New Therapeutic Targets in Neonatal Pulmonary Hypertension

Julie A Dillard, Claire Murray, Amit A Mathur

Abstract
Persistent pulmonary hypertension of the newborn (PPHN) is a significant cause of morbidity and mortality in neonates. Despite advances in medical care, mortality remains high. In the United States, inhaled nitric oxide is the gold standard treatment in patients with PPHN. However, while it decreases the need for extracorporeal membrane oxygenation, many patients do not respond to inhaled nitric oxide, and it does not improve overall mortality in those with PPHN. Furthermore, its use is cost-prohibitive in many parts of the world. Thus, there is a critical need to research alternative therapies to improve neonatal outcomes. In this review, we present the animal and human data of some emerging therapeutic targets for pulmonary hypertension, prioritizing pediatric and neonatal data when available. Specifically, we discuss the role of soluble guanylate cyclase stimulators and activators, prostacyclin and analogues, phosphodiesterase 3, 4, and 5 inhibitors, rho-kinase inhibitors, endothelin receptor blockers, PPARγ agonists, and antioxidants in the treatment of neonates with PPHN.

Keywords: Extracorporeal membrane oxygenation, Neonate, Persistent pulmonary hypertension of the newborn, Pulmonary hypertension.

Introduction
In utero, the lungs are fluid-filled and pulmonary vascular resistance (PVR) is high, with only 10–20% of cardiac output reaching the lungs. At birth, with the establishment of ventilation and removal of the low resistance placental circuit, PVR falls dramatically, resulting in the establishment of pulmonary blood flow (PBF). Persistent pulmonary hypertension of the newborn (PPHN) results when the normal circulatory transition at birth fails to occur and pulmonary vascular pressures remain elevated. This leads to right-to-left shunting of blood across the foramen ovale and ductus arteriosus (DA), with resulting hypoxemia. The need for extracorporeal membrane oxygenation (ECMO) in these infants is high, and despite advances in medical care, mortality remains up to 10% in the United States. Inhaled nitric oxide (iNO), the only FDA-approved treatment for PPHN, continues to be the mainstay of therapy, and there is a lack of evidence-based alternative therapies for this population. Furthermore, iNO does not clearly reduce mortality or improve neurodevelopmental outcomes among survivors. Thus, there is a critical need to identify novel therapeutic targets to improve patient outcomes. In this paper, we will review the preclinical and clinical research of some emerging therapeutic targets for neonatal pulmonary hypertension.

Soluble Guanylate Cyclase Stimulators and Activators
Nitric oxide (NO) exerts its vasodilatory effects through activation of the enzyme, soluble guanylate cyclase (sGC), resulting in greater cyclic guanosine monophosphate (cGMP) concentrations. The native form of sGC contains a prosthetic heme group that serves as the location of the NO-binding site and is required for NO–sGC activation. In the heme-free state, sGC is dysfunctional and ultimately degraded, which occurs under conditions of oxidative stress. In disease states characterized by reduced NO bioavailability or the development of NO tolerance, therapeutics that are able to modulate sGC in the absence of NO may prove beneficial in clinical practice. Two such classes of drugs, sGC stimulators and sGC activators, bind directly to sGC to activate the enzyme via an NO-independent mechanism.

Soluble guanylate cyclase stimulators and activators directly bind and stimulate the heme-containing form of sGC, increasing cGMP concentrations. Additionally, these compounds stabilize the binding of NO to sGC, and thus, exhibit synergism with NO. Experimental animal models demonstrate sustained pulmonary vasodilation in the presence of the sGC stimulators, BAY 41-2272 and BAY 41-8543, in fetal lambs, intravenous infusion of BAY 41-2272 resulted in a 75% reduction in PVR with a threefold increase in PBF. Furthermore, the pulmonary vasodilator effects were not attenuated by the addition of an NO synthase inhibitor, suggesting that the effects of BAY 41-2272 were independent of NO. Of concern in this study, systemic effects were observed at higher doses and during prolonged infusion, although the study drug was infused directly into the LPA. In another study of severe PPHN generated by prenatal ligation of the DA, BAY 41-2272 infusion resulted in a 75% reduction in PVR by day 5, greater than that of sildenafil-treated fetal lambs. Moreover, in neonatal sheep, while BAY 41-2272 infusion resulted in greater pulmonary vasodilation than iNO, the combined treatment with both agents resulted in enhanced pulmonary vasodilation and improved oxygenation compared to either treatment alone. In fact, multiple animal studies provide evidence of synergy between NO and sGC.
stimulators. In adult rats, pulmonary vasodilator response to BAY 41-8543 was attenuated if endogenous NO production was inhibited and could be restored by additional treatment with an NO donor. Unfortunately, given as an IV infusion in this study, BAY 41-8543 resulted in similar dose-dependent decreases in systemic arterial pressure, which may limit its clinical applicability as an IV infusion in neonates.

