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REVIEW ARTICLE

Persistent pulmonary hypertension of the newborn

Ru-Jeng Teng*, **Tzong-Jin Wu**

Division of Neonatology, Department of Pediatrics, Medical College of Wisconsin, Wauwatosa, WI, USA

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Persistent pulmonary hypertension of the newborn (PPHN) is a severe pulmonary disorder which occurs at a rate of one in every 500 live births. About 10–50% of the victims will die of the problem and 7–20% of the survivors develop long-term impairments such as hearing deficit, chronic lung disease, and intracranial bleed. Most adult survivors show evidence of augmented pulmonary vasoreactivity, suggesting a phenotypical change. Several animal models have been used to study the pathophysiology and help to develop new therapeutic modality for PPHN. The etiology of PPHN can be classified into three groups: (1) abnormally constricted pulmonary vasculature as a result of parenchymal diseases; (2) hypoplastic pulmonary vasculature; and (3) normal parenchyma with remodeled pulmonary vasculature. Impaired vasorelaxation of pulmonary artery and reduced blood vessel density in lungs are two characteristic findings in PPHN. Medical treatment includes sedation, oxygen, mechanical ventilation, vasorelaxants (inhaled nitric oxide, inhaled or intravenous prostacyclin, intravenous prostaglandin E1, magnesium sulfate), and inotropic agents. Phosphodiesterase inhibitors have recently been studied as another therapeutic agent for PPHN. Endothelin-1 (ET-1) inhibitors have been studied in animals and a case of premature infant with PPHN successfully treated with an ET-I inhibitor has been reported in the literature. Surfactants have been reported as an adjunct treatment for PPHN as a complication of meconium aspiration syndrome. Even with the introduction of several new therapeutic modalities there has been no significant change in survival rate. Extracorporeal membrane oxygenator is used when medical treatment fails and the patient is considered to have a recoverable cause of PPHN.

Introduction

Persistent pulmonary hypertension of the newborn (PPHN) is a frequent cause of hypoxemic respiratory failure in term and late preterm infants affecting 0.43–6.8 per 1000 live

* Corresponding author. Division of Neonatology, Department of Pediatrics, Medical College of Wisconsin, C410, 999 North 92nd Street, Wauwatosa, WI 53226, USA.
E-mail address: rteng@mcw.edu (R.-J. Teng).

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births.[1] Severe hypoxemia usually develops directly after birth, but occasionally can be a consequence of other diseases, such as severe respiratory distress syndrome, or secondary to the management of other perinatal disorders, such as body cooling for hypoxic–ischemic encephalopathy and slowly develops several hours or even days of birth. With the introduction of inhaled nitric oxide (INO) the management of PPHN is easier than it was a decade ago, but the mortality rate remains unchanged and a high percentage of PPHN survivors carry long-term sequelae including chronic oxygen dependence, stroke or impaired hearing. In this review we describe the physiology of the perinatal transition, some commonly used animal models that help us to understand the pathophysiology of PPHN, etiology and risk factors for PPHN, and recent strategies in PPHN management.

Animal models of PPHN

Several animal models have been used to elucidate the pathophysiology of PPHN including intra-uterine ductus arteriosus ligation of fetal lamb or piglet, acute or chronic hypoxic pulmonary hypertension of newborn animals,[2,3] intratracheal meconium instillation,[4] creation of aortopulmonary shunt,[5] and intra-uterine nonsteroidal anti-inflammatory drug (NSAID) or selective serotonin re-uptake inhibitor (SSRI) exposure.[6] Each model has its pros and cons and readers should refer to each published model for details. Most animal models show smooth muscle layer thickening of the pulmonary arteries and thickened right ventricular wall. Other described findings include decreased blood vessel density of the lungs, impaired endothelial nitric oxide synthase (eNOS) activity, increased endothelin-1 (ET-1) formation, increased reactive oxygen species (ROS) formation,[7] and impaired alveolar formation. The increased ROS formation can stimulate the proliferation of smooth muscle cells[8] and increases activity of phosphodiesterases (PDE) that enhance the hydrolysis of cyclic guanosine monophosphate (cGMP).

