment associated with PPHN. These observations indicate that the clinical problem of PPHN and respiratory failure is much more complex than a simple deficiency in NO production. Further, one of these multicenter trials studied the potential benefit of beginning iNO at a milder or earlier point in the disease course (for an oxygenation index of 15-25). Early use of iNO did not decrease the incidence of ECMO and/or death, improve other patient outcomes, or reduce the incidence of severe neurodevelopmental impairment (32, 33).

Respiratory failure of prematurity

Considerable evidence from preclinical models indicates that impaired production of endogenous NO contributes to the pathogenesis of bronchopulmonary dysplasia (BPD), a common and serious complication of infants born with extreme prematurity. Multiple studies have demonstrated that lung eNOS expression is decreased in premature animal models of BPD (34, 35). Recent work also indicated that intervention with 14 days of NO inhalation in preterm baboons improved lung compliance and lung volume, as well as lung growth and architecture (36).

These findings have led to a substantial effort to investigate the clinical role of iNO in preterm infants. Early case reports of NO inhalation in premature newborns with severe respiratory failure demonstrated improvement in pulmonary hypertension, accompanied by marked improvement in oxygenation. However, these early reports also raised speculation about potential adverse effects such as intracranial hemorrhage, based in part on laboratory and clinical studies in adults suggesting that high doses of iNO (30-40 ppm) prolong bleeding times.

Five large randomized, masked clinical trials focusing on iNO administration in preterm infants have now presented their short-term outcomes. It is important to note that these studies focused primarily on preterm infants requiring mechanical ventilation, and further study will be needed to address the potential role for iNO in preterm infants managed with noninvasive modalities such as CPAP or nasal cannulas. However, the data thus far represent data from more than 2800 enrolled infants, and include a wide range of patient populations, disease severity, and treatment strategies. Three of the trials used an early, protective strategy, randomizing infants to receive placebo gas or iNO for 7-21 days (37-39). The smallest, single center trial showed that iNO significantly improved survival without

BPD (37), but this finding was not confirmed in the two larger, multicenter trials. All three trials will continue to report neurodevelopmental outcomes through at least age 5.

The trial conducted by Van Meurs et al (40) also focused on an early enrollment strategy, but only included infants with very 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. In this much sicker population of neonates, iNO did not reduce death or BPD. Further, post hoc analysis of the 316 infants with birth weights of <750 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 very 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.

Ballard et al (41) studied the effects of iNO in older preterm infants still requiring mechanical ventilation, thus targeting a population at particularly high risk for BPD. In this trial, iNO was begun later (7-21 days), given at a higher dose (20 ppm, followed by stepwise weaning), and delivered for a longer time period (24 days). iNO administration resulted in a 23% improvement in survival without BPD at 36 weeks corrected gestational age. Post hoc analysis indicated that age at study entry could be an important factor, in that inhaled NO started between 7 and 14 days resulted in an 77% increase in survival without BPD, while there was no benefit if iNO was started after 15 days. A follow-up study through one year of age showed that infants randomized to iNO were significantly less likely to require home oxygen, or use bronchodilators, steroids, or diuretics after NICU discharge (42). These 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 needed to support lung development.

Despite the large number of babies enrolled in these studies, clear recommendations for the use of iNO in specific populations of preterm infants have not yet emerged. Individual patient data meta-analysis will soon be available to help determine whether certain patient or treatment characteristics predict benefit from iNO in premature infants (43).

Pediatric primary and secondary pulmonary hypertension

Outside of the NICU setting, the most common clinical use of iNO is for the infant or child with congenital heart disease with associated pulmonary hypertension. Postoperatively, iNO is frequently used to prevent or treat pulmonary hypertensive crises, improve oxygenation, and increase cardiac output. Pulmonary hypertension commonly develops in infants with congenital heart lesions associated with pulmonary overcirculation, such as truncus arteriosus or atrioventricular canal. If the heart lesion is not corrected, medial and intimal thickening occur in the pulmonary vasculature over time, which eventually leads to luminal obliteration. Acute life-threatening exacerbation of pulmonary hypertension following cardiopulmonary bypass is a serious clinical problem even in very young infants. In addition, children undergoing cavopulmonary connections for single ventricle lesions require low pulmonary vascular resistance for surgical success. A number of small single-center studies indicate that iNO can attenuate pulmonary hypertension in at-risk postoperative patients, reduce the number of pulmonary hypertension crises, and shorten time on mechanical ventilation (44). In one larger randomized trial of pediatric patients, iNO (20 ppm) significantly decreased PVR and pulmonary hypertensive crises, and shortened the time to extubation readiness (45).