The oral sGC stimulator, BAY 63-2561, or riociguat, is FDA-approved for the treatment of adults with pulmonary arterial hypertension (PAH) and chronic thromboembolic pulmonary hypertension. In phase 3 double-blind study of 443 adults with symptomatic PAH, riociguat improved several clinical outcomes including 6-minute walk distance, PVR, and time to clinical worsening. In the open-label follow-up study, improvements in exercise and functional capacity were maintained for up to 1 year, with a survival rate of 97%. While serious adverse events were rare, hypotension was documented in 9% and syncope in 7% of patients receiving treatment. Interestingly, a clinical trial demonstrated that riociguat was effective in the majority of adult patients who had inadequate response to treatment with PDE5 inhibition. Riociguat is currently being evaluated in children aged 6–18 years with PAH in an open-label, dose adjustment study, PATENT–CHILD, and results are pending (https://clinicaltrials.gov/, NCT 02562235). A single case report of a 3.5-year-old boy with therapy-resistant PAH who was treated with riociguat for 6 months demonstrated improved PVR/systemic vascular resistance (SVR) ratio, right ventricular hypertrophy (RVH), and pediatric functional class without adverse effects of systemic hypotension. Currently, there are no reports of studies utilizing sGC stimulators in infants and neonates, and more research is needed to elucidate the safety and efficacy in this population.

In contrast to sGC stimulators, sGC activators bind to the dysfunctional, heme-free form of sGC that is unresponsive to NO. Considering that pathophysiological conditions of oxidative stress can oxidize the heme moiety of sGC, rendering it unresponsive to NO, the use of sGC activators may have wide-reaching clinical implications, including in neonatal pulmonary hypertension (PH). Experimental animal studies demonstrate that the sGC activator, BAY 58-2667 (cinaciguat), elicits potent and sustained pulmonary vasodilation. In a fetal ovine model, infusion of cinaciguat resulted in dose-dependent and long-lasting increase in PBF and reduced PVR by 80%. Importantly, sGC oxidation by ODQ enhanced the pulmonary vasodilatory effects of cinaciguat in vitro and resulted in a 14-fold increase in cGMP levels in pulmonary artery smooth muscle cells (PASMC) in vivo compared to non-ODQ treated cells. Likewise, in PASM isolated from a lamb model of PPHN, cinaciguat increased cGMP generation by >60-fold following oxidation with ODQ and approximately 20-fold after exposure to moderate hyperoxia. In this PPHN lamb model induced by prenatal DA ligation, newborn sheep treated with cinaciguat had increased PBF and decreased PVR, and the vasodilatory effects were greater than treatment with oxygen or iNO. In heme-deficient sGC mice, the ability of NO to relax precontracted aortas was abolished, whereas the ability of cinaciguat to relax the vessels was enhanced. Not surprisingly, rat studies utilizing intravenous injection of sGC activator, BAY 60-2770 reported potent and long-lived reduction in systemic arterial pressures as well (Fig. 1).

In the COMPOSE studies, a series of three randomized, double-blind, placebo-controlled trials among adults with acute decompensated heart failure, researchers determined that treatment with intravenous cinaciguat did not meaningfully improve cardiac index or dyspnea and was associated with significant reductions in systemic blood pressure. The study was terminated early due to an increased occurrence of hypotension and poor recruitment. At present, no studies have examined the use of sGC activators in the pediatric population. The findings in animal models and adults of substantial systemic hypotension are concerning and may be prohibitive in researching this therapy in neonates. Interestingly, inhalation of microparticles containing sGC stimulators (BAY 41-2272 and BAY 41-8543) or sGC activator (BAY 58-2667) produced pulmonary vasodilation and transpulmonary cGMP production without impacting systemic hemodynamics in an experimental lamb model of pulmonary hypertension, suggesting that inhalational therapy may show efficacy while avoiding adverse effects on the systemic circulation.

Fig. 1: Pulmonary vascular tone is regulated by cAMP and cGMP, which are hydrolyzed by the phosphodiesterases. NO activates sGC to increase cGMP concentrations. Additionally, sGC stimulators (riociguat) and sGC activators (cinaciguat) directly bind and stimulate sGC independent of NO. PDE5 hydrolyzes cGMP, thus PDE5 inhibition with sildenafil or tadalafil results in increased cGMP levels. Prostacyclin and its analogues are concerning and may be prohibitive in researching this therapy in neonates. Interestingly, inhalation of microparticles containing sGC stimulators (BAY 41-2272 and BAY 41-8543) or sGC activator (BAY 58-2667) produced pulmonary vasodilation and transpulmonary cGMP production without impacting systemic hemodynamics in an experimental lamb model of pulmonary hypertension, suggesting that inhalational therapy may show efficacy while avoiding adverse effects on the systemic circulation.
**Prostaglandins**

Prostacyclins and their analogues have pulmonary vasodilatory and immunomodulatory effects. Upon binding to its receptor, prostacyclin activates the enzyme, adenylate cyclase, thereby increasing intracellular cyclic adenosine monophosphate (cAMP) concentrations. This activates protein kinase A which relaxes smooth muscle, leading to vasodilatation of the pulmonary arteries. The use of prostacyclins in the treatment of PPHN is an active area of research and their use in adults and pediatric patients with PH is well-established. However, their use has been somewhat limited by their short half-life. The prostacyclins are available in IV, subcutaneous, and inhaled formulations.

