New approaches for persistent pulmonary hypertension of newborn
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The management of persistent pulmonary hypertension of newborn (PPHN) has seen remarkable advances in the recent years that have led to significant decreases in mortality and morbidity from this condition [1–4]. PPHN occurs when the pulmonary vascular resistance (PVR) fails to decrease at birth. The elevated pulmonary artery pressure decreases pulmonary blood flow and leads to right-to-left shunting across the patent ductus arteriosus or the patent foramen ovale. Because of the presence of these fetal shunts, this condition was also referred to in the past as persistent fetal circulation. However, right-to-left shunting can occur without PPHN, as in congenital heart diseases with left heart hypoplasia or obstruction. PPHN is therefore a more appropriate term for noncardiac causes of cyanosis with elevated PVR in term and near-term infants. The incidence of PPHN is estimated to be around 0.2% of term infants [2], with higher incidence in pregnancies with no prenatal care and with the use of tobacco and illicit drugs [2].

Conditions associated with persistent pulmonary hypertension of newborn

PPHN can occur without associated parenchymal lung disease (idiopathic) or with a variety of lung diseases. PPHN occurs most commonly in association with meconium aspiration syndrome (50%), followed by idiopathic PPHN (20%), pneumonia/sepsis (20%), respiratory distress syndrome (5%), and other causes (asphyxia, maternal diabetes, polycythemia, etc) [2,4]. Alveolar-capillary dysplasia, a developmental defect in the proper alignment of alveoli and pulmonary vessels to form the air-blood barrier, is being increasingly recognized in lethal cases of PPHN [5]. Congenital diaphragmatic hernia (CDH) is also often associated with PPHN [6]. However, infants with CDH have severe underlying
pulmonary hypoplasia, and the course and management are different from other causes of PPHN [6].

Transition of pulmonary circulation at birth

A proper understanding of the balance between pulmonary and systemic vascular pressures is essential for effective management of infants with PPHN. In the normal fetus, pulmonary and systemic pressures are nearly equal (Fig. 1). The placenta is a low-resistance/low-pressure vascular bed. The pulmonary vessels are constricted and allow only 5% to 10% of cardiac output to go through them in utero [7]. This allows the bulk of cardiac output to bypass the lungs, which are not involved in gas exchange in utero. The low arterial oxygen tension in the fetus facilitates this vasoconstriction. During the first few minutes of postnatal life, the pulmonary artery pressure decreases rapidly to 50% of systemic pressure (Fig. 1), and the pulmonary blood flow increases nearly tenfold to match lung perfusion with the onset of ventilation [8]. Failure of this pulmonary vasodilation (Fig. 1) results in an inability to establish oxygenation during postnatal life [1]. The management of these infants is mainly supportive to maintain oxygenation for a period of time to allow the postnatal pulmonary vascular adaptation to occur.