Animal models that closely mimic the conditions associated with congenital heart disease have been developed, which involve placing large aorto-pulmonary shunts in the late gestation fetal lamb (46). Shunted lambs are initially asymptomatic, and gradually develop clinical findings of pulmonary overcirculation and heart failure over a period of 6-8 weeks that are similar to those observed in human infants. The shunt lamb model allows for examination of vascular dysfunction over time, and allows for the testing of clinical strategies that prevent and reverse vascular remodeling. Substantial data indicates that abnormalities of NOS expression and Hsp90 interactions result in NOS dysfunction and decreased NO production in the pulmonary vasculature (47). More recently, mitochondrial dysfunction and increased oxidant stress have been linked to the development of pulmonary hypertension in this model (48), and these findings will likely lead to novel approaches such as L-carnitine supplementation to maintain normal eNOS-HSP90 interactions, NO signaling, and endothelial function.

Inhaled NO has also emerged as an important tool for testing acute pulmonary vascular reactivity during cardiac catheterization. Inhaled NO has several advantages over other pulmonary vasodilators in this setting, including rapid onset of action, and few potential adverse effects such as systemic hypotension. For instance, Balzer et al reported that preoperative evaluation with inhaled nitric oxide in combination with oxygen allowed for identification of a greater number of appropriate candidates for corrective cardiac surgery or transplantation (49). Others have shown that response to iNO during evaluation for pulmonary hypertension provides a safe, effective tool for predicting response to other agents such as calcium channel blockers and sildenafil.

While iNO would theoretically benefit children with chronic primary or secondary pulmonary hypertension, its application in this setting has been limited by the lack of practical home-based continuous delivery devices, and the risk for rebound pulmonary hypertension if it is inadvertently discontinued.

Pediatric respiratory failure

In initial clinical studies of adult and pediatric patients with severe acute respiratory distress syndrome (ARDS), inhaled NO produced selective pulmonary vasodilation and improved systemic oxygenation (50). However, subsequent randomized controlled trials have not shown a beneficial effect on mortality or duration of mechanical ventilation in pediatric or adult patients (51, 52). While a transient improvement in oxygenation was observed in most of these studies, it has been speculated that since most patients dying from acute respiratory distress syndrome suffer from multiple organ systems failure, lung-selective therapy such as inhaled NO may not improve the overall survival rate (53).

These are disappointing results, and likely indicate that the problem of pediatric respiratory failure involves much more than vascular dysfunction, and that there is still much to be learned about the role of NO in primary lung injury, as well as ventilator-induced lung injury (VILI) (54). In a large randomized controlled trial of lower- vs. higher-tidal volume ventilation, urinary nitrate levels were preserved to a higher degree in patients treated with a protective low tidal volume ventilator strategy when compared with patients treated with a higher tidal volumes (55). This suggests that preserved NO production may be a biomarker for better outcomes in acute lung injury, and that protective ventilator strategies could work in part through preserving NO production. However, the problem is clearly complex, and even relatively straightforward animal model studies show conflicting results. For instance, NOS3 deficient mice have been shown to be either protected from lung injury (56), or conversely more susceptible to VILI (57). The reasons for these conflicting findings are not clear, although some have suggested that the protective effects of NOS3 deficiency might be

due to reduced production of superoxide through uncoupled function of the enzyme, as described below (54, 57).

Future Directions

As our understanding of nitric oxide signaling evolves, strategies that will enhance responses to endogenous and exogenous NO are steadily emerging. Several of these promising therapeutic approaches have already reached the point of clinical testing.