Animal models of PH utilizing prostacyclin and analogues have shown promising results. In piglets with acute respiratory failure and PH, investigators compared iNO, IV, and inhaled prostacyclin. Inhaled NO and inhaled prostacyclin significantly increased PaO₂/FiO₂ and decreased mean airway pressure and mean pulmonary artery pressure (PAP) without impacting systemic blood pressure. There was no difference in efficacy between iNO and inhaled prostacyclin; however, IV prostacyclin improved oxygenation parameters to a lesser extent at all doses. In isolated lungs from a fetal lamb model of PPHN induced by antenatal ligation of the DA, protein expression of both prostacyclin synthase and prostacyclin receptor were decreased. Furthermore, precontracted pulmonary arteries showed impaired relaxation to prostacyclin, iloprost, and milrinone. Interestingly, pretreatment with milrinone significantly enhanced the vasorelaxation to both prostacyclin and iloprost, suggesting that dual therapy with these agents may be of benefit in neonates with PPHN.

One challenge with the currently available prostanoids and analogues is their short half-life, limiting delivery strategies. Epoprostenol is one such type of synthetic prostanoid that requires continuous intravenous administration and is unstable at room temperature. Case studies of infants given epoprostenol reported that pulmonary arterial pressure was decreased by an average of 19.4 mmHg and oxygenation index improved by a mean of 32 without the side effect of systemic hypotension. In a recent retrospective review of iNO-unresponsive PPHN, IV epoprostenol resulted in a rapid and sustained reduction in oxygenation index (OI) in a small subset of patients who were considered responders. There was an increased need for volume resuscitation after initiation of treprostinil in the subgroup of infants deemed unresponsive to the drug. Additionally, per institutional unit protocol, treatment with IV milrinone preceded initiation of epoprostenol in 95% of the study patients.

The current prostanoid analogues (treprostinil and iloprost) are more chemically stable than epoprostenol. Treprostinil has subcutaneous, intravenous, and inhalation delivery options with a slightly longer half-life than its sister drug, epoprostenol. In a retrospective cohort study, 17 patients with congenital diaphragmatic hernia (CDH)-associated PH who were treated with IV treprostinil for a median of 54.5 days had a significant reduction in B-type natriuretic peptide and improvement in echocardiographic parameters of PH at one month. Despite this, patients treated with treprostinil were more likely to require ECMO, had a longer length of mechanical ventilation and hospital stay, and had an overall mortality of 35%. There is currently an ongoing placebo-controlled clinical trial enrolling babies with PPHN to receive IV remodulin (treprostinil) vs placebo in addition to standard of care (https://clinicaltrials.gov/, NCT02261883).

Iloprost is available for use as an inhaled medication and has shown pulmonary selectivity. An early randomized controlled trial (RCT) evaluating the efficacy of inhaled iloprost in 203 adults with severe PH, 40% of the iloprost group increased their 6-minute walk distance by greater than 10%, and approximately 25% had improvement in functional class over a 12-week period. In an open-label extension study of 71 adults with PH, long-term treatment with inhaled iloprost improved functional class after 1 and 3 years and had survival rates of 83, 78, and 58% at 1, 3, and 5 years, respectively. Large, prospective studies examining the safety and efficacy of inhaled iloprost in neonates are lacking. In a small, prospective study of neonates with PPHN, inhaled iloprost 4–8 times per day resulted in improved echocardiographic parameters of PH and respiratory severity score in 8 of 9 patients. In another prospective study, 47 neonates with PPHN were given either oral sildenafil or inhaled iloprost as first-line therapy. The group who received inhaled iloprost showed decreased time to clinical response, ventilatory parameters, and length of mechanical ventilation. Furthermore, while there was a significant decrease in systemic blood pressure in the sildenafil group, this was not noted with inhaled iloprost. Inhaled NO was not used in these low resource settings and both authors concluded that inhaled iloprost may be a beneficial first-line agent for this purpose. The original delivery of nebulized iloprost that was studied in ambulatory adults with PH could not be used in a closed ventilator circuit. Recent advances have identified reliable methods of drug delivery in mechanically ventilated adults, but studies are lacking in infants. A recent study of iloprost delivery utilizing a neonatal test lung model found that delivery of iloprost was optimized when using a vibrating mesh nebulizer proximal to the patient airway and was more efficient during high-frequency ventilation than conventional ventilation. Definitive in vivo studies need to be completed to confirm these results.