Vascular biology of pulmonary vasodilation at birth

The regulation of pulmonary vascular tone is a complex process and represents a balance between the constrictor and dilator mechanisms. The key mediators involved in the vasodilation that occurs at birth are prostaglandins and nitric oxide (NO) released by pulmonary vascular endothelium [9,10]. The influence of these dilator stimuli is opposed by several vasoconstrictors, such as endothelin, thromboxane, and products of the cytochrome P450 pathway [11]. The mechanism of pulmonary vasodilation that occurs at birth has been in-
vestigated extensively over the last 50 years. Initial work focused on the effects of physical and chemical stimuli, such as lung distension, establishment of the air/liquid interface, and exposure to oxygen on pulmonary blood flow and resistance [7,8]. Studies in fetal lambs have shown that distension of lung alone causes a fourfold decrease in PVR [9]. Presence of oxygen in the lung is necessary for the nearly 15-fold decrease in PVR that occurs normally at birth [7,9]. The discovery of NO as the endogenous endothelium-derived vasodilator [12] and delineation of the signaling mechanisms involved in the NO-mediated vasodilation [13] led to rapid advances in the understanding of birth-related pulmonary vasodilation. These studies also resulted in major improvements in the treatment of PPHN, such as application of inhaled nitric oxide (INO) therapy and more recent efforts to use prostacyclin or phosphodiesterase inhibitors. The key enzymes involved in the transition of pulmonary circulation at birth, cyclooxygenase and NO synthase (NOS), undergo developmental maturation during late gestation and at birth [14,15]. The activity of these enzymes increases dramatically in response to birth related stimuli, with ventilation and oxygenation having specific effects on the activity of these enzymes [16,17]. In addition, oxygen stimulates the activity of these enzymes by increasing the synthesis and release of ATP from oxygenated red blood cells in the pulmonary circulation during the transition [18,19]. ATP and its metabolites are potent pulmonary vasodilators and increase the activity of NOS and cyclooxygenase [20,21]. NO is released as a byproduct of the conversion of L-arginine to L-citrulline by endothelial NO synthase (eNOS) (Fig. 2). The biologic effects of NO are mediated by soluble guanylate cyclase in the vascular smooth muscle, which promotes conversion of GTP to cGMP. The enzyme phosphodiesterase catalyzes the breakdown of cGMP to limit the duration of vasodilation observed with NO (Fig. 2). Guanylate cyclase and phosphodiesterase undergo developmental maturation, with marked increases in activity at term gestation and in the early postnatal life [22,23]. Prostacyclin (PGI₂), synthesized in endothelial cells from arachidonic acid and endoperoxides,
stimulates adenylate cyclase-mediated conversion of ATP to cAMP (Fig. 3). A cAMP-specific phosphodiesterase catalyzes the breakdown of cAMP and limits the duration of the vasodilator response to prostacyclin. Increases in cGMP and cAMP levels in vascular smooth muscle are associated with relaxation and vasodilation (Figs. 2 and 3). Therefore, current therapeutic approaches to pulmonary vasodilation are focused on increasing the intracellular levels of these cyclic nucleotides. This is accomplished by enhancing the availability of NO or prostacyclin, inhibition of phosphodiesterase activity to preserve the biologic activity of cGMP or cAMP, and by preventing the quenching of NO or cGMP by reactive oxygen species such as superoxide (O$_2^\bullet$-).

Catalytic functions of nitric oxide synthase

NOS is a complex oxidoreductase enzyme that functions physiologically as a dimer [24]. The electron transfer to O$_2$ occurs at the arginine oxygenase domain, which receives electrons from the NADPH-reductase domain [24]. In the presence of l-arginine in a stable configuration, electron transfer is coupled to oxidation of the terminal guanidino nitrogen of arginine to release NO and citrulline (Fig. 4). Interaction of eNOS with a number of cofactors facilitates this coupled catalytic activity, with the release of NO as a final product [25]. Among the cofactors, association of heat shock protein 90, an intracellular molecular chaperone, with eNOS (Fig. 4) favors NO release [25]. In addition, tetrahydrobiopterin, a bioactive form of folic acid, and l-arginine facilitate the coupled activity of eNOS [26]. In vitro studies have shown that depletion of these cofactors or inhibition of hsp90-eNOS interactions lead to uncoupled NOS activity [25–27]. Stimulation of NOS under these conditions leads to release of O$_2^\bullet$- instead of NO from NOS activity (Fig. 4). The biologic effects of O$_2^\bullet$- on vascular tone are opposite to those of NO, resulting in vasoconstriction and hypertrophy of vascular smooth muscle. The effects of O$_2^\bullet$- on vascular tone are partly related to the quenching of available NO and in part to the formation of
peroxynitrite (ONOO⁻) from the reaction of NO with O₂⁻. ONOO⁻ can nitrate target proteins, such as guanylate cyclase [28] or K⁺ channels [29], preventing their activation by NO or other physiologic signals. Evidence for increased synthesis of reactive oxygen species (ROS) and ONOO⁻-mediated nitration of proteins has been demonstrated in a number of vascular diseases, including diabetes and hypertension [30].

