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Pharmacologic Strategies in Neonatal Pulmonary Hypertension other than Nitric Oxide

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[1]

Abstract

Inhaled nitric oxide (iNO) is approved for use in persistent pulmonary hypertension of the newborn (PPHN) but does not lead to sustained improvement in oxygenation in a third of patients with PPHN. Inhaled NO is less effective in the management of PPHN secondary to congenital diaphragmatic hernia (CDH), extreme prematurity and bronchopulmonary dysplasia (BPD). Intravenous pulmonary vasodilators such as prostacyclin, alprostadil, sildenafil and milrinone have been successfully used in PPHN resistant to iNO. Oral pulmonary vasodilators such as endothelin-receptor antagonist bosentan and phosphodiesterase-5 inhibitors such as sildenafil and tadalafil are used both during acute and chronic phase of PPHN. In the absence of infection, glucocorticoids may also be effective in PPHN. Many of these pharmacologic agents are not approved for use in PPHN and our knowledge is based on case reports and small trials. Large multicenter randomized controlled trials with long-term follow-up are required to evaluate pharmacologic strategies in PPHN.

Keywords

hypoxia; oxygen; nitric oxide; prostacyclin; sildenafil; bosentan; iloprost; milrinone

Introduction

The fetus is in a state of physiologic pulmonary hypertension with low pulmonary blood flow, while the placenta functions as the site for gas exchange. At birth, successful adaptation to extra-uterine life requires a rapid increase in pulmonary blood flow to establish

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Bobby Mathew and Corinne Leach declare that they have no relevant competing interests

Off label use:

This paper contains information about unapproved use of certain pharmacologic agents (prostaglandins, bosentan, sildenafil, milrinone, riociguat, ciniciguat and rhSOD are not approved for use in newborn period).

the lungs as the site of gas exchange. Persistent pulmonary hypertension of the newborn (PPHN) is a syndrome in which normal circulatory transition at birth fails to occur, and pulmonary blood flow remains low with right-to-left shunting at the patent foramen ovale (PFO) and/or patent ductus arteriosus (PDA). Pulmonary vasoconstriction, and vascular proliferation and remodeling contribute to elevated pulmonary vascular resistance (PVR) in PPHN. The incidence of PPHN has been reported as 1.9 per 1000 live births (range: 0.4–6.8) in the United States and 0.43 – 6 per 1000 live births in the United Kingdom with mortality rate ranging between 4–33%[12].

Management of PPHN includes supportive therapies, lung recruitment strategies, and pharmacologic pulmonary vasodilation. Inhaled NO (iNO) has been the primary agent studied in large randomized clinical trials, and is currently the only FDA approved specific pulmonary vasodilator therapy for infants. The response to iNO, however, remains suboptimal. A meta-analysis of randomized trials of iNO in newborns with PPHN revealed that almost a third to half of near-term and term infants with hypoxic respiratory failure/ PPHN had a suboptimal response to iNO[3].

Alternative agents for iNO-resistant PPHN are under active investigation based on their potential physiologic effects and complementary action with NO. These include systemic and inhaled vasodilators such as PDE 5 inhibitors, prostaglandins, the PDE 3 inhibitor milrinone, and ET-1 receptor antagonists. These promising therapeutic strategies are used by clinicians and centers with expertise in pulmonary vasodilator therapy. In addition, novel areas of investigation include emerging agents such as recombinant human superoxide dismutase (rhSOD), L-citrulline, sGC stimulators and activators, Rho-kinase inhibitors, and proliferator-activated receptor-γ agonists.

Endothelium Derived Mediators

The pulmonary vascular endothelium releases vasoactive mediators that play an important role in cardiopulmonary transition at birth. In PPHN, the function of the endothelium is impaired and the balance between vasodilators and vasoconstrictors is altered. There is decreased production of vasodilators such as prostacyclin and nitric oxide (NO) and increased production of vasoconstrictors such as endothelin (Fig 1).

Many of these mediators and their derivatives or inhibitors are effective pulmonary vasodilators and are beneficial in the treatment of PPHN. These mediators are broadly classified into three categories based on their action via the cGMP, cAMP, and endothelin pathways.

Pulmonary vasodilators acting via the cGMP pathway

Pulmonary endothelial NO production increases markedly at the time of birth. The shear stress resulting from increased pulmonary blood flow and increased oxygenation induces endothelial nitric oxide synthase (eNOS) expression, contributing to NO-mediated pulmonary vasodilation after birth[4].

Nitric oxide exerts its action through soluble guanylate cyclase (sGC) and the important second messenger, cGMP (Fig 1). The enzyme phosphodiesterase 5 (PDE 5) breaks down cGMP to inactive GMP. Hyperoxia and superoxide anions may inactivate eNOS, oxidize sGC and decrease cGMP production and stimulate PDE 5 to enhance breakdown of cGMP[5]. Natriuretic peptides (ANP, BNP and CNP) stimulate particulate guanylate cyclase (pGC) and produce cGMP. Plasma BNP levels are elevated in PPHN[6–8] and may be an alternate source of cGMP. Inhaled NO and PDE 5 inhibitors are commonly used agents in the management of PPHN.

Sildenafil (Viagra®, Revatio®, Pfizer) is the prototype PDE 5 inhibitor. Considerable research in pulmonary arterial hypertension (PAH) and rebranding with a new trade name Revatio® has resulted in sildenafil being a common agent in the chronic management of PAH in adults[9]. Tadalafil (Adcirca®) is also a PDE 5 inhibitor approved for use in pulmonary arterial hypertension in adults. Sildenafil is the most common enteral pulmonary vasodilator used to treat infants, although this has been controversial.

(http://www.cbsnews.com/news/viagra-for-kids/). The FDA recently reignited the controversy by issuing a warning and recommending against its use in children (http://www.fda.gov/Drugs/DrugSafety/ucm317123.htm). Subsequent publications by experts[10] and a clarification from FDA (http://www.fda.gov/Drugs/DrugSafety/ucm390876.htm) have acknowledged that there may be situations in which the risk-benefit profile of sildenafil may be acceptable in individual children, especially when treatment options are limited. It is important to note that sildenafil is not approved in neonates, and that the study that triggered this controversy did not enroll any neonates or infants under the age of 1 year[11]. Sildenafil is available in intravenous form, oral tablets and recently as a 10mg/ml suspension.

Currently, sildenafil is used for the following indications in neonates: (a) as an acute adjuvant to iNO in NO-resistant PPHN or to facilitate weaning of iNO; (b) as an acute primary treatment of PPHN where iNO is not available or is contraindicated and (c) in chronic primary treatment of pulmonary hypertension in conditions such as BPD and CDH.

There are no randomized trials evaluating sildenafil in neonates with PPHN. The available evidence from animal models and neonatal reports is summarized below.