**The Phosphodiesterases**

The cyclic nucleotide phosphodiesterases (PDEs) are composed of a superfamily of 11 enzymes with various tissue and cellular distribution, and cell-specific function and regulation. PDEs degrade the ubiquitous second messengers, cAMP and/or cGMP, and thus, are effectors in many cellular processes including vascular tone and remodeling, and inflammation. While nearly all of the PDE families have been identified in the pulmonary vasculature, this review will focus on the most well-studied PDEs in neonatal PH: PDE3, PDE4, and PDE5.

**PDE3**

PDE3 hydrolyzes both cAMP and cGMP with high affinity. It is known as the cGMP-inhibited PDE, as its rate of hydrolysis for cAMP is greater than that of cGMP. Increased PDE3 activity has been reported in pulmonary arteries from a rat model of PH as well as in isolated PASM-C from patients with PH. Moreover, the PDE3 inhibitor, milrinone has beneficial effects in animal models of PH and in case reports of neonates with PH. This review will focus on the most well-studied PDEs in neonatal PH: PDE3, PDE4, and PDE5.

**PDE3**

PDE3 hydrolyzes both cAMP and cGMP with high affinity. It is known as the cGMP-inhibited PDE, as its rate of hydrolysis for cAMP is greater than that of cGMP. Increased PDE3 activity has been reported in pulmonary arteries from a rat model of PH as well as in isolated PASM-C from patients with PH. Moreover, the PDE3 inhibitor, milrinone has beneficial effects in animal models of PH and in case reports of neonates with PH. Recent data suggest that treatment with NO leads to an increase in PDE3 expression and activity. In experimental animal models, PDE3 expression and/or activity is increased in the pulmonary vasculature following treatment with NO. Pulmonary arteries of newborn sheep treated with iNO and 100% oxygen had the highest PDE3 activity and the greatest relaxation response to milrinone. Interestingly, in this study, the second-highest PDE3 activity
was seen in one-day-old spontaneously breathing lambs, which suggests a role for PDE3 in the transitional circulation. We recently demonstrated that treatment of neonatal human PASMC with an NO donor resulted in increased PDE3 activity and decreased cAMP concentrations.56 Moreover, there is evidence of synergy between iNOS and milrinone. In an experimental model of PH induced by a thromboxane mimetic, rabbits who received the combination of iNOS + IV milrinone had a greater drop in the PAP and PVR compared to either treatment alone.57 These data strongly support a role for PDE3 in neonatal PH. Moreover, as NO treatment appears to increase PDE3 activity, we speculate there is a role for PDE3 inhibition in neonates with PPHN that is unresponsive to iNO.

Milrinone has inotropic, lusitropic, and vasodilatory properties and is researched for use in multiple disease conditions. Currently, milrinone is FDA-approved for short-term use in adults with acute decompensated heart failure. Additionally, in children and infants, milrinone is used for the treatment and prevention of low cardiac output syndrome.57–59 Case reports of the use of milrinone in neonates with iNO-unresponsive PPHN have found promising results, with evidence of improved oxygenation.50,51,60,61 Some reports have found no impact on systemic hemodynamics.50,51 While others have found a decrease in systemic arterial pressures.52,60,62 In a prospective study of 11 neonates with PPHN resistant to iNO, the addition of milrinone led to improved oxygenation, decreased iNO dose, and decreased PAP.52 While there was a statistically significant reduction in systolic arterial pressure following the milrinone bolus, there was an overall improvement in cardiac output and markers of systemic perfusion, including lactate and base deficit.52 In a recent RCT evaluating the use of milrinone + sildenafil (a PDE5 inhibitor) in neonates with PPHN in a resource-limited setting, the investigators reported that combination therapy with milrinone and sildenafil resulted in a greater decrease in the PAP and OI than either monotherapy, further evidence of synergy between the two treatments.63 Importantly, there was no statistically significant difference in either the systolic or diastolic blood pressures before and after treatment in any of the three groups.63 Despite the promising animal and human data supporting the use of milrinone in neonates with PPHN, large RCTs in this population have not been performed. This is likely secondary to low enrollment of a rare disease process, as well as difficulty in adherence to study arms given the critically ill-nature of the patients.64