**Pathophysiology of persistent pulmonary hypertension of newborn**

Hypoxia, acidosis, and alveolar atelectasis promote pulmonary vasoconstriction and maintain pulmonary hypertension. The presence of meconium or blood in the airway or lung from perinatal aspiration can interfere with the onset of ventilation and cause pulmonary vasoconstriction. PPHN is more common in full-term and near-term (>34 weeks gestation) neonates compared with premature babies (<34 weeks gestation). The development of smooth muscle around the small pulmonary arterioles in late gestation (>28 weeks) may predispose near-term infants to increased resistance to the pulmonary flow [31]. A well-recognized cause of PPHN is constriction of the fetal ductus arteriosus in utero from exposure to non-steroidal anti-inflammatory drugs (NSAIDs) during the third trimester [32,33]. Constriction of the ductus arteriosus leads to a sustained increase in fetal pulmonary artery pressure [34,20], pulmonary vascular remodeling, and failure of vasodilation in response to birth-related stimuli [34,35]. Recent studies using sensitive analytical techniques to detect drug exposure...
in utero demonstrated that NSAID exposure is a more frequent association with PPHN than previously recognized [36]. In this study, the presence of ibuprofen and naproxen in the meconium of the infant (implying in utero exposure) and the concentration of these drugs in the meconium correlated with incidence and severity of PPHN [36]. However, the nature of biologic susceptibility that predisposes some but not all infants that had prenatal NSAID exposure to PPHN remains unknown. Prenatal constriction of the duc tus arteriosus in fetal lambs reproduces the hemodynamic and structural features of PPHN, such as smooth muscle hypertrophy and the extension of smooth muscle to the small arterioles around the alveoli [37]. A decrease in expression [38] and activity [39] of NOS enzyme have been documented in babies with PPHN and in the fetal lamb model of PPHN induced by prenatal ductal constriction [40–42].

**Disruption of NO-cGMP signaling and prostacyclin signaling in persistent pulmonary hypertension of newborn**

There is increasing evidence for derangement of the NO-cGMP pathway in PPHN. In lambs with PPHN induced by prenatal ductal constriction, eNOS activity and expression are significantly reduced [40–42]. In addition, the sensitivity of guanylate cyclase on vascular smooth muscle to NO seems to be decreased [43]. Recent studies have demonstrated an uncoupling of eNOS in the pulmonary arteries in this model due to a decrease in its association with heat shock protein (hsp) 90 [20]. Decreased eNOS-hsp90 interactions lead to decreased synthesis of NO and increased release of O$_2^\cdot$ from NOS in PPHN [20]. In addition to eNOS, NADPH oxidases in vascular smooth muscle may also serve as a source of O$_2^\cdot$ in pulmonary hypertension [44]. The addition of superoxide dismutase (SOD) improves the NO-dependent vasodilation in the ductal ligation model of PPHN [20,44]. The improved vasodilation observed with quenching of superoxide may be due to improved bioavailability of endogenous NO or due to a decrease in ONOO$^-$ formation and its effects on downstream targets on the vascular smooth muscle.

Few studies have addressed the alterations in prostacyclin metabolism in PPHN. Although administration of NSAID to pregnant animals increases the incidence of PPHN phenotype in the offspring [45], it is unclear whether these effects are due to inhibition of prostaglandin synthesis in the pulmonary circulation or due to ductal constriction and its secondary effects on the NO signaling in pulmonary arteries.

**Therapeutic approaches to persistent pulmonary hypertension of newborn**

*Inhaled nitric oxide*

Few recent advances in neonatology rival the impact that INO made on the management of PPHN. INO was initially tested in animal models and adults with...
pulmonary hypertension [46,47]. INO administered in doses <100 parts per million (ppm) causes selective pulmonary vasodilation [46]. NO diffuses across alveolar space (Fig. 5) to pulmonary artery smooth muscle where it initiates vasodilation by increasing cGMP levels. As it continues to diffuse to the lumen of the pulmonary artery, it is inactivated by hemoglobin in red cells (Fig. 5). There are several reasons why inhaled NO is an ideal pulmonary vasodilator: (1) Its effect is confined to the pulmonary vascular bed due to its rapid inactivation by hemoglobin in the pulmonary circulation, (2) its vasodilator effect is not altered by extra-pulmonary shunts, (3) it has the ability to improve ventilation-perfusion matching because the pulmonary vasodilation occurs in the ventilated segments of the lung, and (4) NO causes vasodilation even in the presence of endothelial cell injury or dysfunction frequently noted in PPHN. Pilot studies have validated these advantages of inhaled NO therapy in term and near-term newborn infants with respiratory failure and PPHN [48,49]. Several randomized clinical trials have shown a clinically significant improvement in oxygenation and decrease in use of extra-corporeal oxygenation (ECMO)/mortality with the use of INO therapy in infants with hypoxic respiratory failure [3,4,50–52]. Based on these trials, INO therapy was approved by the FDA for use in newborn infants >34 weeks of gestational age with hypoxic respiratory failure [53]. However, the response of infants with respiratory failure to INO therapy is not uniform, with approximately 70% of treated infants showing a >20 mm Hg increase in PaO2. Several adjunctive therapies used in infants with respiratory failure have an impact on the response to INO [54,55], and a number of questions remain regarding the use of INO therapy.