Physiology of perinatal transition

Many structural and functional changes occur during fetal lung development to prepare the lung for the transition to air breathing. The development of pulmonary vasculature is genetically controlled and pulmonary vessels acquire increased vasoreactivity with advancing gestation. An extensive review was recently published and readers who are interested in this topic are encouraged to read this article.[9] Before birth, the lungs are filled with fluid and the pulmonary artery resistance is very high, because of low oxygen tension in the alveoli, and most of the blood returning to the right atrium goes through the foramen ovale into the left atrium. Blood entering the right ventricle also shunts through the ductus arteriosus into the aorta leaving only a small amount of blood flowing into the lungs. The first breath after birth allows air to enter into the alveoli with a dramatic reduction in pulmonary artery resistance secondary to increased oxygen tension. The sudden increase of oxygen tension from 20 torr to 150 torr (0.15 to 1.125 Pascal) increases mitochondrial oxidative phosphorylation and adenosine triphosphate (ATP) production. The surge in blood ATP levels during the postnatal transition can stimulate eNOS function with production of NO and pulmonary vasorelaxation.[10]

Several vasoactive substances are known to modulate the vasomotor tone of the pulmonary artery. Endothelin-1 (ET-1), nitric oxide (NO) and prostacyclin (PGI2) are the most extensively studied in PPHN. Thromboxane A_2 is another product of the cyclo-oxygenase and thromboxane synthase system that plays some role mainly during infections associated with PPHN. Voltage-gated potassium (Kv) channels which modulate vascular smooth muscle contraction also play an important role in PPHN.[11] ET-1 is a vasoconstrictor to the pulmonary arteries and enhances O_2^- formation that depletes NO bioavailability and promotes the growth of the pulmonary artery muscular layer. When pulmonary artery resistance fails to decrease during the perinatal transition the deoxygenated blood shunts from right to left through either the foramen ovale (no differential cyanosis) or ductus arteriosus (with differential cyanosis) with PPHN. Most venous return from the inferior vena cava goes through the foramen ovale whereas most blood returning from the superior vena cava tends to go into the main pulmonary trunk (Fig. 1).

Endothelial NOS, or nitric oxide synthase type 3 (NOS3) is the most extensively studied enzyme in PPHN. Unstimulated eNOS is believed to bind to caveolin-1.[12] When activated by shear stress or ATP, eNOS departs caveolin and become phosphorylated to form a dimer. The eNOS dimer then associates with heat shock protein 90 (hsp90) in the presence of tetrahydrobiopterin (BH4), calcium, flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), iron and L-arginine to be "coupled" to convert L-arginine into NO and L-citrullin (Fig. 2). BH4 depletion and hypocalcemia can both uncouple eNOS and lead to O_2^- formation. Hypoglycemia leads to low FAD or FMN levels in endothelial cells which may also uncouple eNOS. L-Arginine is not only the substrate for eNOS: its presence can also help eNOS coupling. If arginase-II activity is increased, L-arginine is converted into asymmetric dimethylarginine (ADMA) which inhibits eNOS function.

Etiology of PPHN

The etiology of PPHN can be classified into three main categories (Table 1). The most common is PPHN secondary to parenchymal diseases, including meconium aspiration syndrome (MAS), severe respiratory distress syndrome, RDS and pneumonia. This is mainly caused by poor oxygen entry into the alveolar space, especially in MAS with obstruction in the airways. Inadequate blood vessel density with decreased total cross-section of pulmonary vasculature and increased pulmonary vascular resistance is the cause of PPHN in congenital diaphragmatic hernia. The least common etiology is normal parenchyma with remodeled pulmonary vasculature, such as idiopathic PPHN, congenital heart disease, and chronic intrauterine hypoxia. Some congenital heart diseases are associated with obstructed pulmonary venous return which can lead to secondary increased pulmonary artery resistance. Hypoxic–ischemic encephalopathy caused by chronic intra-uterine hypoxia may remodel the