Animal models of PPHN. Intravenous sildenafil was noted to be as effective as iNO in piglets with PPHN induced by intratracheal instillation of meconium [12]. Intravenous sildenafil (2 mg/kg) administered to the same model while receiving iNO resulted in systemic hypotension demonstrating that sildenafil-induced vasodilation is not limited to the pulmonary circulation[13]. In a nitrofen-induced rat model of CDH, antenatal sildenafil administration improved lung structure, increased pulmonary vessel density, reduced right ventricular hypertrophy and improved postnatal NO-mediated pulmonary vasodilation [14]. In a rat model of BPD induced by antenatal lipopolysaccharide (LPS) and postnatal hyperoxia exposure, intraperitoneal sildenafil improved alveolarization, and increased vascular distribution in the lung tissue by acting through the hypoxia-inducible-factor (HIF) pathway[15]. Exposure to hyperoxia increases PDE5 expression and activity in pulmonary vasculature and reduces cGMP impairing pulmonary vasodilation [16, 17]. Hence, sildenafil

may be an effective agent during management of neonates with PPHN with prolonged exposure to hyperoxic ventilation.

Infants with PPHN. A randomized trial of oral sildenafil in term infants with severe PPHN without access to iNO demonstrated improved survival (6/7) compared to placebo (1/6)[18]. A pharmacokinetic trial involving 8 different dosing regimens showed that a loading dose of sildenafil − 0.4 mg/kg over 3 hours (0.14 mg/kg/h), followed by 0.07 mg/kg/h (or approximately 1.6 mg/kg/day) continuous infusion provided the best therapeutic levels and was effective in improving oxygenation (Figure 2)[19].

Infants with BPD. Small retrospective studies suggest that sildenafil decreases right ventricular pressures [20, 21] and potentially improves right ventricular function over time in infants with BPD-associated pulmonary hypertension. When compared to historic rates, mortality in this population may be decreased with sildenafil[22]. Sildenafil has not been shown to improve gas exchange in these infants however, and as a nonselective pulmonary vasodilator, it can lead to systemic hypotension and worse ventilation-perfusion mismatch[21]. A randomized trial to evaluate the efficacy of sildenafil in BPD-associated pulmonary hypertension is very much needed but may be difficult to conduct given the widespread off-label use and reasonable safety profile [23].

Infants with CDH. Sildenafil is used in many units for treatment of chronic pulmonary hypertension associated with CDH [24, 25]. In one center, 17% of CDH patients had been discharged on sildenafil therapy and slowly weaned after discharge. A trial evaluating chronic sildenafil for severe CDH was recently terminated due to the change in clinical practice (allowing routine administration of sildenafil beyond 6 weeks of age) incompatible with the possibility of placebo enrollment (NCT00133679 – clinicaltrials.gov).

Pharmacokinetics. In adults, sildenafil is 41% bioavailable (90% confidence interval - 36–47%) after oral administration[26] and has a half-life of 3.7 hours[27]. In neonates, the volume of distribution is 4 times higher and the clearance is slower resulting in a longer terminal half-life of 48 – 56 hours. Sildenafil clearance increases threefold in the first week of life and likely reflects maturation of the CYP mediated N-demethylation[28].

Glucocorticoids. Recent evidence derived from animal models of PPHN suggests a potential role for glucocorticoids in restoring normal pulmonary vascular function. In the lamb model of PPHN induced by ductal ligation, hydrocortisone significantly improved arterial-to-alveolar ratios and attenuated oxidative stress, in part by increasing SOD activity [29, 30]. Hydrocortisone increased cGMP by normalizing sGC and PDE 5 activity and by attenuating abnormalities induced by oxidative stress.

The glucocorticoids have potent anti-inflammatory properties and intravenous methylprednisolone (0.5 mg/kg/day in two divided doses) has been shown to reduce the duration of oxygen dependence in neonates with MAS [31]. There is anecdotal evidence that hydrocortisone improves oxygenation in neonates with PPHN, and it is used in some centers as a rescue strategy prior to ECMO. Generally favorable results from studies have indicated that glucocorticoids may be beneficial, particularly in severe MAS in the presence of lung edema, pulmonary vasoconstriction, and inflammation. Caution must be exercised when

considering hydrocortisone therapy, as it could mask the signs of infection. Hydrocortisone also stabilizes systemic blood pressure and reduces right-to-left shunting in PPHN.

Pulmonary vasodilators acting via the cAMP pathway

The arachidonic acid-prostacyclin pathway also plays an important role in pulmonary vascular transition at birth; prostaglandins activate adenylate cyclase (AC) to increase cAMP concentrations in vascular smooth muscle cells (Figure 1). Prostacyclin derivatives are the mainstay of pulmonary hypertension management in adults. In vascular smooth muscle, PDE 3A is an important enzyme that breaks down cAMP and this enzyme is inhibited by milrinone (Primacor®). Exposure to NO appears to enhance PDE 3A in animal studies[32, 33] and may explain the increased efficacy of milrinone in iNO-resistant PPHN[34–38].

Prostaglandins: Two classes of prostaglandin have therapeutic applications for treatment of PPHN: prostacyclin (Prostaglandin I_2, PGI_2) and prostaglandin E_1 (PGE_1). *Prostacyclin (PGI_2)* mediates vasodilation by activating adenylate cyclase and increasing cAMP in the pulmonary arterial smooth muscle cell (figure 1). In newborns, prostacyclin partly mediates pulmonary vasodilation at birth in response to ventilation of the lungs; it does not play a significant role in vasodilation in response to oxygenation [394041]. In a study of lambs with PPHN induced by antenatal ductal ligation, pulmonary prostacyclin synthase and PGI_2 receptor protein levels in the lung were decreased, but the adenylate cyclase levels were not altered[42].

PGI_2 analogs are the mainstay of pulmonary vasodilator therapy in adults and children with pulmonary arterial hypertension (PAH), and the intravenous route is the most studied. All PGI_2 analogs have the limitation of an extremely short half-life. Prostacyclins are currently approved in multiple forms and are listed below:

- **a.** Epoprostenol (intravenous)
- **b.** Treprostinil (oral, intravenous, subcutaneous)
- **c.** Iloprost (intravenous, inhaled)
- **d.** Beraprost (oral)

Epoprostenol (Flolan®, Glaxo-Wellcome, Middlesex, UK) is commonly used as a continuous intravenous infusion in the adult intensive care unit setting for pulmonary arterial hypertension [434445]. However, the associated systemic hypotension has limited intravenous use in infants with PPHN. In addition, epoprostenol has a short half-life of 6 minutes and needs to be administered continuously. To minimize the systemic effects, the localized delivery of epoprostenol through inhalation has been achieved by aerosolizing the intravenous formulation. Inhaled epoprostenol has reduced pulmonary hypertension and improved oxygenation in animal studies and clinical trials without decreasing systemic blood pressure[46–49].