Fig. 5. Mechanism of selective pulmonary vasodilation caused by inhaled NO. As NO diffuses from the alveolus to the adjacent pulmonary artery, relaxation of vascular smooth muscle occurs. NO that diffuses into the lumen of the artery is bound to hemoglobin and inactivated in the red cell to nitrite and nitrate (NO₂ + NO₃).
What is the optimum dose of inhaled nitric oxide to be used in hypoxic respiratory failure?

The current recommended starting dose of INO therapy in term infants with respiratory failure is 20 ppm, based on randomized trials that demonstrated the efficacy of this dose in improving oxygenation [53]. Doses higher than 20 ppm are not more effective in improving oxygenation and are associated with a higher incidence of methemoglobinemia and exposure to nitrogen dioxide [4,56]. Other studies have shown that doses as low as 5 ppm were effective in improving oxygenation in these infants [55,57]. A randomized, placebo-controlled trial of INO therapy given early in the course of respiratory failure in term and near-term infants demonstrated that starting INO at a dose of 5 ppm improves oxygenation by >20 mm Hg in 58% of infants [55]. When infants with <20 mm Hg increase were tried on the 20 ppm dose, 36% had a >20 mm Hg increase in PaO2, for an overall response rate of 73%. Initiation of INO at lower than the current recommended dose has the advantage of weaning the dose faster and exposing the developing lung in the newborn to lower concentrations of nitrogen oxides that cause oxidant stress. Cornfield et al [58] reported that an initial dose of 2 ppm of INO was ineffective in improving the oxygenation on a sustained basis. These data indicate that the optimum initial dose to improve oxygenation may be close to 5 ppm. Studies in premature newborn infants have generally initiated INO<20 ppm with significant improvement in oxygenation [59,60] in a majority of the infants. The lower doses are probably important for this population of infants because the safety of INO therapy in the premature lung remains unknown.

How early is inhaled nitric oxide therapy indicated in hypoxic respiratory failure?

Initial randomized trials of INO have focused on the use of INO therapy in neonates with severe respiratory failure (Table 1) and in infants that are eligible for ECMO therapy [3,4,50,51]. These trials have shown that when infants present

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Abbreviations: INO, inhaled nitric acid; OI, oxygenation index; ECMO, extra-corporeal oxygenation.

* Oxygen index calculated as mean airway pressure × FiO2 ÷ PaO2. These trials were designed to test INO effects on ECMO use/mortality as primary outcome.

* Babies in the control group received INO when their OI reached ≥25 as standard therapy, and babies randomized to early INO received this therapy when their OI reached 15 to 25 in this trial.
with an oxygenation index (OI = mean airway pressure × FiO₂/PaO₂) > 25, INO therapy decreased the use of ECMO, although mortality was not significantly altered. The incidence of ECMO/mortality with INO therapy in these trials was still close to 40%. There are potential benefits to starting INO early in the course of respiratory failure at an oxygenation index < 25. Severe respiratory failure in neonates is often associated with alveolar atelectasis from progression of underlying disease or from iatrogenic lung damage caused by aggressive ventilation [61]. Strategies that improve alveolar ventilation in neonates with respiratory failure enhance the response to INO [54]. Introduction of INO before alveolar atelectasis supervenes may improve the response to INO. A randomized clinical trial of INO given early in respiratory failure, at an oxygenation index of 15 to 25, demonstrated that early INO improves oxygenation and decreases the progression to severe respiratory failure (OI > 40) compared with standard INO therapy initiated at an OI of 25 [55]. The incidence of ECMO use, which was similar in both groups (11%), was lower than the observed outcome in previous clinical trials with INO (Table 1). Infants were enrolled in the trial at a mean OI of 19.2, and standard INO therapy was initiated at an OI of 25 for infants whose respiratory failure has progressed. In addition, for the 176 infants enrolled at an OI of 15 to 20, a trend for lower incidence of ECMO use/mortality (10% with early INO versus 18% with control group, P = 0.12) was noted [55]. These data indicate that the earlier use of INO in respiratory failure may be associated with further reductions in short-term morbidity compared with previous randomized trials (Table 1). Infants whose respiratory failure is rapidly deteriorating may benefit from INO therapy given at an OI of 15 to 20.