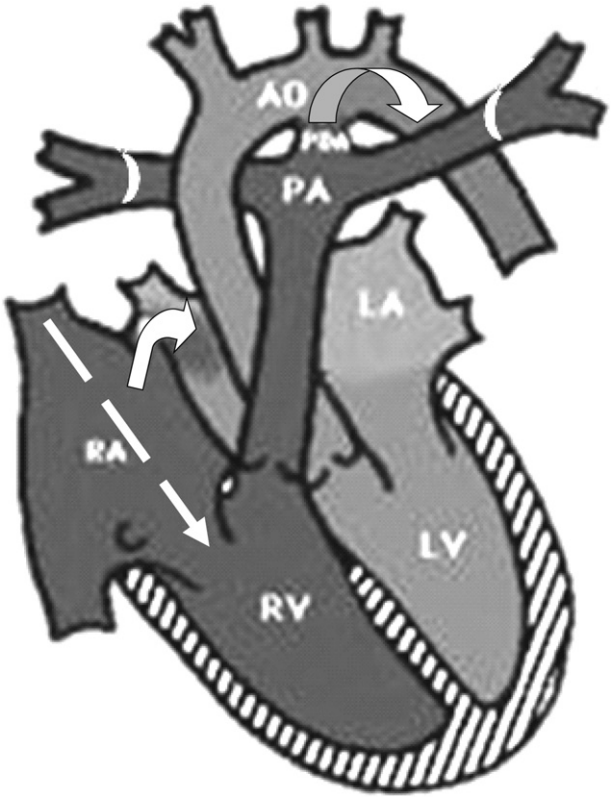

Figure 1 Blood flow pattern of persistent pulmonary hypertension of the newborn (PPHN). Two potential right-to-left shunts, either through the foramen ovale or ductus arteriosus, can be observed in PPHN. There is a differential cyanosis if the right-to-left shunt is through the ductus. Venous blood from the superior vena cava preferentially flows through the right ventricle into the main pulmonary artery so an intravenous vasorelaxant should be given via an intravenous catheter placed in the upper part of the body. Most venous blood from the inferior vena cava flows directly through the foramen ovale when PPHN is present so inotropic agents should be given through a catheter placed in the lower part of the body.

pulmonary vasculature with either eNOS uncoupling or increased ET-1 production that increases pulmonary vascular resistance. Idiopathic PPHN is the rarest cause of PPHN, usually with normal chest X-ray findings. There are some metabolic, or genetic, disorders that can present with PPHN. Pearson et al reported heterozygote T1405N genotype for carbamoyl-phosphate synthetase, an enzyme that determines the blood levels of arginine and citrulline, and is associated with PPHN possible because of a lack of substrate for eNOS.[13] Epidemiologic study demonstrated that Black and Asian maternal race is associated with a significant higher risk for PPHN. Male gender also leads to higher incidence of PPHN.[14] Although it is hard to decipher the observations, the evidence suggests some genetic predisposition to PPHN and deserves further exploration.

Pathology of PPHN

Decreased vascular density and a thickened smooth muscle layer of the pulmonary artery are the two most common pathological findings in PPHN. Morphometric analysis of PPHN lungs reveals extension of muscle into small pulmonary arteries: all alveolar duct and wall arteries ($<$30 μm external diameter), normally nonmuscular, are fully musularized. The medial wall thickness of the normally muscular intra-acinar arteries is doubled; arterial size and number, however, are normal in all.[15] A decreased number of alveoli is seen in lung hypoplasia and congenital diaphragmatic hernia.

Symptoms and signs

In developing countries post-term infants and intrauterine growth restriction (IUGR) are the major groups to have PPHN, so dry and peeled skin is commonly seen. Meconium passage or lack of subcutaneous fat tissue is commonly observed in IUGR and post-term neonates. General cyanosis is the typical presentation of PPHN, but sometimes we can observe so-called differential cyanosis with preductal skin less cyanotic than the postductal skin unless transposition of the great arteries (TGA) is also present. When PPHN is associated with TGA then reversed differential cyanosis can be seen. The routine hyperoxia challenge usually cannot help the diagnosis, because severe PPHN can behave like typical cyanotic congenital heart disease and does not respond to high oxygen challenge.

Diagnosis and evaluation

Echocardiography is the most convenient and reliable method for establishing the diagnosis. Poor myocardial contractility, poor movement of the interventricular septum, deviation of the interatrial septum to the left, turbulent flow for tricuspid regurgitation, or shunt through the ductus arteriosus can be used to evaluate the cause and severity of the PPHN. Pulmonary arterial accelerating time, and maximal velocity of the tricuspid regurgitation can be used to estimate the pulmonary artery pressure. Major complex congenital heat disease has to be ruled out by echocardiography prior to extracorporeal membrane oxygenator (ECMO) treatment. Infusion of normal saline through a venous line placed below the diaphragm which creates a microbubble picture can be used to demonstrate the interatrial right-to-left shunt, either through a patent foramen of ovales or a patent ductus arteriosus, as supportive evidence for PPHN. Very rarely PPHN can be the consequence of obstructive type total anomalous pulmonary venous return, which does not respond to usual PPHN treatment, and this can only be ruled out with echocardiography. Echocardiography is also a mandatory study before initiation of ECMO because it is necessary to rule out possible lethal cyanotic congenital heart disease that cannot be corrected by heart surgery.