Enhancement of endogenous NOS activity

Tetrahydrobiopterin (BH_4) is an important cofactor for NOS, and is essential for its dimerization and synthesis of NO. Without BH_4, NOS becomes 'uncoupled' and contributes to oxidant stress through synthesis of superoxide anion. The rate-determining step for BH_4 is its synthesis by GTP-cyclohydrolase (GTP-CH1). For instance, mice with impaired GTP-CH1 activity exhibit reduced lung BH_4 levels and spontaneous development of vascular remodeling and pulmonary hypertension. Conversely, congenital overexpression of GTP-CH1 in vascular endothelium protects mice from hypoxic PH (58). In a piglets with pulmonary hypertension induced by chronic hypoxia, combined therapy with BH_4 and a superoxide dismutase mimetic restores endothelial function (59). Additional recent evidence indicates that GTP-CH1 may be a redox sensitive enzyme, and that antioxidant treatment may restore NOS activity in part by restoring GTP-CH1 expression and BH_4 synthesis (60). Others have proposed that oxidative stress may reduce BH_4 activity by conversion to dihydrobiopterin (61). Sapropterin hydrochloride, a synthetic form of BH_4, was recently approved for treatment of patients with phenylketonuria. This observation could lead to clinical testing in children with pulmonary hypertension.

Sufficient availability of the substrate L-arginine is also important for eNOS activity, and recent data from human infants suggest that sufficient synthesis of L-arginine is necessary for optimal NOS function during the neonatal period (62). Exogenous L-arginine supplementation enhances NOS activity *in vitro*, but has been less successful when attempted *in vivo*. However, L-arginine can be endogenously synthesized from L-citrulline by a recycling pathway consisting of two enzymes, argininosuccinate synthase (AS) and argininosuccinate lyase (AL). Recent studies indicate that providing exogenous L-citrulline may reverse NOS dysfunction in animal models of neonatal pulmonary hypertension (63). L-citrulline has also been used in several patient populations with some success. For example, in children undergoing cardiopulmonary bypass at risk for development of postoperative pulmonary hypertension, oral supplementation with L-citrulline increased both plasma citrulline and arginine levels, and plasma citrulline levels greater than 37 μM/L appeared to protect against pulmonary hypertension (64). Intravenous L-citrulline has been shown to be safe and well tolerated in children undergoing cardiopulmonary bypass (65), and clinical trials are underway.

Modulation of oxidant stress

There is mounting evidence that oxidant stress plays an important role in the pathogenesis of PPHN, and reactive oxygen species (ROS) such as superoxide and hydrogen peroxide likely diminish the effects of NO through direct and indirect mechanisms. For instance, superoxide and hydrogen peroxide production is increased in the smooth muscle and adventitia of pulmonary arteries from lambs with chronic intrauterine pulmonary hypertension (66-68), as well as in young piglets exposed to chronic hypoxia (69). Possible sources for elevated concentrations of reactive oxygen species include increased expression and activity of NADPH oxidases, 'uncoupled' eNOS activity as described above, as well as reduced superoxide dismutase (SOD) activity (66, 68). Once present in the lung, elevated

concentrations of ROS can promote vascular smooth muscle cell proliferation in PPHN, and alter abnormal vascular reactivity through mechanisms that blunt cGMP accumulation (Figure 2).

Inhaled NO is usually delivered with high concentrations of oxygen. While hyperoxic ventilation continues to be a mainstay in the treatment of PPHN, we know surprisingly little about what oxygen concentrations will maximize benefits and minimize risk. Recent evidence suggests that increasing oxygen concentration beyond greater than 50-60% will not lead to greater pulmonary vasodilation in the normal or remodeled pulmonary circulation (70, 71). The extreme hyperoxia routinely used in PPHN management may in fact be toxic to the developing lung by the formation of ROS (60, 68, 72). Superoxide may react with arachadonic acid to increase concentrations of isoprostanes, and may also combine with NO to form peroxynitrite (73). Both are potent oxidants with the potential to produce vasoconstriction, cytotoxicity, and damage to surfactant proteins and lipids, and peroxynitrite can directly induce NOS uncoupling. New data indicate that even brief (30 minute) periods of exposure to 100% O2 are sufficient to increase reactivity of pulmonary vessels in normal lambs (70, 74), diminish the response of the pulmonary vasculature to endogenous and exogenous nitric oxide (70), and increase activity of cGMP-specific phosphodiesterases (72).