Use of adjunctive therapies with inhaled nitric oxide in hypoxic respiratory failure

The importance of achieving optimum lung expansion in neonates with respiratory failure has been demonstrated [54,62]. Alveolar recruitment probably enhances the distribution of INO to the pulmonary circulation. The strategies that were used to improve lung expansion were surfactant therapy and high-frequency oscillation (HFO). Surfactant therapy was shown to decrease the ECMO use/mortality in term infants with hypoxic respiratory failure, particularly when given at an OI < 22 [62]. Although the use of surfactant was not tested in conjunction with INO, there is a physiologic basis for using these therapies in combination in the presence of parenchymal lung disease. Meconium is known to inactivate surfactant [63], and a subgroup analysis of the randomized trial of surfactant in respiratory failure demonstrated an improvement in the outcome for babies with meconium aspiration syndrome [62]. The combination of HFO and INO was shown to be better than conventional ventilation plus INO in infants that had significant parenchymal lung disease in a randomized trial of 205 infants with PPHN [54]. The overall use of surfactant therapy and HFO in the randomized trial of early INO in hypoxic respiratory failure was nearly 80% and may have contributed to the low incidence of ECMO and death in this trial [55]. These
data suggest that a lung recruitment strategy is important during application of INO therapy in PPHN.

Rebound pulmonary hypertension after the discontinuation of inhaled nitric oxide therapy

The occurrence of significant oxygen desaturation and elevation of pulmonary artery pressure after discontinuation of INO therapy has been noted in the initial studies of INO therapy. Studies in newborn lambs demonstrated a significant decrease in NOS activity in endothelial cells after treatment with INO [64]. The gradual weaning of INO to doses ≤ 1 ppm before discontinuation ameliorates the sudden drop in oxygenation [65]. Clinically significant desaturations rarely occur with this strategy in neonates with PPHN [65]. Oral sildenafil (0.3 mg/kg), a phosphodiesterase inhibitor, was successfully used to allow withdrawal of INO therapy in a 7-week-old infant with severe PPHN secondary to CDH [66] and in infants with pulmonary hypertension after cardiac surgery [67]. However, the use of inhaled prostacyclin or phosphodiesterase inhibitors to attenuate the rebound vasoconstriction during INO withdrawal has not been tested in prospective randomized trials in PPHN. The use of alternate strategies, such as folic acid or l-arginine, to preserve or stimulate the endogenous eNOS activity during INO withdrawal has not been studied.

Use of inhaled nitric oxide therapy in congenital diaphragmatic hernia

INO therapy does not consistently improve oxygenation and does not decrease the use of ECMO/mortality in infants with CDH [68]. There is no evidence that the use of adjunctive therapies such as HFO or surfactant improve the response to INO in this population of infants. The appropriate indication for INO therapy in babies with CDH is to stabilize the infant as a bridge to ECMO cannulation.

Use of inhaled nitric oxide therapy in premature infants with respiratory distress syndrome

INO therapy improves oxygenation in premature newborns with severe respiratory distress syndrome (RDS) [69,70]. However, the efficacy of INO in reducing the incidence of chronic lung disease/mortality and its long-term safety in premature infants remains unclear. The reasons for using INO in premature infants with RDS are (1) many infants with severe RDS have echocardiographic evidence of pulmonary hypertension with decreased pulmonary blood flow [59,69]; (2) INO can improve ventilation/perfusion mismatch, which is the primary cause of hypoxemia in premature RDS; and (3) recent studies have shown that NO is a signaling molecule involved in parenchymal lung growth [71] and that treatment with INO decreases lung injury and chronic lung disease in the premature baboon model of RDS [72]. INO also decreases the inflammatory cell influx and cytokine production in premature lambs on ventilator support for RDS [73]. There are several concerns about the use of INO therapy in premature newborns: (1) premature infants have a higher risk of intraventricular hemorrhage than term infants, and the potential inhibition of platelet aggregation by INO may
have an adverse impact on this morbidity; (2) the premature lung has lower antioxidant defenses and may be vulnerable to oxidant stress from NO or other nitrogen oxides; and (3) there is a potential for increased left-right shunting across the patent ductus arteriosus with the rapid pulmonary vasodilation in response to INO. A recent randomized trial conducted in a single center has shown that INO improves the probability of survival without chronic lung disease in premature infants with RDS [60]. There was a decrease in the incidence of severe IVH with the use of INO in this trial [60], although some preliminary studies have reported a higher incidence of IVH with INO [74]. Although the results of the study are promising, they need to be confirmed in a larger trial before INO therapy can be recommended for premature infants with RDS. There are three randomized multi-center trials of INO therapy in progress for premature newborn infants with RDS and BPD. These trials may provide more definitive answers on the safety and impact of INO therapy on the chronic lung disease and mortality in premature infants.