Risk factors

Some risk factors for PPHN have been reported in the literature. However, the true mechanism remains obscure for most of them.

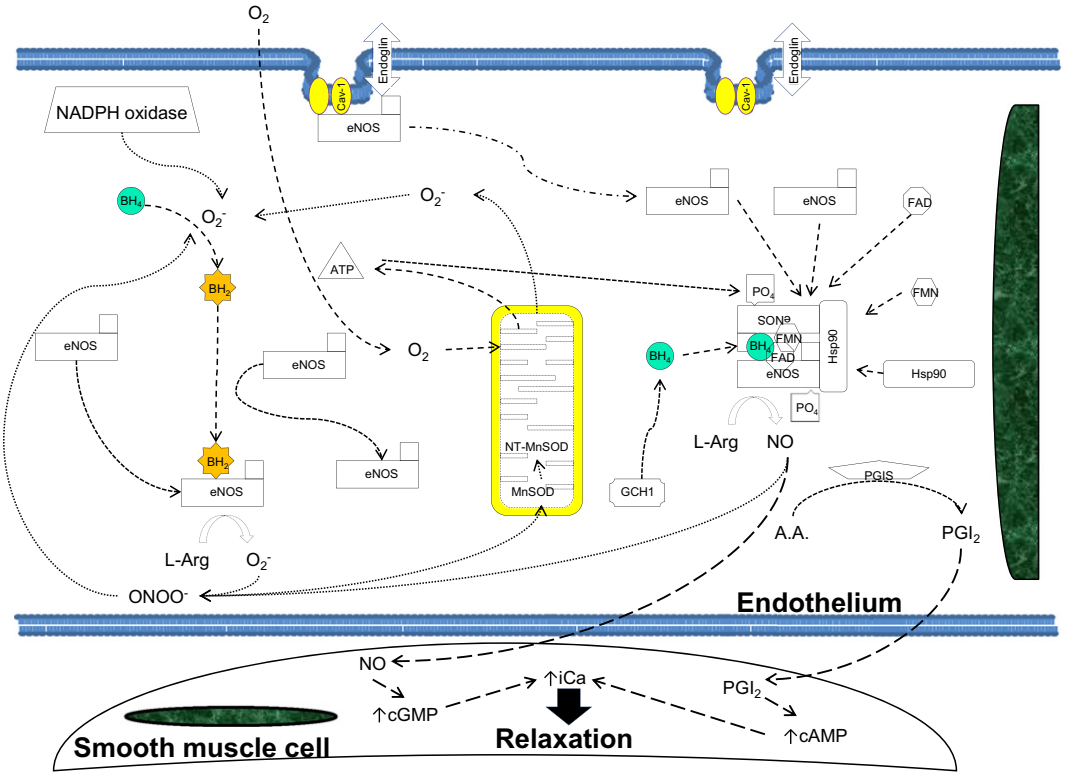

Figure 2 Controls of the endothelial nitric oxide synthase (eNOS) function. Shear stress in a blood vessel activates eNOS expression and activity. When activated, eNOS is free from the association with caveolin-1 and forms a dimer and becomes phosphorylated. eNOS then binds to heat shock protein 90 (hsp90), calmodulin, and in the presence of tetrahydrobiopterin (BH4), calcium, flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN) is activated. Once activated, eNOS converts L-arginine into citrullin and nitric oxide.

Intra-uterine growth restriction

Intra-uterine growth restriction has been reported to be associated with increased risk of PPHN.[16] It is believed that utero-placental insufficiency may lead to postnatal pulmonary hypertension by two mechanisms: oligohydramnios and chronic fetal hypoxia. Hypoxia increases endothelial synthesis of vasoconstrictors and smooth muscle mitogens such as endothelin-1, platelet-derived growth factor-β and vascular endothelial growth factor; it also inhibits eNOS. Increased thickness of right ventricular wall, a hallmark of pulmonary hypertension, is also commonly seen in animal models of intra-uterine growth restriction.