PDE4

PDE4 consists of four isoforms that are ubiquitously expressed and hydrolyze cAMP with high affinity.43,65 PDE4 isoforms are highly expressed in inflammatory cells, and therefore have been implicated for a role in respiratory disorders characterized by chronic inflammation, including chronic obstructive pulmonary disease (COPD) and asthma.66,67 The PDE4 inhibitor, GPD-1116, attenuated the increase in RV systolic pressure and RVH (as measured by Fulton’s Index) in rats with monocrotaline-induced PH and resulted in a 57% increase in pulmonary cAMP concentrations in non-diseased rats.68 Furthermore, the effects of GPD-1116 on the above measures were greater than that seen with the PDE5 inhibitor, tadalaflit.68 However, it was discovered that GPD-1116 also potently inhibits PDE1, so the effects seen from this molecule may not be attributed solely to PDE4. Several experimental animal studies of bronchopulmonary dysplasia (BPD) evaluating the effects of PDE4 inhibition on neonatal lung injury have shown promising results.69–72 In a hyperoxia model of acute lung injury in newborn rats, treatment with the PDE4 inhibitor, rolipram, improved survival and decreased lung inflammatory cell count and cytokine expression compared to controls.70 Similar findings were reported in a preterm rat model of hyperoxic lung injury in which PDE4 inhibitors prolonged survival, reduced capillary alveolar protein leakage, alveolar fibrin deposition, and influx of neutrophils and macrophages into the lung. PDE4 inhibition also resulted in reduced expression of inflammatory genes.71 In another experimental BPD study in neonatal rats, researchers investigated the effects of prophylactic and rescue PDE4 inhibition on hyperoxia-induced lung injury. Prophylactic treatment with the PDE4 inhibitor, piclamilast improved mortality and prevented the development of PH assessed by increased pulmonary vessel density, reduced arteriolar medial wall thickness, and attenuation of RVH.72 While rescue treatment with piclamilast on day 6 reduced arteriolar wall thickness and attenuated RVH, it did not restore lung angiogenesis or alveolar development.72 In the majority of studies, PDE4 inhibition did not significantly improve alveolarization, and we speculate that this may be related to the growth retardation seen in animals treated with PDE4 inhibitors, which may have important clinical implications for the neonatal population.70–72

The PDE4 inhibitor, rolflumilast is FDA-approved for use in adults with COPD. In patients with moderate-to-severe COPD, rolflumilast improves lung function and lowers the risk of exacerbation in some patients.73,74 However, in these studies, rates of gastrointestinal adverse events were high, including diarrhea and weight loss. Thus, this represents a major barrier to utilizing PDE4 inhibitors in practice, especially in the pediatric and neonatal populations. One approach to improve tolerability and mitigate adverse effects is to develop inhaled formulations, which should avoid systemic effects, and is currently being evaluated in animal models and adult COPD patients.75 Furthermore, PDE4 is comprised of 4 isoforms: A-D. Studies have shown that PDE4B inhibition is predominantly responsible for the anti-inflammatory effects of the PDE4 inhibitors, while PDE4D may be the primary cause of emesis and poor weight gain,76,77 and thus, it may be beneficial to study isoform-specific PDE4 inhibition.

PDE5

PDE5 hydrolyzes cGMP. Thus, agents that inhibit PDE5 lead to increased cGMP concentrations, resulting in vasorelaxation and inhibition of cellular proliferation. PDE5 is the most abundant PDE in the pulmonary vasculature, and therefore is the most well-studied for its role in PH.77,78 PDE5 expression is increased in the lungs of patients with PH79 in animal models of PH.80 and following exposure to hyperoxia.81,82 Multiple experimental animal models of PPHN demonstrate that PDE5 inhibition decreases PVR and improves oxygenation.83–85 In chronic hypoxia-induced PH in newborn piglets, a single dose of oral sildenafil decreased PAP and PVR compared to control and did not impact systemic hemodynamics.86 Notably, prophylactic sildenafil did not prevent the development of PH in hypoxia-exposed piglets, which contrasts with the findings in adult animal models of hypoxia-induced PH.87,88

Sildenafil is a well-established therapeutic option in adults with symptomatic PH, with notable improvement in pulmonary hemodynamics and enhanced exercise capacity.89,90 Despite the compelling adult and animal data, and although sildenafil is commonly utilized as a second-line agent in neonates with PPHN,91 no large RCTs evaluating the use of sildenafil in this population have
been published. In an open-label dose-escalation trial of 36 neonates with PPHN, IV sildenafil was associated with a sustained reduction in OI.52 Hypotension occurred in five patients, necessitating discontinuation of the drug in three cases, one patient went on ECMO, and one died. Most patients received iNO + sildenafil which was demonstrated to be safe, while six neonates never required iNO after initiation of sildenafil.53 In resource-limited settings where iNO is cost-prohibitive and ECMO is not readily available, oral sildenafil appears to be a reasonable alternative based on the results of three RCTs in neonates with PPHN.55–57 In the largest of the three studies, an RCT of 51 term infants with PPHN, oral sildenafil given every 6 hours resulted in improved oxygenation compared to placebo.55 Moreover, oral sildenafil improved mortality, which was 40% in the placebo group, compared to only 6% in the sildenafil group.56 A 2017 meta-analysis evaluating the use of sildenafil for PPHN, utilizing data from 166 enrolled patients in five eligible trials, concluded that sildenafil may be of benefit in improving oxygenation and reducing mortality, specifically in resource-poor environments in which iNO is not readily available.57 However, they did not find a survival benefit when sildenafil was compared to a control active (magnesium sulfate) or when combined with iNO.58 Two recent RCTs evaluating the use of sildenafil alone compared to sildenafil + milrinone63 or sildenafil + bosentan99 in neonates with PPHN both concluded that combination therapy was more effective than either monotherapy. A few retrospective chart reviews evaluating the use of sildenafil in BPD-associated PH have reported reduction in PAP100,101 but no RCT has been reported in this population despite its widespread use.102

The use of sildenafil in neonates with PH is still being actively investigated. A large, international, multicenter trial evaluating the use of IV sildenafil or iNO in infants with CDH-associated PH is currently enrolling.103 Finally, a large, multicenter RCT evaluating the use of IV sildenafil in PPHN completed enrollment with 59 patients, and results are pending (https://clinicaltrials.gov/, NCT01720524).