Alternate approaches to inhaled nitric oxide therapy in persistent pulmonary hypertension of newborns

INO therapy improved oxygenation in nearly 70% of neonates with PPHN in previous randomized trials. Infants who do not show an initial response to INO and those that deteriorate subsequently while on INO therapy may continue to have significant pulmonary hypertension. Investigation of alternate and complementary approaches to INO therapy is needed. The alternatives include (1) vasodilator prostaglandins such as prostacyclin or PGE1, (2) the NO precursor l-arginine, (3) phosphodiesterase inhibitors such as sildenafil, and (3) the free radical scavenger SOD. Other agents that were investigated in pediatric and adult pulmonary hypertension include adenosine, ATP-MgCl2, magnesium sulfate, and the endothelin receptor antagonist Bosentan. All these therapies remain investigational at this point, with limited or no data in neonates with PPHN, particularly for endothelin receptor antagonists. This section presents the available evidence and future direction of the clinical research. Studies with l-arginine, sildenafil, and SOD are presented first because they work on the NO-cGMP pathway for pulmonary vasodilation, followed by the use of vasodilator prostaglandins and other approaches.

L-Arginine infusion to augment endogenous nitric oxide

The rationale for using l-arginine, a precursor for NO, is twofold: l-arginine is a required substrate for NO synthesis, and it promotes coupled activity of NOS under conditions of stress [25]. l-arginine causes vasodilation even in the presence of sufficient endogenous stores of this amino acid [75]. This “arginine paradox” has been observed initially in studies conducted in the endothelial cells [75] and in lambs with pulmonary hypertension [76]. l-Arginine has been shown to ameliorate pulmonary hypertension and right ventricular hypertrophy
in animal models [77]. There is also evidence that plasma levels of L-arginine are decreased in neonates with PPHN compared with infants requiring ventilation for other causes [39]. Plasma concentrations of asymmetric dimethyl L-arginine, a competitive inhibitor of eNOS activity, are elevated in patients with vascular disease [78]. In addition to being a substrate for NOS, L-arginine promotes coupled NOS activity and causes \( \cdot NO/\cdot O_2^- \) release under conditions of oxidant stress [25]. Increased dietary intake of L-arginine improves exercise tolerance in adults with pulmonary hypertension [79]. Despite the many benefits of L-arginine in improving NOS function and endothelium-dependent vasodilation, it has not been investigated in a randomized trial in PPHN. Although the vasodilator effect tends to be small compared with INO, it may help preserve the endogenous NOS activity and permit a smoother weaning of INO therapy.

**Sildenafil**

Sildenafil causes vasodilation by inhibition of type 5 phosphodiesterase, which is the major pathway for breakdown of cGMP in the vascular smooth muscle. Because type 5 PDE appears in large concentration in pulmonary [23] and penile vasculature, sildenafil has been investigated in experimental and clinical pulmonary hypertension. There are currently over 90 studies on the use of sildenafil in animal models and in patients with pulmonary hypertension. Most of the patients included in these studies are adults or older children with pulmonary hypertension [80,81]. The major concern about the use of sildenafil is that it can cause systemic hypotension, which can worsen the right-to-left shunting and hypoxemia in PPHN [82]. In addition, the pulmonary vasodilation caused by sildenafil is not confined to the ventilated segments of the lung, which can potentially worsen the VQ mismatch and hypoxemia. A recent study done in a porcine model of meconium aspiration syndrome illustrates its potential for improving pulmonary vasodilator response to INO and its adverse effects on systemic vascular resistance and VQ mismatch [83]. Stocker et al [82] have reported the use of intravenous sildenafil (0.35 mg/kg over 20 minutes) given before or after the initiation of INO therapy in 16 infants with pulmonary hypertension after cardiac surgery. Although sildenafil increased the pulmonary vasodilator response to INO, it caused significant decreases in systemic blood pressure and oxygenation. Sildenafil use has been associated with retinal dysfunction in adults [84] and with the progression of retinopathy of prematurity in a preterm infant [85]. These concerns need to be addressed in randomized trials. Although the potential adverse effects may limit the use of sildenafil as the drug of choice in the initial management of hypoxic respiratory failure with PPHN, the use of this agent in babies whose hypoxemia is unresponsive to INO warrants further investigation. A phase I trial to investigate the safety and to identify its effective dose in babies with PPHN is underway at five north American centers (John Kinsella, MD, Denver, CO, personal communication, March 2004). In addition, the use of sildenafil in babies with CDH and
pulmonary hypertension persisting after ECMO therapy and repair of CDH has been described in case reports [66] and requires further investigation.