Table 1 Classification of persistent pulmonary hypertension of the newborn (PPHN).

- Abnormally constricted pulmonary vasculature caused by parenchymal diseases:
  - Meconium aspiration syndrome
  - Respiratory distress syndrome[30]
  - Pneumonia
- Hypoplastic pulmonary vasculature:
  - Congenital diaphragmatic hernia
  - Lung hypoplasia
- Normal parenchyma with remodeled pulmonary vasculature:
  - Idiopathic PPHN
  - Congenital heart disease
  - Hypoxic–ischemic encephalopathy, chronic
  - Others

Maternal SSRI exposure

SSRIs, which are commonly prescribed antidepressants, have been reported to be associated with PPHN, especially during the late trimester, in a case–control study.[17] Some later studies also demonstrated the increased risk of PPHN in SSRI-exposed neonates. A similar finding was seen in a rat study and it is believed to be the result of smooth muscle cell proliferation in pulmonary arteries.[18]

In utero NSAID exposure

In utero NSAID exposure is considered a risk factor for PPHN because most NSAIDs inhibit prostaglandin synthesis and *in utero* exposure is believed to close the ductus arteriosus prenatally. Alano et al studied meconium and found an association between the existence of NSAID metabolites and the development of PPHN.[19]

Genetic risk factors

The only genetic risk factor reported is the association between heterozygote T1405N genotype of carbamoyl-

phosphate synthetase and PPHN, possibly because of a lack of substrate for eNOS.[13] Epidemiologic studies suggest Black maternal race and male newborns have a higher chance of developing PPHN.

Management

Current therapies for PPHN include mechanical ventilation, muscle paralysis/relaxation, sedation, alkalosis and vasorelaxants.[20] Inhaled nitric oxide (INO) is currently regarded as the gold standard therapy, but as many as 30% of patients are nonresponsive to INO treatment.[1](Table 2)

General management

A quiet environment with minimal stimulation is recommended for PPHN management. It is known that bright light or loud noise can affect the oxygenation. Body temperature should be maintained at a thermoneutral range (37.0 ± 0.5 °C). Appropriate hydration and hematocrit (40—50%) should be maintained. Polycythemia (hematocrit $>$ 55%) can increase blood viscosity and pulmonary vascular resistance. Hypoglycemia and hypocalcemia should be avoided. Hypoglycemia may lead to reduced ATP formation and ATP is a known agonist for eNOS. Calcium is one of the critical cofactors for eNOS activity and hypocalcemia may impair eNOS function and should be corrected. Preductal oxygenation should be used to adjust the ventilator support and SpO_2 above 95% may be appropriate. Some centers prefer to monitor the difference between pre- and postductal SpO_2 as an indicator for the severity of PPHN, although we do not regularly use this. For severe PPHN shunting through the foramen ovale we can observe fluid infusion through an intravenous catheter below the diaphragm through the foramen ovale, so it is reasonable to provide pressor(s) via such a line in order to reduce the effect on the pulmonary arteries. Empirical antibiotics, such as ampicillin and gentamicin, are recommended before infection (especially group B streptococcus) can be ruled out as the cause of PPHN.

Table 2 Management of persistent pulmonary hypertension of the newborn (PPHN).

- General:
  - Reduce stimulation: noise control, dim ambient light, thermoneutral control
  - Sedation and/or muscle relaxation
  - Empirical antibiotics
  - Avoid hypoglycemia
  - Avoid hypocalcemia
  - Nutritional support
  - Alkalosis: questionable benefit
  - Inotropic agent
- Mechanical ventilation:
  - Conventional mechanical ventilation: pressure limited or volume-controlled ventilator
  - High-frequency (oscillator or jet) ventilator
- Vasorelaxant:
  - Inhaled nitric oxide
  - Prostaglandin: PGE_1 or PGI2
  - Others: sildenafil, $MgSO_4$, milrinone
- Extracorporeal membrane oxygenator: VA- and VV-type