The experience in infants with PPHN treated with inhaled epoprostenol thus far has been limited to case reports or small series. Inhaled epoprostenol improved oxygenation in infants with PPHN who had failed iNO therapy and also who had not received iNO. In one series,

four preterm infants with PPHN received epoprostenol as an endotracheal bolus or continuous endotracheal infusion [50]. Their OI improved significantly without systemic vascular compromise. Another series looked at 4 term neonates with hypoxic respiratory failure and PPHN that was refractory to I NO [51]. Inhaled epoprostenol rapidly improved oxygenation in all four neonates although one neonate with alveolar capillary dysplasia subsequently deteriorated. The authors suggest that neonates with PPHN and an inadequate response to NO may have impaired cGMP-mediated pulmonary vasodilation and might benefit from PGI_2, which acts through cAMP (figure 1). Inhaled epoprostenol is commonly nebulized at a dose of 50 ng/kg/min [51]. The intravenous formulation Flolan® is dissolved in 20 ml of manufacturer's diluent (a glycine buffer, pH −10). Fresh solution is added to the nebulization chamber every 4 hours [51]. The effect of such alkaline pH on neonatal respiratory tract is not known.

Treprostinil (Remodulin®, United Therapeutics) is a stable prostacyclin analog with a half-life of 3 hours in adults and can be administered by oral, subcutaneous or intravenous route. There is limited data on its use in infants [52].

Beraprost sodium (BPS, Dorner® 20μg tablet, Toray Industries Inc, Tokyo, Japan) is an oral formulation developed in Japan with a half-life of 35–40 min, and has been shown to improve pulmonary hypertension in adults [53, 54] and children with congenital heart disease [55, 56]. In a case series of 7 infants with PPHN refractory to alkali therapy and high frequency ventilation, beraprost reduced OI but also decreased systemic blood pressure by an average of 11 mm Hg by 6 hours[57]. In this study, infants received a 1μg/kg of beraprost every 6 hours. One quarter of the 20μg tablet was crushed and dissolved in 5 ml of sterile water (1μg/ml) and this suspension was administered to the infant via an orogastric tube. Further studies to evaluate the appropriate dose and minimize the risk of systemic hypotension are warranted.

Iloprost (Ventavis®, Actelion Pharmaceuticals, US) administered in intravenous or inhaled form, and alone or in combination with NO has resulted in improvements in PPHN in a number of reports [58–60]. In a study of 47 infants with PPHN, inhaled iloprost appeared to be more effective than sildenafil in time to, and duration of clinical response, and the iloprost group had less need for inotropic support[60]. In a study of 33 infants with severe PPHN, intravenous iloprost significantly reduced the OI, although the need for inotropic support was increased [59]. lloprost was administered by inhalation using a jet nebulizer at doses of 1 – 2.5 μg/kg with an interval of 2–4 hours between doses. In intubated patients, the jet nebulizer was adapted to the respiratory circuit with a T-connector. In the US, iloprost is available as 2.5 and 5 μg discs to be administered using an l-neb adaptive aerosol delivery device (AAD).

PGE_1 - The prostaglandin PGE_1, Alprostadil (Prostin VR Pediatric, Pharmacia and Upjohn Company) is widely used as a continuous intravenous infusion to maintain ductal arteriosus patency in newborns as a bridge to operative correction or palliation of cyanotic congenital heart lesions. In one retrospective analysis of infants with PPHN but without ductal dependent cardiac lesions, in whom PGE_1 was initiated during transport, PGE_1 treatment was associated with significantly shortened length of stay. The proposed mechanisms

include the pulmonary vasodilator effects of PGE_1 and the advantage ductal patency offers in providing a bypass or pop-off that permits improved function in the otherwise volume and pressure loaded right ventricle [61].

PGE_1 is also available in inhalation form. Aerosolized alprostadil has been used to treat pulmonary hypertension in adults as well as in experimental animal models. In infants with PPHN, a small pilot phase I-Il study demonstrated that inhaled PGE_1 was a safe, selective pulmonary vasodilator in hypoxemic respiratory failure [62, 63]. The commonly used dose in iNO-resistant PPHN is at 150–300 ng/kg/min diluted in saline to provide 4 ml/hour as a continuous nebulization [63].

Milrinone is a phosphodiesterase 3 (PDE3) inhibitor with inotropic and lusitropic (myocardial relaxation) effects that was approved in the 1980;s for intravenous use in decompensated congestive heart failure. Vascular PDE 3 breaks down cAMP in arterial smooth muscle cells and myocardium (figures 1 and 3). By inhibiting PDE 3, milrinone also functions as a vasodilator, independent of β1-adrenergic receptor stimulation and has been called an “inodilator” because of these dual effects [64–66]. Milrinone increases cAMP levels in cardiac muscle and vascular cells, improving ventricular function both directly and by reducing afterload. The common indications of milrinone in pulmonary hypertension include:

- **i.** Ventricular dysfunction – especially if associated with pulmonary venous hypertension or high left atrial pressures; these infants demonstrate a left to right shunt at PFO because of left ventricular dysfunction and high left atrial pressure in spite of a right to left shunt at PDA due to high pulmonary arterial pressures (this situation is a contraindication for iNO and is common in asphyxia, CDH and sepsis associated with PPHN – figure 3).
- **ii.** As an adjuvant to iNO to promote pulmonary vasodilation and provide synergy

There are no randomized trials evaluating milrinone in neonates with PPHN. The available evidence from animal models and pediatric trials and neonatal case reports is summarized below.

Animal models of PPHN: In the ovine model of PPHN induced by antenatal ductal ligation, milrinone relaxed pulmonary arterial rings in a dose dependent manner[42]. In the same model, an intravenous loading dose of 10 μg/kg followed by 1μg/kg/min for an hour reduced pulmonary vascular resistance from 0.503 ± 0.06 mmHg/mL/kg/min to 0.383 ± 0.03 mmHg/mL/kg/min ($p<0.05$) and was not associated with a significant decrease in systemic mean blood pressure (54 ± 3 to 51 ± 4mmHg)[67]. In lungs isolated from lambs with PPHN, protein levels of adenylate cyclase and PDE3A (the predominant PDE3 isoform in vascular tissue) were similar to control lambs without any change in PDE3A activity suggesting that the target enzymes for milrinone were unchanged by vascular remodeling in PPHN [42]. In addition, exposure to iNO and oxygen both in vivo[33] and at a cellular level[32] markedly increases PDE 3 expression and may potentially increase the efficacy of milrinone.

Clinical studies: The benefits of milrinone in children following surgery for congenital heart disease have been well established in several studies (including the randomized, double-

masked PRIMACORP study, n=238)[68–71]. In addition, anecdotal reports have shown that milrinone can be an effective therapeutic option in PPHN.[35, 72] These retrospective case reports from two hospitals in Ontario, Canada looked at 24 critically ill late preterm/term infants (except one infant at 26 weeks postmenstrual age PMA) with HRF or PPHN unresponsive to iNO. In the first report, Bassler et al reported 4 infants with PPHN. Some of the infants were "primed" with normal saline (15ml/kg) and administered a loading dose of 50 μg/kg over 30 min followed by 0.33 μg/kg/min. None of the infants developed systemic hypotension, and all of them showed consistent improvement in oxygenation. One of the infants was born at 26 weeks PMA and developed bilateral intraventricular hemorrhage (IVH) with moderate dilation of all ventricles. A term infant also developed IVH, and a third infant (39 weeks PMA) showed a small left subependymal hemorrhage.