Tadalafil, another PDE5 inhibitor is FDA-approved for use in adults with PH and has a longer half-life than sildenafil, allowing for the convenience of once-daily dosing. In a prospective, open-label study of 25 patients aged 2 months to 5 years who were started on daily tadalafil either as initial therapy or transitioned from sildenafil, tadalafil improved mean PAP and was well tolerated.104 In two recent RCTs, comparing the efficacy of tadalafil and sildenafil in infants with PH, investigators found no difference between the two treatments.105,106

**Rho-kinase Inhibitors**

Emerging evidence implicates rho-kinase (ROCKs) for a role in vascular tone and remodeling, and thus, an important contributor to neonatal pulmonary hypertension. The small GTP-binding protein Ras homolog gene family member A (RhoA) acts on the serine-threonine kinase, ROCKs, which inhibits myosin light chain phosphatase, resulting in sustained smooth muscle cell contraction.107 The RhoA/ROCK pathway activity has been found to be increased in experimental animal models of PH, including in pulmonary arteries of neonatal rats.108 In fetal lambs, brief intrapulmonary infusions of the ROCK inhibitors, Y-27632 and HA-1077, resulted in potent pulmonary vasodilation.109 Furthermore, treatment with Y-27632 prevented vasoconstriction induced by inhibition of endogenous NO production.110 These data are compelling in that they elucidate a potential role for RhoA kinase in the maintenance of high PVR in utero, and the rapid drop in PVR in the transitional circulation, although mechanistic pathways remain unknown. Furthermore, several studies have demonstrated complex reciprocal interactions between NO and the RhoA/ROCK pathway. In a rat model of bleomycin and hypoxia-induced PH, the elevated PVR did not respond acutely to inhaled or systemic NO yet normalized completely after giving a ROCK inhibitor.111 These data suggest that the RhoA/ROCK pathway may be responsible for the suboptimal response to iNO seen in some neonates with PPHN. Furthermore, RhoA/ROCK pathway is involved in both the serotonin and PPARy pathways, which are also undergoing interrogation for their role in the pathophysiology of PH (Fig. 2).

The ROCK inhibitor, fasudil, is currently being investigated in clinical trials in adult and pediatric PH patients in China and Japan. Overall, studies in adults utilizing fasudil for PH have shown promising results.111–114 In prospective studies in adults with PAH, IV fasudil decreased PVR by 17%112 and long-acting oral fasudil improved cardiac index from baseline.113 A randomized clinical trial in 209 hospitalized adults with PH and right heart failure demonstrated markedly improved in-hospital mortality and 30-day rehospitalization rates.114 In these studies, adults were receiving maximal therapeutic treatment of PH, including multiple other pulmonary vasodilators. Interestingly, fasudil was well tolerated and had no significant impact on systemic hemodynamics, suggesting that it is at least somewhat selective for the pulmonary vasculature.112 In a prospective study of 12 pediatric patients with a mean of 12.3 years diagnosed with congenital heart disease (left-to-right shunt) and mild-to-moderate PH, treatment with IV fasudil led to a significant decrease in PAP and PVR, and an increase in cardiac output, PBF, and mixed venous oxygen saturation.115 While the investigators did note a small drop in systemic arterial blood pressures and SVR, there was an overall decrease in the PVR/SVR ratio.115 No studies have been done in neonates with PH.

**Endothelin Receptor Blockers**

In PH pathophysiology, there is a decrease of vasodilator mediators and an increase of vasoconstrictor mediators. One such vasoconstrictive mediator is endothelin (ET-1) which acts on...
endothelin receptors in the smooth muscle cell and increases ionic calcium concentration, resulting in vasoconstriction. ET-1 has been found to be elevated in infants with PPHN. Early on, medications classified as endothelin receptor antagonists were discovered in lamb models to block the binding of endothelin to its receptors, thereby negating endothelin's deleterious effects on the pulmonary vasculature (Fig. 3). Bosentan, an endothelin receptor antagonist, was FDA approved in 2017 for the treatment of pulmonary hypertension for patients ages 3 years and older. In an open-label prospective study of 19 pediatric patients between 10 and 40 kg, pharmacokinetics were found to be similar to that of adults. Improvements in PAP and PVR were significant. Bosentan was well tolerated, and while there were small decreases in systemic blood pressure, no symptomatic hypotension was observed. Fifty-eight percent of the study patients were receiving IV epoprostenol and the use of the two drugs together appeared to be safe. A database search in 2009 included 21 studies, both retrospective and prospective, examining the evidence on the effectiveness and safety of bosentan in the treatment of pediatric arterial hypertension. The authors state that bosentan appears to improve long-term functional status and hemodynamics in children with PAH without safety concerns. Adverse events include liver enzyme elevations were seen less frequently than in studies utilizing bosentan in the adult population. Several retrospective studies examining long-term outcomes of pediatric patients with PAH treated with bosentan report stabilization or improvement in WHO functional class, with no major safety concerns.