**Superoxide dismutase to enhance response to inhaled nitric oxide**

An attractive strategy to reverse the impaired vasodilation in PPHN is to reduce the production of ROS and ONOO\(^{-}\) formation. Several laboratory studies have suggested that accumulation of ROS occurs in PPHN. Superoxide scavengers improve the vasodilator response of isolated pulmonary arteries or intact lambs in the ductal ligation model of PPHN [20,44]. In addition, SOD protects the lung from oxidant damage caused by the combination of exogenous NO and high inspired oxygen concentrations in newborn piglets, conditions that neonates with PPHN are often exposed to [86]. There is evidence for impairment of eNOS function and an increase in \(O_2^{•−}\) and decreased NO production in the ductal ligation model of PPHN [20]. Steinhorn et al [87] have shown that intratracheal administration of 5 mg/kg recombinant human SOD improves the efficacy of INO in causing pulmonary vasodilation and improving oxygenation in lambs with PPHN induced by prenatal ligation of ductus. These studies suggest that increased superoxide formation in PPHN not only impairs the ability of pulmonary arteries to dilate at birth, but also inhibits their response to exogenous NO. Recombinant SOD has been shown to improve oxygenation in doses of 2.5 to 10 mg/kg in premature lambs with RDS and ventilator induced lung injury [88]. In addition, IV SOD (1000 U/kg every 6 hours) has been shown to prevent rebound pulmonary vasodilation after discontinuation of INO therapy in lambs [89]. There are no studies of the use of SOD in neonates with PPHN alone or in conjunction with INO. The issues of identifying a safe and effective dose and frequency of administration of SOD need to be addressed before human trials can begin.

**Prostacyclin**

Prostacyclin is a potent vasodilator, and its effects on vascular tone are complementary to that of NO, which increases the cGMP level in vascular smooth muscle (Fig. 3). cAMP and cGMP may compete as substrates for phosphodiesterase, enhancing the effects of NO and PGI\(_2\) when they are used together [90]. Interaction between NO and PGI\(_2\) signaling pathways has been demonstrated in endothelial and smooth muscle cells [91]. Therefore, there is a potential synergistic effect on the vascular tone with the combined use of these two drugs, as shown in studies performed in monocrotaline-induced pulmonary hypertension in rats [92]. Prostacyclin infusion has been shown to decrease pulmonary artery pressure and PVR in adults and older children with primary pulmonary hypertension, and continuous infusion of prostacyclin has become the mainstay of vasodilator therapy in this condition [81]. Intravenous and aerosol administration of prostacyclin have been investigated in PPHN. Bos et al [93] have reported a significant improvement in oxygenation without a decrease in
systemic pressure with IV PGI2 (5–20 ng/kg/min) in neonates with CDH and pulmonary hypertension [93]. Eronen et al [94] reported that infusion of PGI2 in doses of 20 to 60 ng/kg/min in eight term and near-term infants with PPHN resulted in significant improvement in oxygenation. Although a drop in the systemic pressure was noted in these babies, the pulmonary/systemic pressure ratio estimated by echocardiography also decreased, potentially reducing the right-left shunting [94].

Although IV PGI2 has been shown to decrease pulmonary artery pressure and improve oxygenation in neonates with PPHN, systemic hypotension and a potential decrease in oxygenation from worsening VQ mismatch remain concerns with this therapy. The aerosol administration offers the potential for more selective pulmonary vasodilation. The half-life of PGI2 is short because it undergoes a spontaneous hydrolysis to its stable metabolite, 6-keto-PGF1-alpha [92]. Thus, aerosolized PGI2 has been shown to cause a selective decrease in pulmonary artery pressure, whereas IV PGI2 caused decreases in pulmonary and systemic pressures in a 4-month-old infant with primary pulmonary hypertension [95].