If reactive oxygen species promote vasoconstriction, scavengers of reactive oxygen species such as superoxide dismutase (SOD) and/or catalase may augment responsiveness to iNO and promote pulmonary vasodilation. In lambs with pulmonary hypertension, a single dose of recombinant human SOD (rhSOD) given by intratracheal delivery was found to dilate the pulmonary circulation and enhance responsiveness to inhaled NO (75), as well as improve oxygenation over a 24-hour period to a degree that was similar to iNO (73). Further, rhSOD treatment appeared to block formation of oxidants such as peroxynitrite and isoprostanes, and restore activity of endogenous nitric oxide synthase (60). Thus, use of antioxidants may have multiple beneficial effects: scavenging superoxide may increase the availability of both endogenous and inhaled NO, and may also reduce oxidative stress and limit lung injury (76).

Modulation of cGMP signaling

Alterations in downstream signaling mechanisms in pulmonary vascular smooth muscle cells may also lead to inadequate vascular responses to NO. Nitric oxide stimulates soluble guanylate cyclase to increase cGMP, which is the central and critical second messenger that regulates contractility of the smooth muscle cell by modulating the activity of cGMP-dependent kinases, phosphodiesterases, and ion channels. Therefore, multiple critical points in the pathway downstream from NO production serve as attractive targets for manipulating cellular cGMP concentrations. For example, expression and activity of soluble guanylate cyclase are decreased in the abnormally remodeled pulmonary vessels of lambs with chronic intrauterine hypertension, which could potentially diminish responses to both endogenous and exogenous NO (77). This finding would indicate that new compounds that directly stimulate sGC at a NO-independent but heme-dependent site may be helpful, a hypothesis that appears to be promising in pre-clinical testing (78).

Recent evidence indicates that the low cGMP concentrations associated with PPHN may also be associated with increased activity of cGMP-specific phosphodiesterases (PDE5). Inhibition of PDE5 activity would be expected to increase cGMP concentrations, dilate the pulmonary vasculature, and/or increase the efficacy of iNO. 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 (79, 80). Intravenous sildenafil was found to be a selective pulmonary vasodilator with efficacy equivalent to iNO in a piglet model of meconium aspiration. When combined with iNO,

sildenafil enhanced the reduction in pulmonary vascular resistance, but also worsened systemic hypotension and oxygenation (81, 82), raising concerns about its selectivity for the pulmonary circulation Data are now emerging on the use of sildenafil in clinical populations of critically ill infants and children. A recent report demonstrated that enteric sildenafil improved oxygenation and survival in human infants with PPHN compared to placebo (83). 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 (84). This study also noted that seven infants received sildenafil prior to initiation of iNO, and showed similar improvements in oxygenation (Figure 3). Of these, six survived to discharge without need for additional therapy with iNO or ECMO, which suggests that inhibition of PDE5 activity may independently decrease pulmonary vascular resistance and improve oxygenation in human infants with PPHN.

Summary

Inhaled NO is now well established as a highly effective pulmonary vasodilator for newborn infants with PPHN or hypoxemic respiratory failure. Because of this tremendous success, the use of iNO has rapidly expanded into other diseases that affect infants and children. Similarly, our understanding of the role and mechanism of action of NO in pediatric and cardiac disease continues to steadily increase. Current and future research in areas such as enhancement of eNOS activity, inhibition of cGMP phosphodiesterases and reduction of oxidant stress will undoubtedly produce new strategies that will amplify the vascular effects of both exogenous and endogenous NO.