In a subsequent study, McNamara et al reported 9 term infants with PPHN with poor response to iNO who received intravenous milrinone. Because of the potential risk of systemic hypotension, a loading dose was avoided in these patients with PPHN. The infusion was started at 0.33 μg/kg/min and increased in increments of 0.33 according to clinical response to a maximum of 0.99 μg/kg/min. There was a significant improvement in oxygenation after commencement of milrinone, particularly in the first 24 h of infusion and there was no systemic hypotension. The same authors performed pharmacokinetic studies in 11 late preterm and term infants with PPHN resistant to iNO with a loading dose of 50 μg/kg over 60 min followed by an infusion of 0.33 to 0.99 μg/kg/min and demonstrated an improvement in oxygenation and cardiac output and a reduction in pulmonary arterial pressure by echocardiography without any IVH [73].

Preterm infants: Recently, James et al have described 7 preterm infants with PPHN resistant to iNO and treated with milrinone infusion [37]. An echocardiogram was obtained one hour (median) prior to milrinone infusion. Milrinone infusion increased left ventricular output, right ventricular output and reduced right ventricular pressure. These echocardiographic changes were associated with a reduction in iNO dose and oxygen requirement over the subsequent 72 hours [37].

Infants with CDH: Patel recently reported improved right ventricular diastolic function and oxygenation in 6 neonates with CDH following milrinone infusion [74]. All of them were treated with iNO or intravenous sildenafil or both. Four of these patients had undergone surgical repair prior to initiation of milrinone. Oxygenation index decreased from 10.6 ± 5.6 to 7.9 ± 6.2 by 12–24 hours and to 5.1 ± 2.6 by 48–72 hours after commencement of milrinone infusion [75]. Milrinone is commonly used during the management of CDH without any randomized trials conducted to show benefit. Thirty percent of infants with CDH in the Children's Hospital Neonatal Database (CHND)[76] and 22% of late-preterm and term infants with CDH in the Pediatrix database [77] received milrinone. Milrinone appears to be an effective therapeutic option in infants with PPHN resistant to iNO and/or in the presence of cardiac dysfunction. Randomized trials evaluating its use in PPHN are warranted[75].

Pharmacokinetics. The half-life, total body clearance, volume of distribution and steady state concentration of milrinone in neonates with PPHN are shown in table 1. Similar values were obtained from 48 neonatal, post-op cardiac patients in the PRIMACORP study (table

1) which also showed that the clearance in neonates is only 25% of that in children [68]. With a constant-rate infusion, neonates take a much longer time to achieve steady-state concentration. Without a loading dose, neonates reached 50% of steady-state concentration by 2 hours (compared to 45 minutes in older children). However, for the same infusion rate, the steady-state concentration will be higher in neonates than older patients because of decreased renal function. These findings underscore the necessity of a loading dose for rapid achievement of a therapeutic blood concentration in neonates, but lower infusion rate to avoid higher levels secondary to poor clearance [68]. Milrinone pharmacokinetics have also been evaluated in preterm infants[78, 79], infants following cardiac surgery [71, 80] and more recently in neonates with iNO resistant PPHN [73] (table 1).

Pulmonary vasodilators acting via the Endothelin pathway

Endothelin-1 (ET-1) synthesized by vascular endothelial cells is a potent vasoconstrictor[81] and acts through two receptors; ET_A and ET_B (figure 1). The ET_A receptor plays a critical role in vasoconstriction while the ET_B receptor promotes vasodilation[8283] mediated by endothelium-derived nitric oxide [84, 85]. Selective blockade of the ET_A receptor causes fetal pulmonary vasodilation[86]. ET-1 gene expression and levels are increased[87] in the lungs and pulmonary arterial endothelial cells in the fetal lamb model of PPHN [87,88] while ET_B protein is decreased in pulmonary artery endothelial cells isolated from lambs with PPHN induced by antenatal ductal ligation[88]. This model of PPHN is associated with significant remodeling of the pulmonary artery with smooth muscle hypertrophy and muscularization of small, distal pulmonary arteries [89] similar to that described in infants with PPHN and was used in preclinical studies for iNO [90].

Chronic intrauterine ET_A receptor blockade following ductal ligation decreases pulmonary arterial pressure and distal muscularization of small pulmonary arteries *in utero*, decreases right ventricular hypertrophy, and increases the fall in PVR at delivery in newborn lambs with PPHN[91]. Elevated plasma ET-1 levels are observed in infants with CDH and PPHN and maybe a marker of disease severity and poor prognosis [92, 93]

Bosentan (Tracleer™ tablets, Actelion Pharmaceuticals) is an ET-1 antagonist acting at both endothelin A and B receptors (ET-A and ET-B, figure 1). PPHN has been shown to be associated with increased ET-A mediated vasoconstriction and loss of ET-B mediated vasorelaxation in a newborn lamb model of PPHN [86, 91, 94]. Elevated plasma levels of ET-1 has been documented in newborn infants with PPHN [92, 93].

Bosentan has proven efficacy in adults with pulmonary hypertension [95] and is commonly used in chronic therapy of primary pulmonary hypertension and thromboembolic PAH. In neonates, studies of bosentan in PPHN is limited mostly to case reports and two small randomized controlled trials. Successful use of bosentan has also been reported in pulmonary hypertension associated with congenital heart disease in newborn infants. Mohamed et al in a prospective, randomized controlled trial in newborn infants with PPHN showed both improved short-term outcomes and longer term outcomes at 6 months in infants who received bosentan as compared to placebo [96]. A study by Steinhom et al with bosentan as adjuvant therapy in patients receiving inhaled nitric oxide for PPHN did not

show an additive effect[97]. Bosentan is well absorbed following enteral administration (~98% in adults), is metabolized in the liver and is eliminated by biliary excretion. The dose of bosentan used in newborns is 1–2 mg/kg twice daily (usually prepared from 32 mg dispersible tablets).

Bosentan is available only in oral formulation and this limits its use in the acute stage of PPHN in the immediate newborn period. However it may be used as chronic therapy in infants with pulmonary hypertension associated with BPD or CDH. It may also have a role in the management of PPHN in resource-poor settings without access to inhaled nitric oxide.

Adverse effects associated with bosentan therapy include elevation of transaminases and liver failure. Hence liver function should be checked prior to onset of therapy and during treatment with bosentan. As per manufacturer recommendations bosentan should not be initiated in patients with transaminase levels more than 3 times the upper limits of normal and must be discontinued with elevation of bilirubin more than twice the upper limit of normal. Other reported side effects include angioedema, anemia, leukopenia and thrombocytopenia.