Bindl et al [96] reported that aerosol PGI2, in a dose of 20 to 28 ng/kg/min, improved oxygenation and decreased pulmonary artery pressure measured by Doppler echocardiography without affecting the systemic pressure. Kelly et al [97] reported a pilot study of aerosol PGI2 administered in conjunction with INO therapy in four neonates with PPHN. PGI2 was given at a dose of 50 ng/kg/min when oxygenation failed to improve with INO or deteriorated while on INO. Three infants had significant improvement in oxygenation without a change in heart rate or systemic pressure. PA pressures were not measured at the time of initiation of PGI2. These studies demonstrate a potential role for aerosol PGI2 in babies with hypoxic respiratory failure that failed to respond adequately to INO or in centers where INO is not available as a rescue therapy. Randomized controlled trials are needed to establish the safety and efficacy of this therapy. However, initiation of randomized trials in this population becomes more difficult because a single center is unlikely to have large enough number of infants unresponsive to INO. In addition, some INO-unresponsive infants are unstable and do not tolerate additional interventions and a delay in initiation of ECMO therapy.

**Role of PGE1 in the management of pulmonary hypertension**

PGE1, a vasodilator prostaglandin, has been used to maintain ductal patency in congenital heart disease. PGE1 was found to be an effective vasodilator in experimental pulmonary hypertension [98] and in adults with primary or post-operative pulmonary hypertension [99,100]. This effect was noted with inhaled and IV administration of PGE1, although the response to PGE1 was not as striking as the response to PGI2 in a canine model of pulmonary hypertension [101]. The potential use of PGE1 as a pulmonary vasodilator in neonates was suggested by the observation of improved oxygenation in babies with PPHN that were receiving it to maintain ductal patency for suspected congenital heart disease. However, no clinical studies have been published on the use of IV or inhaled PGE1 as a pulmonary vasodilator in neonates with PPHN. Its weaker vasodilator
effect compared with PGI2 [101] and its potential to delay closure of ductus arteriosus make it a less desirable option than PGI2 for the future investigation of pulmonary vasodilators.

Adenosine and ATP

The purine nucleoside adenosine and its tri-nucleotide ATP are potent pulmonary vasodilators in fetal and neonatal lambs [102]. They cause selective pulmonary vasodilation when infused in low doses IV because of their rapid uptake and inactivation by the pulmonary vascular endothelium [103,104]. Adenosine infusion given IV at 25 to 50 μg/kg/min was shown to improve oxygenation in babies with PPHN in a randomized placebo-controlled pilot study [105]. There were no systemic hypotension or arrhythmias observed in this study. However, the improvement in oxygenation was not sustained, and some infants had a decrease in oxygenation presumably related to worsening of VQ mismatch due to an increase in total pulmonary flow. Ng et al [106] reported that adenosine infusion (50 μg/kg/min) improved oxygenation and decreased pulmonary artery pressure in six of nine infants with PPHN already receiving INO therapy. ATP-MgCl2 infusion was shown to cause selective pulmonary vasodilation in children with post-operative pulmonary hypertension [107]. However, the use of this agent has not been investigated in PPHN. The presence of extra-pulmonary right-left shunts in PPHN may result in systemic hypotension with these agents.

Magnesium sulfate

IV magnesium sulfate in a dose of 200 mg/kg given as a bolus followed by an infusion of 20 to 100 mg/kg/hr improved oxygenation [108] and decreased the oxygenation index [109] in two uncontrolled trials done in babies with PPHN who were not receiving other vasodilators [108,109]. There are no randomized controlled trials of this agent in PPHN. The potential for causing systemic hypotension and depression of central nervous system may limit the use of this therapy in neonates despite the low cost and availability of this drug.

Summary

The management of PPHN entered a new era with the development of inhaled NO therapy for the relief of pulmonary hypertension. The wider application of INO therapy and improved ventilation strategies led to a decrease in the need for invasive life-sustaining therapies such as ECMO. The remarkable advances in the understanding and treatment of PPHN were made possible by the extensive investigations in the laboratory using animal models. Further decreases in morbidity and mortality are possible with specific strategies targeted to correct the alterations in NO and prostacyclin biology and strategies to reduce lung injury. Further research is needed to understand the basis for the biologic susceptibility of some infants to environmental insults such as intra-uterine stress or exposure to NSAIDs in utero.
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