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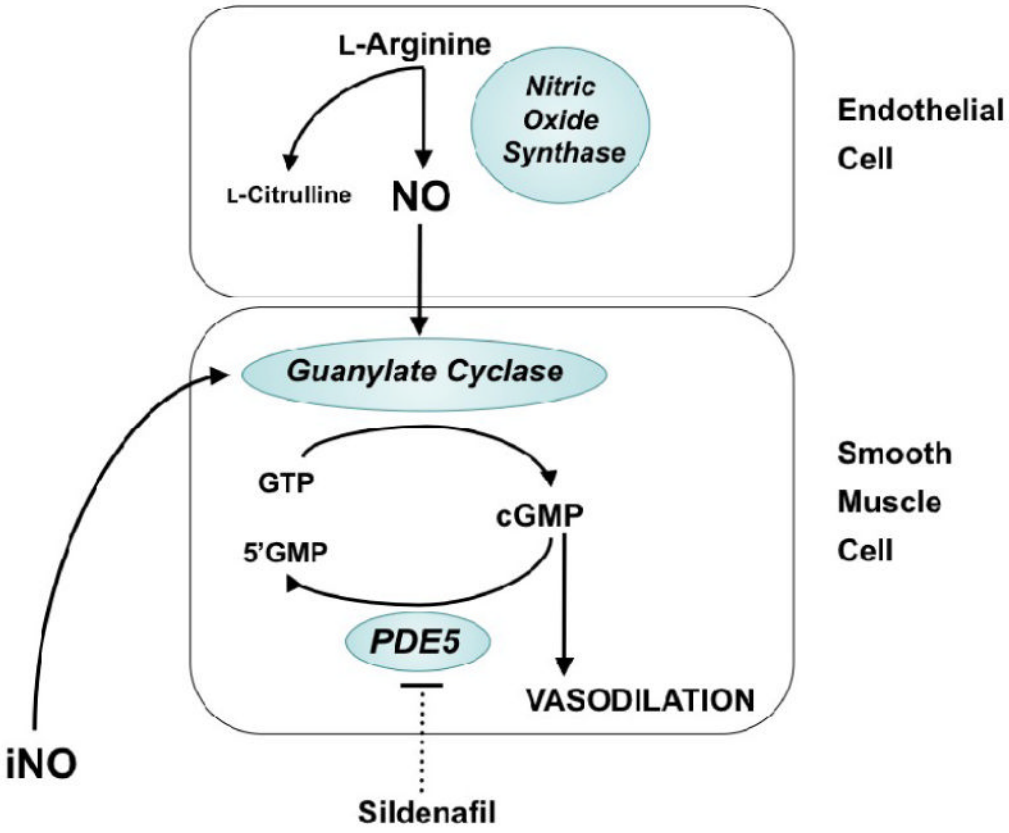

Figure 1.

Nitric oxide (NO) 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 from endogenous sources, or delivered as an inhaled gas, stimulates soluble guanylate cyclase (sGC) to increase intracellular cGMP, which indirectly decreases free cytosolic calcium, resulting in smooth muscle relaxation. Specific phosphodiesterases such as PDE5 hydrolyze cGMP, thus regulating the intensity and duration of its vascular effects. Inhibition of cGMP phosphodiesterases with agents such as sildenafil may enhance pulmonary vasodilation.