Bosentan is not currently approved for treatment of pulmonary arterial hypertension in children or for PPHN. Bosentan is currently available only through a restricted distribution program and requires physician certification and enrollment in the program. Other endothelin receptor antagonists including the selective ET-A blockers have not been carefully evaluated in PPHN.

Combination Therapy

The combination of pharmacologic agents with different mechanisms of action in treatment of PPHN may offer the benefit of an additive effect or may induce the same effect at lower doses of each agent. For example, inhaled epoprostenol may augment the response to NO in infants because its mechanism of action is different from and complementary to that of NO. When combined with NO, it also may prevent the rebound hypertension seen with NO weaning.

A number of clinical studies in adults have examined the effect of combining prostacyclins (epoprostenol, treprostinil, iloprost) with the phosphodiesterase inhibitor, sildenafil or endothelin receptor antagonist, bosentan. These have shown promising results with improvement in hemodynamic parameters and exercise tolerance [98]. In a case report of a preterm infant following repair of CDH with right ventricular failure and severe PPHN, the combination of PGE_1 infusion and oral sildenafil normalized right ventricular pressure and function[99].

Emerging targets and therapies

Emerging targets and therapies currently under investigation for PPHN are shown in Fig 4.

L-citrulline: With increased understanding of the pathobiology of pulmonary vascular disease in infants, and specifically the role of NO and prostaglandin signaling has revealed

novel pharmacologic approaches to treat PPHN. Fike et al have demonstrated an impaired L-citrulline–L-arginine–nitric oxide pathway is involved in the pathogenesis of hypoxia-induced pulmonary hypertension[100]. Hypoxia reduces NO production in pulmonary arterial endothelial cells by uncoupling endothelial nitric oxide synthase, which is responsible for synthesizing NO from L-arginine. It is interesting that although L-arginine supplementation can improve NO signaling, oral citrulline is more effective than arginine in increasing serum L-arginine concentrations with fewer side effects. Rescue therapy with L-citrulline has been shown to ameliorate hypoxia-induced-pulmonary hypertension in newborn piglets[101]. In adult patients with idiopathic pulmonary hypertension or Eisenmenger syndrome, oral L-citrulline malate reduced pulmonary arterial pressure and improved six minute walking distance[102]. To date, no clinical trials of L-citrulline have been published in the neonatal population.

Soluble guanylate cyclase stimulators and activators: The target enzyme for iNO is sGC (figure 1 and 3). However, resistance to NO and tolerance may limit cGMP production in pulmonary arterial smooth muscle cell and limit vasodilation. Such circumstances have led to development of heme-dependent sGC stimulators and heme-independent sGC activators. Riociguat (Adempas®) is a stimulator and was approved by FDA in 2013 for the treatment of adults with chronic thromboembolic pulmonary hypertension (CTEPH) and some forms of pulmonary arterial hypertension (PAH). A related agent, cinaciguat is a sGC activator and has been shown to be effective as a pulmonary vasodilator in a lamb model of PPHN induced by ligation of the ductus arteriosus[103, 104]. The effectiveness of NO donors to increase cGMP is maximal in 21% oxygen in the absence of oxidative stress. Cinaciguat increased cGMP production in pulmonary artery smooth muscle cells from control and PPHN lambs even in the presence of oxidative stress induced by exposure to 50% oxygen or 1H-[1,2,4] oxadiazolo[4,3-a] quinoxalin-1-one (ODQ), an sGC oxidizer or hydrogen peroxide. In the presence of oxidized sGC, unlike NO donors, cinaciguat may still be effective in increasing cGMP production and vasodilation. Thus, cinaciguat may provide a novel treatment option for severe PPHN treated with prolonged hyperoxia.

Rho-kinase inhibitors: Vascular smooth muscle contraction is regulated by cytosolic Ca^{2+} levels $[Ca^{2+}]_i$. With an elevation of $[Ca^{2+}]_l$, formation of the calcium-calmodulin (CaM) complex increases, and myosin light-chain kinase (MLCK) is activated. MLCK phosphorylates the myosin light chain (MLC), enhancing cross-bridge cycling and inducing vascular smooth muscle contraction[105]. A small GTP-binding protein RhoA increases Rho-kinase (ROCK) activity leading to phosphorylation of MLC and vascular contraction. The RhoA-Rho-kinase pathway “sensitizes” the contractile proteins to $[Ca^{2+}]_l$, and plays an important role in hypoxic pulmonary vasoconstriction[106]. RhoA-Rho-kinase is an important mediator of elevated myogenic tone contributing to high PVR in fetal lambs[107].

PPHN induced by partial constriction of the ductus arteriosus in fetal lambs is associated with increased ROCK activity in pulmonary arterial endothelial cells and contributes to impaired angiogenesis[108]. In a fetal sheep model, ROCK inhibition increased left pulmonary artery blood flow and decreased PVR[107]. ROCK inhibitors such as fasudil and Y27632 may have therapeutic potential in infants with PPHN

PPAR γ agonists: Both ROCK and peroxisome proliferator-activated receptor-γ (PPARγ) regulate smooth muscle cell proliferation and contribute to vascular remodeling in pulmonary hypertension. PPAR-γ is an essential regulator of pulmonary arterial smooth muscle cell proliferation and vascular tone[109]. PPARγ agonists produce vasodilation through inhibition of ROCK and there may be a potential role for PPARγ agonists in the management of PPHN.

Antioxidants: Oxidant stress appears to play an important role in pulmonary hypertension. The use of intratracheal recombinant human superoxide dismutase (rhSOD) induces pulmonary vasodilation, improves oxygenation and decreases oxidative stress in lambs with PPHN[110].

Conclusion

Unavailability of iNO in developing countries coupled with its high cost and lack of efficacy in almost a third of patients with PPHN has triggered research evaluating newer pharmacologic approaches to PPHN. In addition, two remaining challenges where large knowledge gaps persist include management of pulmonary hypoplasia and pulmonary hypertension in CDH and BPD-associated pulmonary hypertension in the premature infant. Newer pulmonary vasodilators outlined in this chapter are currently under investigation. Further research to develop appropriate cost-effective strategies to ameliorate pulmonary vascular disease associated with conditions such as pneumonia and asphyxia and meconium aspiration, common in developing countries is warranted. Lack of equipoise among clinicians, ethical considerations (such as providing placebo to a preterm infant with BPD and pulmonary hypertension) and rapid progression of disease and clinical deterioration following failure of iNO in acute PPHN (limiting time for consent and randomization) have impaired our ability to conduct randomized control trials to evaluate pharmacological therapies outlined in this manuscript leading to significant knowledge gaps in PPHN management.