In a 2018 retrospective chart review of infants with PPHN, the combination of iNO + bosentan or bosentan alone both improved oxygenation. Additionally, several RCT utilizing treatment with bosentan have been performed in neonates with PH. In a randomized, double-blind, placebo-controlled trial of 24 infants given bosentan for PPHN, OI decreased by an average of 10 and mechanical ventilation days decreased by 7.2 days. In the FUTURE-4 trial, an exploratory trial designed to assess feasibility of enteral bosentan as adjuvant to iNO in neonates with PPHN, the investigators assessed the safety and pharmacokinetics of bosentan in 21 neonates who received the study drug. Blood concentrations of bosentan were variable in the first 12 hours after administration and did not reach steady state until day 5. Overall, bosentan was well tolerated, and no adverse effects on systemic hemodynamics or liver transaminases were noted. While the study was not powered to evaluate efficacy, the investigators reported that oxygenation and time on iNO and mechanical ventilation were not improved in the bosentan group. Overall, while several studies have demonstrated the safety and feasibility of bosentan use in neonates with PPHN, the efficacy remains largely unknown. Mixed results could be due, in part, to trials with small numbers of participants and the use of multidrug therapy. Notably, bosentan is only available orally, and thus in critically ill neonates, there may be delayed or variable absorption of the medication.

PPARγ

 Peroxisome proliferator-activated receptors (PPARs) are members of the superfamily of nuclear receptors. They are ligand-activated transcription factors that exert their effects by binding to DNA and altering gene expression. PPARs play a major role in energy homeostasis, specifically lipid metabolism. The most well-studied of the three subtypes, PPARγ, has also been studied for its role in inflammation and cellular proliferation and is highly expressed in both pulmonary vascular endothelial cells and smooth muscle cells, as well as adipocytes. Importantly, metabolic derangements are common in PH and significantly worsens the disease course. Thus, treatment of metabolic dysfunction in PH patients may be of value to prevent disease progression. Epithelial cell PPARγ-deficient mice develop airspace enlargement with decreased tissue resistance and increased lung volumes, suggesting a role for PPARγ in alveolar development. Many experimental animal models have implicated a role for PPARγ in the development of PH. In a rat model of PPHN and in adults with PAH, PPARγ expression was decreased. Mice with targeted deletion of PPARγ in smooth muscle cells spontaneously developed PAH, characterized by RVH, elevated RV systolic pressure, and distal pulmonary artery muscularization. In a rat model of chronic hypoxia-induced PH, the PPARγ agonist, rosiglitazone, attenuated PA remodeling and prevented muscularization of the distal arterioles. Moreover, it reversed the vascular remodeling and arteriole muscularization in mice previously exposed to chronic hypoxia. However, while this agent attenuated RVH, PA pressures remained significantly elevated in this model, suggesting PH. In an experimental BPD model in which newborn rats were exposed to chronic hyperoxia, both antenatal and neonatal administration of rosiglitazone enhanced lung maturation and ameliorated lung injury in pups compared to controls (Fig. 4). The pathogenic mechanisms of PPHN are complex and likely involve the interaction of multiple key pathways. In a fetal lamb model of PPHN, inhibition of the RhoA/ROCK pathway results in restoration of PPARγ activity, whereas PPARγ inhibition increased ROCK activity and proliferation in PASMC. Additionally, it has been shown that ET-1 decreases PPARγ activity, leading to pulmonary artery endothelial cell (PAEC) dysfunction and impaired angiogenesis. In a fetal lamb model of PPHN, ET-1 decreased PPARγ activity and reduced endothelial cell tube formation of isolated PAEC. The addition of a PPARγ agonist restored endothelial cell tube formation and increased endothelial nitric oxide synthase (eNOS) activity and NO production. Thus, PPARγ agonists may be most beneficial in PPHN when used in combination with a bosentan, endothelial receptor antagonist or fasudil, a ROCK inhibitor.
Two PPARγ agonists, rosiglitazone and pioglitazone, were approved by the FDA in 1999 for the treatment of adults with Type 2 diabetes mellitus. However, their use was later restricted after it was discovered that they can cause or exacerbate congestive heart failure. While preclinical PH models utilizing PPARγ agonists show promising results, human studies are lacking for this clinical indication. Moreover, no studies have been done on children or neonates.