Severity of PPHN

Two parameters, alveolar—arterial oxygen difference ($AaDO_2$) and oxygenation index (OI), are used most frequently in PPHN management to judge the severity and progress of the disease. $AaDO_2$ is the difference between alveolar oxygen content and arterial oxygen content. The formula to calculate $AaDO_2$ is:

$$AaDO_2 = (ATM - P_{H_2O}) \times FiO_2 - PaO_2 - PaCO_2/RQ$$

ATM is the atmospheric pressure, which is usually equal to 760 torr (5.7 Pascal) at sea level but needs to be adjusted in high altitude. P_{H2O} is the pressure of water vapor in 1 ATM, which is usually considered to be 47 torr (0.35 Pascal). FiO_2 is the fraction of inspired air provided by the ventilator. The reason to correct for $PaCO_2$ is because some oxygen used to produce CO_2 is from the nutrient inside the body. RQ is the respiratory quotient and RQ = 1 if the energy source is purely sugar or RQ = 0.8 when the nutritional source is a combination of glucose, protein and lipid. The clinical decision to use ECMO usually depends on $AaDO_2$. When $AaDO_2$ is above 600 twice with maximal support then ECMO can be considered. The oxygenation index is more commonly used during medical management of PPHN because it also considers ventilator support. The oxygenation index is calculated as:

$$OI = MAP \times FiO_2 \times 100/PaO_2$$

MAP is the mean airway pressure provided by the ventilator. OI below 10 is usually considered to be mild PPHN, OI between 10 and 20 is considered to be moderate PPHN, whereas OI above 20 is considered to be severe PPHN. If the OI remains above 20 under maximal medical support, we usually recommend ECMO treatment if it is available.

Mechanical ventilation

Mild PPHN can be managed by nasal cannula whereas moderate and severe PPHN requires positive pressure ventilator. High oxygen concentration and low $PaCO_2$ are commonly used for PPHN in the belief that both can help to relax the pulmonary arteries. However, it is recommended that $PaCO_2$ levels should not be lower than 35 torr (0.263 Pascal) since CO_2 also controls cerebral perfusion. Aggressive hyperventilation with hypocapnia is known to be a significant risk factor for hearing impairment in PPHN survivors.[21] Both a conventional mechanical ventilator and a high-frequency (oscillator or jet) ventilator (HFOV or HFJV) can be used. In severe PPHN most centers prefer HFOV because this model usually will remove the patient's spontaneous breathing.

Muscle paralysis/relaxation

Agitation usually aggravates PPHN with initial transient increase of oxygenation followed by a precipitous drop in oxygenation. To remove this flip-flop phenomenon in PPHN management some centers will paralyze PPHN patients.

Sedation

Continuous sedation, either by benzodiazepine or a narcotic agent, is a common practice in PPHN management. Sedation can decrease the frequency of desaturations.

Alkalosis

Induction of alkalosis by either infusion of sodium bicarbonate or hyperventilation has frequently been used as part of the PPHN treatment. However, there is no solid evidence to show that this practice is effective. A multicenter observational study demonstrated an increased ECMO requirement for patients receiving $NaHCO_3$ infusion before the INO era. It is hypothesized that $NaHCO_3$ infusion increases CO_2 formation and leads to increases in ventilator support.[20]

Inotropic agents and vasopressors

An increased right-to-left shunt is believed to be the main reason for the severe hypoxemia in PPHN, so decreasing the right-to-left shunt may be beneficial in PPHN. β-Adrenergic agonists can decrease pulmonary vascular resistance more than systemic vascular resistance and might have a more favorable effect in PPHN especially in cases with poor myocardial function such as in birth asphyxia associated with PPHN.[22] Dopamine increases both systemic and pulmonary vascular resistance and reduces left-to-right ductal shunting in preterm infants which suggests that it may not be a good choice for premature infants with patent ductus arteriosus and PPHN.[23]

Vasorelaxants

The most effective vasorelaxants for PPHN work specifically on the pulmonary vasculature. However, unfortunately, there is so far no specific vasorelaxant for the pulmonary arteries. Several vasorelaxants have been used over the past 4 decades, including epinephrine (β-adrenergic receptor agonist), tolazoline (nonselective competitive α-adrenergic receptor antagonist or histamine release), magnesium sulfate etc. Prostanoids (PGE1 or PGI2) have been suggested recently when the cause of severe hypoxemia remains uncertain before ductal-dependent cyanotic heart disease can be ruled out. Prostanoids help to relax the vascular smooth muscle cells, and maintain the patency of ductus arteriosus, though cAMP formation. However, because of their nonspecificity, and potential side effect of apnea, a secure airway is needed and the possibility of low blood pressure should always be considered. Prostacyclin (PGI_2) and its analogues have been used more commonly than PGE_2 recently. Epoprostanol is an intravenously administered analogue of PGI_2, whereas iloprost is an inhaled analogue. Presently the experience of PGI_2 analogues for PPHN remains limited, but inhaled iloprost is considered to be a more appropriate route to provide more selective effect.