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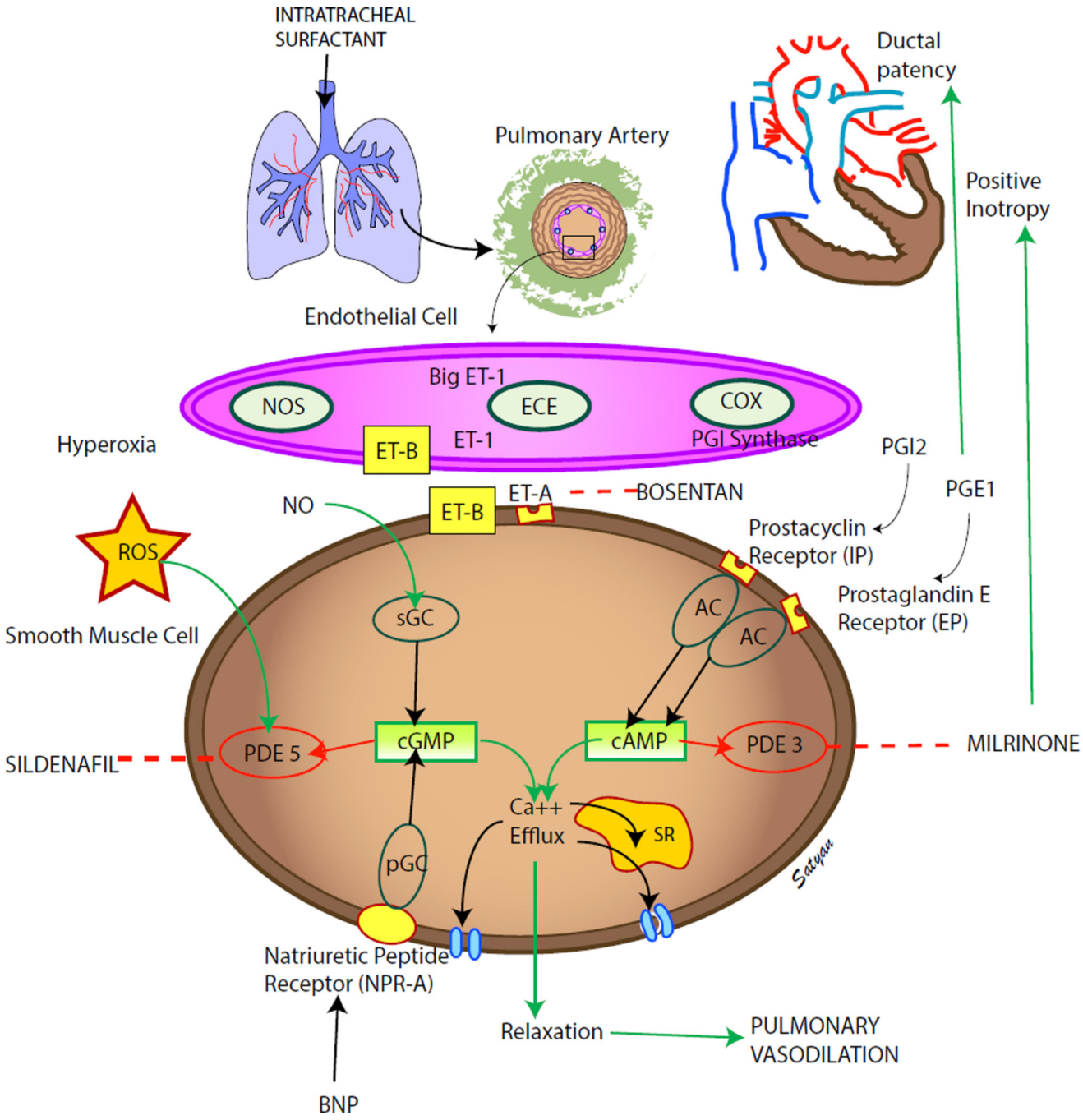

Figure 1. Endothelium derived mediators: vasodilators – prostacyclin (PGI_2) and nitric oxide (NO) and vasoconstrictor (endothelin, ET-1)

Cyclooxygenase (COX) and prostacyclin synthase (PGIS) are involved in the production of prostacyclin. Prostacyclin acts on its receptor (IP) in the smooth muscle cell and stimulates adenylate cyclase (AC) to produce cyclic adenosine monophosphate (cAMP). Cyclic AMP is broken down by phosphodiesterase 3A (PDE 3A, the enzyme most prevalent in vasculature) in the smooth muscle cell. Milrinone inhibits PDE 3A and increases cAMP levels in arterial smooth muscle cells and cardiac myocytes resulting in pulmonary (and systemic) vasodilation and inotropy. Nitric oxide (NO) stimulates PDE 3A. Endothelin is a

powerful vasoconstrictor and acts on ET-A receptors in the smooth muscle cell and increases ionic calcium concentration. A second endothelin receptor (ET-B) on the endothelial cell stimulates NO release and vasodilation. Endothelial nitric oxide synthase (eNOS) produces NO which diffuses from the endothelium to the smooth muscle cell and stimulates soluble guanylate cyclase (sGC) enzyme to produce cyclic guanosine monophosphate (cGMP). Cyclic GMP is broken down by PDE5 enzyme in the smooth muscle cell. Sildenafil inhibits PDE5 and increases cGMP levels in pulmonary arterial smooth muscle cells. Cyclic AMP and cGMP reduce cytosolic ionic calcium concentrations and induce smooth muscle cell relaxation and pulmonary vasodilation. Nitric oxide is a free radical and can avidly combine with superoxide anions to form a toxic vasoconstrictor, peroxynitrite. Hence, the bioavailability of NO in a tissue is determined by the local concentration of superoxide anions. Hyperoxic ventilation with 100% oxygen can increase the risk of formation of superoxide anions in the pulmonary arterial smooth muscle cells and limit the bioavailability of NO and stimulate PDE5 activity. Medications used in PPHN are shown in black boxes (*modified from ref*[114] *copyright Satyan Lakshminrusimha*).