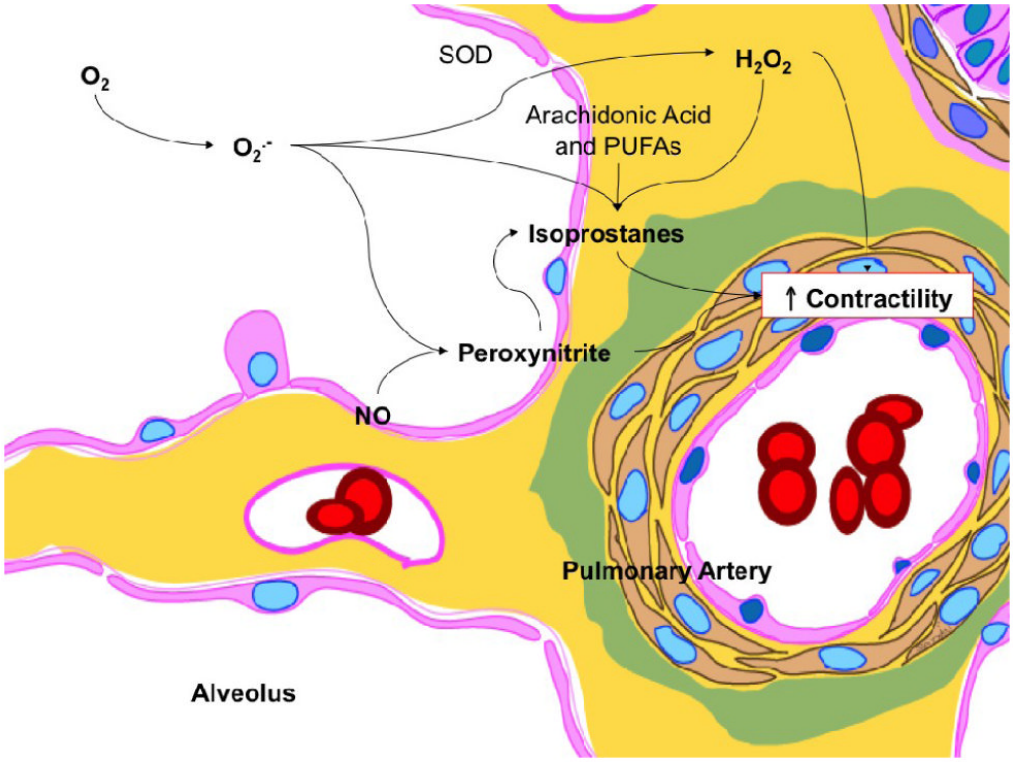

Figure 2.

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 ($O2^{\cdot-}$). 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 (90), with permission.

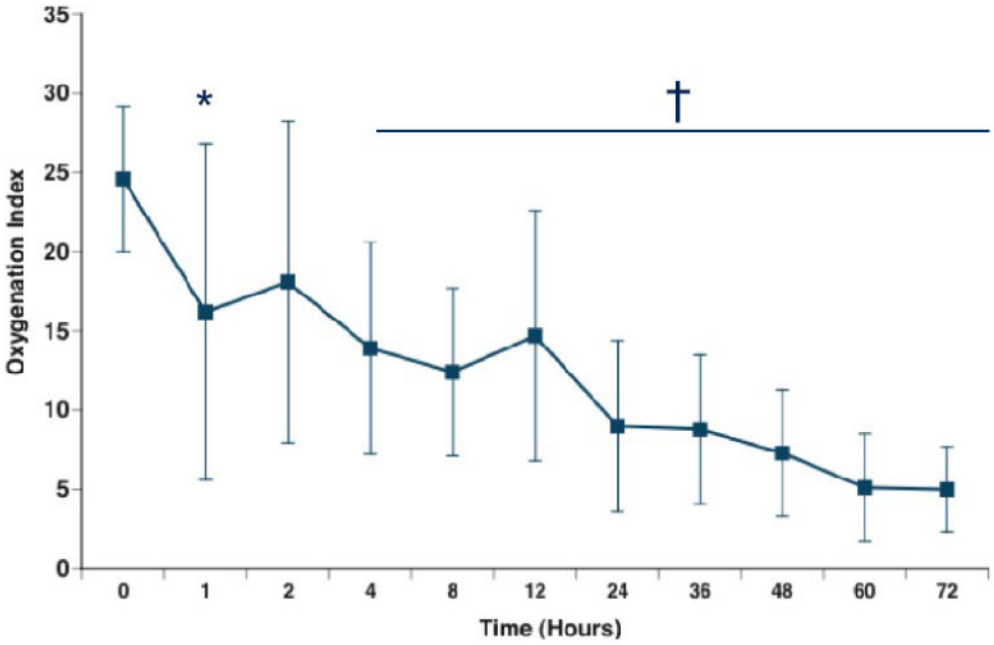

Figure 3.
Response to sildenafil infusion without iNO. Seven infants were enrolled before the need for iNO. OI was improved by 1 hour (24.6±4.6 to 16.1±9.9; *P=0.0502), with significant and sustained improvement by 4 hours after the initiation of sildenafil (14.7±6.4, p=0.0088). From (91), with permission.

Table 1

Summary of the large, multicenter randomized trials of inhaled NO in term newborns with hypoxemic respiratory failure and/or PPHN, showing the effect of iNO on ECMO utilization and mortality. Table adapted from (85), * denotes significant reduction ($p < 0.05$).

			% ECMO		% Mortality	
	n	**Initial OI**	**Control**	**iNO**	**Control**	**iNO**
NINOS (86)	235	44	55	39*	17	14
Roberts (87)	58	44.4	71	40*	7	7
Clark (88)	248	39	65	48*	8	7
Davidson (89)	155	24.7	34	22	2	8
Konduri (32)	299	19.2	12	10	9	7