Nitric oxide (NO) is considered to be the most specific pulmonary artery vasodilator because of its method of administration. By increasing the intracellular cGMP in the smooth muscle cells of the pulmonary arteries NO can decrease the pulmonary vascular resistance. Inhaled nitric oxide (INO) at a dose of $\geq$5 ppm significantly reduces the combined outcome of death and need for ECMO by 35% in infants with oxygen index of $\geq$25. However, long-term follow-up studies (12—24 months) indicate that INO does not alter either the incidence of chronic lung disease or neurodevelopmental impairment.[24,25] Early use of INO in the disease course does not reduce the use of ECMO, mortality, or improve other outcomes. INO above 20 ppm does not provide more benefit and should be avoided. Special arrangement is needed if INO is provided when the patient is on an HFJV. NO can oxidize hemoglobin into methemoglobin and levels of methemoglobin should be monitored regularly because some patients may not detoxify it as a result of enzyme deficiency. INO is not universally effective and about 30% of patients with severe PPHN do not respond to INO.

Original approved INO use is when OI is above 25, but a recent trend is to start it when OI reaches 20. There is no consensus regarding the OI threshold to initiate INO and most centers establish their own criteria according to their own experience and the availability of the ECMO facility. When ECMO locates far away from the care center then some centers will initiate INO when OI reaches 15 twice, several (2—6) hours apart. The initial dose is usually 10 ppm and it is increased or decreased, according to the clinical response. Lower concentrations of INO (5—10 ppm) have been studied, but the results remain unclear.

Extracorporeal membrane oxygenator (ECMO)

When all medical treatment fails then ECMO should be considered when the PPHN's cause is reversible. Chromosomal anomaly, lethal congenital malformation, uncorrectable heart defect, and major intracranial bleed prohibit the use of ECMO. The course of ECMO is usually set between 10 and 14 days according to individual institutional guidelines. If the patient does not respond to ECMO during this period then they will be decannulated and put back on the original management plan. Two types of ECMO can be used, including veno-venous (VV) and veno-arterial (VA). VA-ECMO needs cannulation of one vein and one artery, usually one external jugular vein and one internal carotid artery. The problem with VA-ECMO is that an internal carotid artery is sacrificed and there is an increased chance of intracranial bleeding. VV-ECMO can be performed using a double-lumen catheter without sacrificing any arteries. However, a bigger catheter is required for VV-ECMO and a good pumping heart is mandatory for the technique. The patient's size should be considered for ECMO use: they need to be at least 2 kg in weight and usually more than 34 weeks' gestation in order to be cannulated. During ECMO treatment the patient has to be maintained on a low ventilator setting to keep the alveoli open. Coagulation

profiles should be checked several times a day to avoid massive bleeding. The chance of having an intracranial bleed is about 10—15% for ECMO, and this should be explained to the family before the procedure. Well-trained perfusionists are required and regular wet-runs should be performed to maintain skill levels in ECMO centers. Since the introduction of INO the number of ECMO centers has declined dramatically, because of the cost of maintenance.

Others

L-Arginine is the substrate for eNOS and has been shown to improve eNOS function *in vitro,* but it fails to show benefit *in vivo*. However, L-citrulline, a precursor of L-arginine, can enter endothelial cells effectively and has been shown to ameliorate the severity of hypoxia-induced pulmonary hypertension in an animal model.[26] It is believed the differential effect is because L-arginine cannot enter endothelial cells to recouple the eNOS function. Endothelin receptor antagonists have been investigated as potential vasorelaxants, but their clinical usefulness remains unknown. Bosentan is the only commercially available endothelin receptor antagonist and a few case reports are available in the literature. Magnesium inhibits calcium entry into smooth muscle cells and, as a result, it is an effective vasorelaxant.[27] Tolazoline is an active vasorelaxant, especially when administered by way of inhalation. There are also case series, or reports, about the effect of magnesium and tolazoline use in PPHN, but large-scale randomized controlled trials are lacking for both. Adenosine is a vasorelaxant with an extremely short half-life and is an eNOS agonist that increases the formation of NO. Because of its short half-life there may be fewer systemic side effects with adenosine. However, presently there is limited experience with adenosine in PPHN.[28]