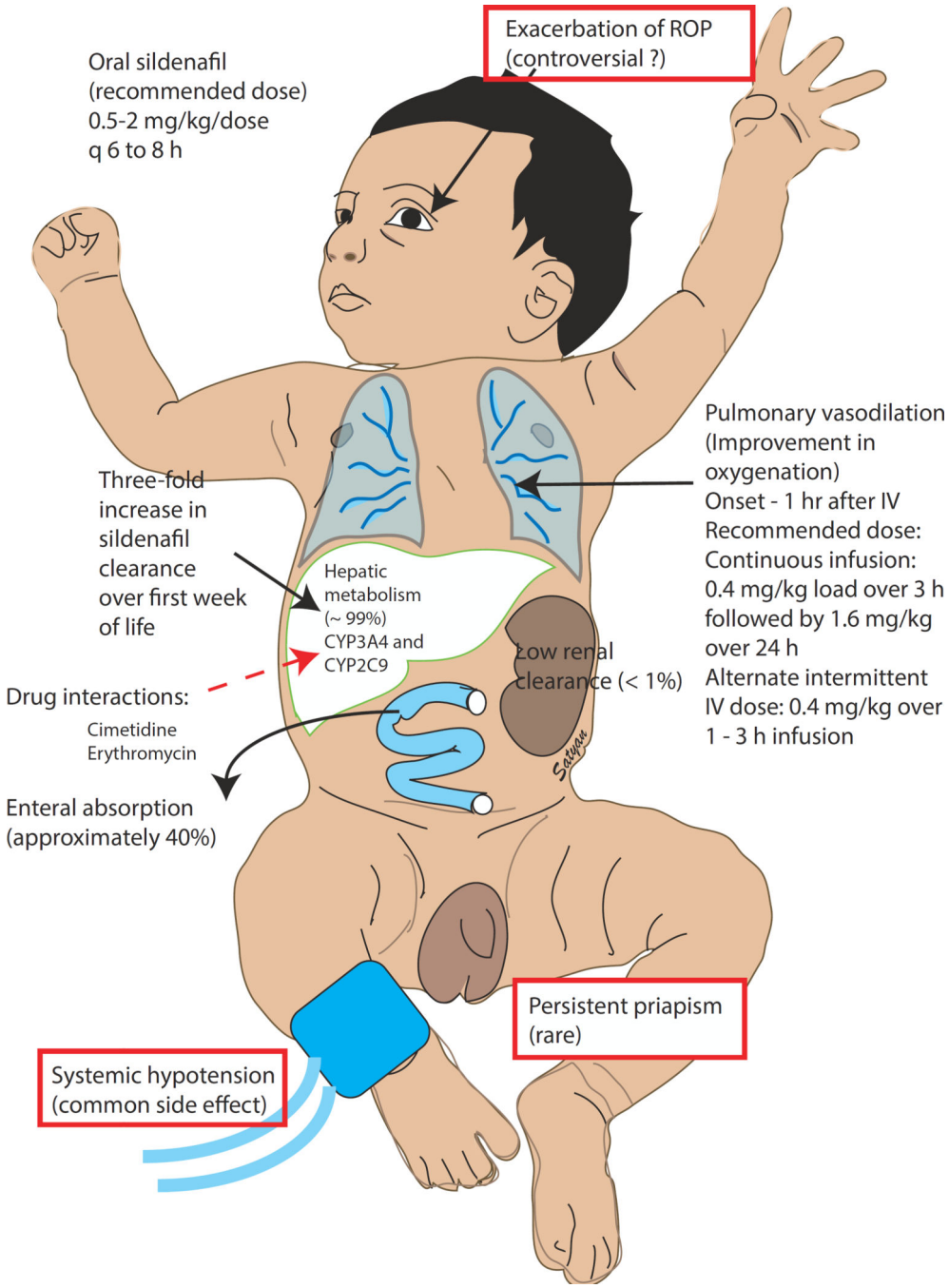

Figure 2. Sildenafil use in PPHN

Sildenafil can be administered orally or by intravenous route. In adult volunteers approximately 40% of orally administered sildenafil is bioavailable. The recommended doses are shown in the figure. Sildenafil is predominantly metabolized in the liver through the CYP pathway (and its metabolism can be inhibited by cimetidine and erythromycin). The metabolism of sildenafil is low in a newly born infant due to hepatic immaturity but increases rapidly over the first week of life in term infants. The most common side-effect is systemic hypotension although priapism has been rarely reported. Deterioration of

preexisting retinopathy of prematurity (ROP) has been described but not confirmed by other studies (these side effects are shown in boxes) (*copyright Satyan Lakshminrusimha*).

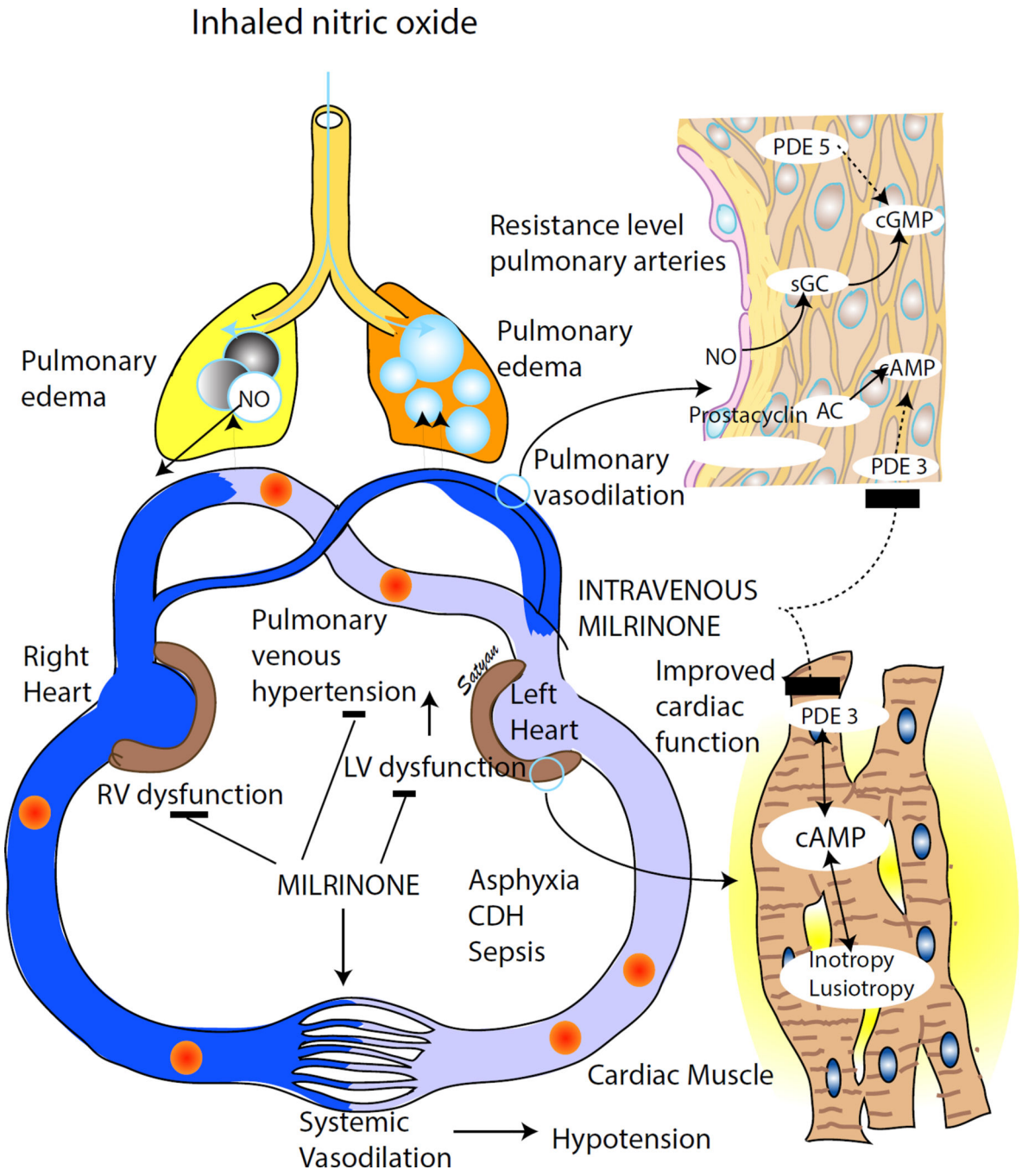

Figure 3. Milrinone in PPHN

Pulmonary hypertension with ventricular dysfunction is common in neonates with asphyxia, congenital diaphragmatic hernia (CDH) and sepsis. Left ventricular dysfunction leads to pulmonary venous hypertension and pulmonary edema. Administration of inhaled NO in the presence of pulmonary venous hypertension can worsen pulmonary edema and deterioration of oxygenation. Milrinone improves left ventricular function through its cAMP mediated lusitropic (augment cardiac relaxation) and inotropic (enhance cardiac contraction) effect and decreases pulmonary venous hypertension. In addition, milrinone causes pulmonary and

systemic vasodilation and reduces afterload on both ventricles, (*copyright Satyan Lakshminrusimha*).