**Antioxidants**

Pathologic changes in PH can be mediated by free radical damage from reactive oxygen species (ROS) and reactive nitrogen species (RNS). Oxidative stress results when there is an overproduction of ROS and RNS that overwhelms the antioxidant defenses and can result in alterations in energy metabolism, inflammation, cellular proliferation, DNA injury, and vascular dysfunction. NO and hyperoxia, two common treatments for PPHN, may alter these homeostatic conditions. Under normal conditions, NO combines with oxyhemoglobin to form nitrate. However, in diseased states, NO can combine with superoxide to form the damaging oxidant, peroxynitrite. Peroxynitrite leads to vascular endothelial dysfunction by multiple methods, including oxidation of the NOS cofactor, tetrahydrobiopterin (BH4), resulting in eNOS uncoupling. Additionally, it can lead to inactivation of prostacyclin synthase, decreasing levels of the vasodilator, prostacyclin, and increasing levels of vasoconstrictors. Oxidant stress and hydrogen peroxide and superoxide generation have been implicated in animal models of PPHN. As has eNOS uncoupling. Overall, these studies suggest that there is an increased burden of oxidant stress and a deficiency in antioxidant activity in neonates with PH (Fig. 5).

N-acetylcysteine (NAC) is a precursor to glutathione, important in antioxidant defense, and acts as a direct ROS scavenger. It is FDA-approved for the use of acetaminophen overdose resulting in hepatotoxicity and is trialed in adults with COPD. In a model of acute lung injury created by intratracheal administration of meconium, adult rabbits who received NAC had a reduction in lung inflammation and peroxidation and improvement in oxygenation compared to controls. In fetal sheep PASMC, the administration of NAC prevented the hyperoxia-induced increase in PDE5 activity and restored cGMP concentrations.

Despite the promising results in animal data, studies in neonates have been less positive. An RCT of 391 extremely low birth weight infants examining the impact of NAC on death or BPD showed that there was no difference between the two groups, with 51% of infants in the NAC group meeting criteria for BPD or death compared to 49% of infants in the control group. However, a recent RCT has garnered significant attention and placed an emphasis on the need for further research of NAC for BPD prevention in extremely preterm infants. In the study, antenatal administration of NAC to pregnant women with impending preterm birth resulted in less resuscitation at birth and was protective against the development of BPD, which was only 3% in the NAC-exposed group vs 32% in the control group.

Superoxide dismutase (SOD) catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. SOD, a predominant antioxidant enzyme in the pulmonary vasculature, is decreased in pulmonary arteries from neonates with PH, which results in reduced availability of NO secondary to NO-superoxide interactions. In a lamb model of PPHN, mechanical ventilation with 100% oxygen increased ROS burden, blunted the expected rise in eNOS expression, and decreased BH4 levels. While treatment with either iNO or recombinant human SOD (rhSOD) decreased ROS production and increased eNOS expression compared to PPHN lambs ventilated with 100% oxygen alone, only rhSOD restored eNOS function. In a few studies utilizing lamb models of PPHN, investigators demonstrated that intratracheal rhSOD improves oxygenation and causes pulmonary vasorelaxation. Importantly, this was enhanced by the combination use of rhSOD and iNO. Interestingly, two placebo-controlled RCT published in 1996 and 1997, enrolled a total of 59 preterm infants and evaluated the use of single and multiple intratracheal doses of rhSOD given 30 minutes after surfactant. Both studies demonstrated enhanced SOD activity in the serum, urine,
New Therapeutic Targets in Neonatal Pulmonary Hypertension

and tracheal aspirates and decreased neutrophil chemotactic activity and albumin concentration in tracheal aspirates of treated infants.\textsuperscript{156,157} A large RCT involving 302 premature infants randomized to receive rhSOD vs placebo every 48 hours for up to one month found no difference in the incidence of death or BPD at 28 days or 36 weeks corrected gestational age.\textsuperscript{158} However, follow-up data on 80% of the enrolled infants at one year of age showed a 36% reduction in wheezing that necessitated treatment with asthma medications. Furthermore, in infants <27 weeks, there was a 55% decrease in emergency room visits and a 44% decrease in hospitalizations.\textsuperscript{158}

**Conclusion**

In conclusion, despite advances in medical care for the neonate with PPHN, overall morbidity and mortality remain high. Our understanding of the pathobiology of PH in neonates continues to evolve, and with it, the emergence of new therapeutic compounds. However, definitive trials in this population are lacking. Sildenafil, the most well-studied of the adjuvant therapies, appears to be a safe and effective alternative when iNO is not available, although it is less clear if it provides benefit when used in combination with iNO or as a rescue therapy after failed iNO. Unfortunately, for many of the other therapies discussed in this review, while the animal data are compelling, studies in adult and pediatric patients have not clearly demonstrated efficacy, as in the case of endothelin receptor blockers and antioxidants, or safety, as in the case of PDE4 inhibitors and PPAR\(\gamma\) receptor blockers and antioxidants, or safety, as in the case of endothelin receptor blockers and antioxidants.

**ORCID**

Julie A Dillard https://orcid.org/0000-0003-3391-5154

**References**


New Therapeutic Targets in Neonatal Pulmonary Hypertension