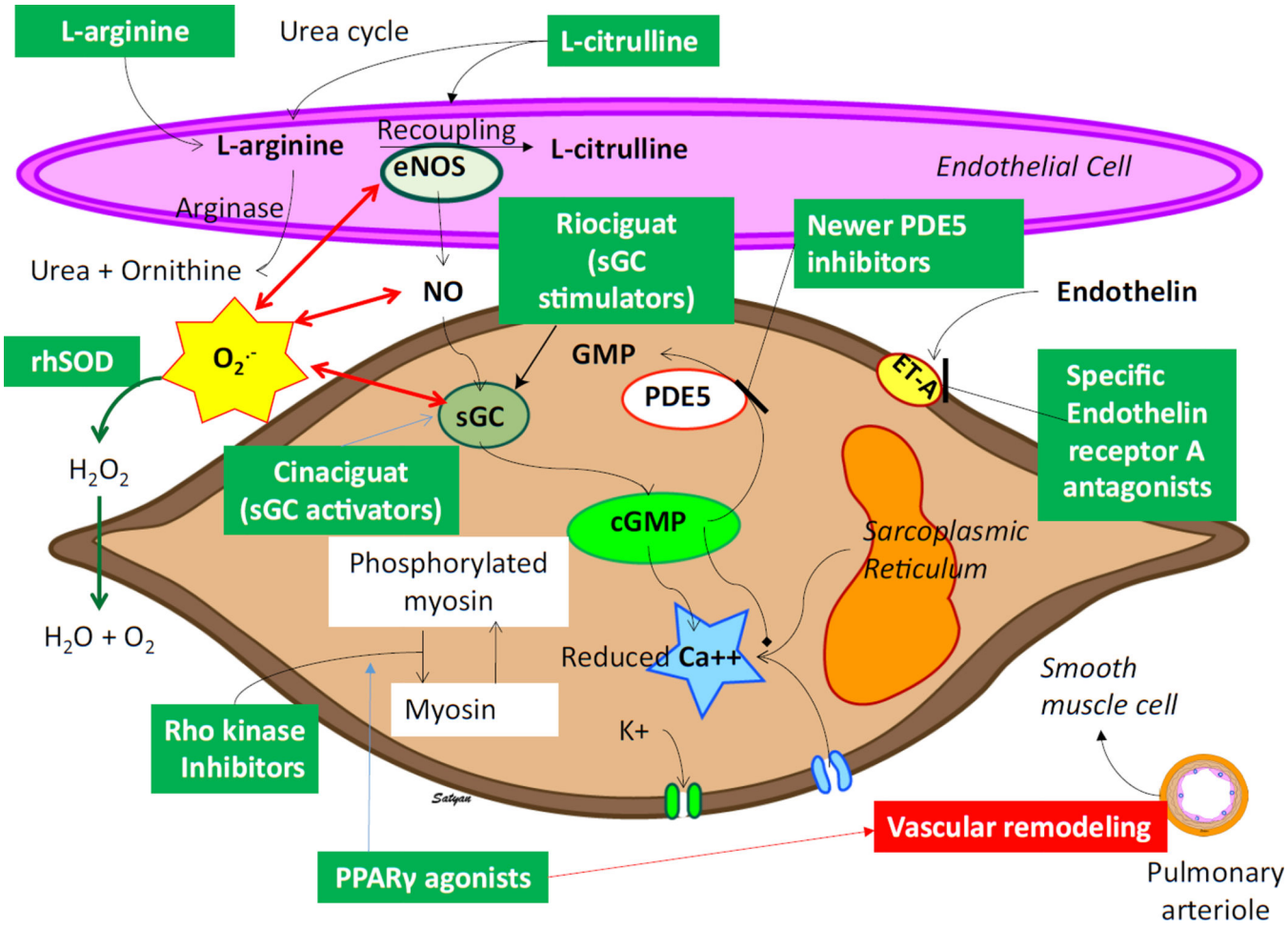

Figure 4. Emerging targets and therapies for PPHN

L-citrulline, soluble guanylate cyclase (sGC) stimulators and activators, Rho-kinase inhibitors, PPAR γ agonists, antioxidants, newer phosphodiesterase (PDE) 5 inhibitors (e.g., tadalafil) and specific endothelin receptor – A antagonists (Sitaxsentan and Ambrisentan) are potential new therapies in PPHN that need further evaluation and clinical studies (see text for details). *Copyright Satyan Lakshminrusimha*

Table 1

Pharmacokinetic data on milrinone in adults, children, term and preterm neonates

Study	Adult[111]	Child [112]	Neonate (post-op CHD)[68]	Neonate (PPHN)[113]	Preterm neonate[79]
Gestational age/age	Healthy adult volunteers	Pediatric patients	Neonates	39.2 ±1.3 weeks 14h (10–30h)	26 weeks (23-28)
Weight (kg)		5.9 ±4*		3.481 ± 0.603	0.85 (0.52-1.26)
Half-life (h)	0.8 ± 0.22	3.7		4.1 ±1.1	10.3
Total body clearance (mL/kg/min)	6.1 ±1.3	2.5 to 10.6 (increases with age)	1.64 ±0.37	1.83 ±0.17	0.64
Volume of distribution (L/kg)	0.32 ± 0.08	0.7–0.9	0.523 ± 0.028	0.56 ±0.19	0.576
Steady-state concentration (ng/mL)				290.9 ±77.7	195 (78–257) at 21h after infusion
Dose regimen	12.5 to 75 μg/kg load followed by 0.5 μg/kg/min	25 to 75 μg/kg load followed by 0.25 to 0.75 μg /kg/min infusion	25 μg /kg over 60 min followed by a 0.25 μg/kg/min (low dose) or 75 mcg/kg load and 0.75 μg/kg/min (high dose)	50 mcg/kg load over 1h followed by 0.33 (to 0.99) μg /kg/min	0.75 mcg/kg/min for 3h followed by 0.2 μg/kg/min

* This weight is an approximate value - based on high dose arm in PRIMACORP study