Recently there is an interest in phosphodiesterase (PDE) inhibitors, especially the type 5 inhibitors.[7] There are at least more than 11 types of PDE that hydrolyze cAMP, cGMP or both. Type 5 is the main PDE in the pulmonary vasculature, genital organ and auditory system. Inhibiting PDE-5 can maintain intracellular cGMP levels and prolong the effect of NO in pulmonary arteries. There have been several case series and one randomized controlled trial using sildenafil (0.5—2.0 mg/kg every 6 hours), a PDE-5 inhibitor, in managing PPHN with success.[29] Milrinone is a type 3 PDE inhibitor that decreases hydrolysis of cAMP. Dosage between 0.2 and 1 μg/kg/min with or without loading (20—50 μg/kg) of milrinone has been reported when patients fail to respond INO. PGI_2 relaxes the pulmonary artery via increasing intravascular cAMP levels, so milrinone may be helpful in treating PPHN. There are a few case series that demonstrated the efficacy of milrinone use in PPHN. However, since milrinone also decreases the systemic vasculature resistance, it may aggravate the right-to-left shunt so its use is recommended only when poor systemic perfusion is considered to affect the PPHN treatment. ET-1 is another important pulmonary vasoconstrictor that contributes to PPHN and use of an ET-1 dual antagonist (Bosentan) has been reported in a few neonates with severe PPHN. A recent single-center, randomized, blinded, controlled trial of Bosentan 1 mg/kg twice daily via a feeding tube was reported against placebo without inhaled NO treatment and showed a dramatic decrease in mortality and better neurological outcome.[30]

Surfactants have been studied in animal models of PPHN induced by meconium aspiration. They have also been combined with an NO donor, or sildenafil, in other types of PPHN with some success. A clinical trial of surfactants in PPHN has been reported in human neonates with MAS under the belief that meconium can inhibit surfactant protein function or used as an airway toilet to wash out the meconium in the airway.[31] A recent Australian multicenter study reconfirmed the efficacy of surfactant in MAS especially in places where ECMO is not available.[16] Superoxide dismutase (SOD), or its mimetics, has been studied by both Steinhorn and Black groups. Both groups show some promise of using SOD in animal models of PPHN.[32] The mechanism may be secondary to the removal of O_2^- that leads to an increased bioavailability of NO and increased apoptosis of pulmonary artery smooth muscle cells.[33—35]

Tetrahydrobiopterin (BH4) is not only a vital cofactor for eNOS function but also an important intracellular antioxidant. Depletion of BH4, or oxidation of BH4 into dihydrobiopterin (BH2), can uncouple eNOS function and shift eNOS from NO formation into O_2^- formation. NO not only relaxes arteries but also mediates blood vessel formation (angiogenesis). Methods to increase endothelial cell BH4 content might improve eNOS function and help angiogenesis. We recently demonstrated that sepiapterin, a precursor for BH4, can recouple eNOS and correct the impaired angiogenesis in PPHN pulmonary artery endothelial cells.[36] Some other antioxidants, such as *N*-acetylcyeteine, apocynin and ascorbate, can either decrease intracellular ROS formation or increase BH4 levels which may help to improve NO bioavailability but their efficacy has not been demonstrated in human infants.

Outcome

Survivors have high morbidity in the form of neurodevelopmental and audiological impairment, cognitive delays, hearing loss, and a high rate of rehospitalization. A low $PaCO_2$ level, usually because of hyperventilation, is considered to be a contributing factor for hearing loss especially when combined with the prolonged use of aminoglycoside treatment.

Conclusion

Even with advances in PPHN treatment the mortality rate of this lung disease remains high. Animal models of PPHN provide insight into potential treatment modalities and pathophysiology, but cannot completely reflect the disease. Decreased ambient stimulation, continuous sedation, appropriate mechanical ventilation, and inhaled NO treatment are the cornerstones of PPHN management. ECMO should be provided when maximal medical treatment fails. Head ultrasound and echocardiography should be performed before the discussion about ECMO use. VV-ECMO is the preferred mode of ECMO when myocardial function is adequate. Potential therapeutic modalities such as PDE inhibitors, intravenous citrullin infusion, or BH4 treatment require more studies before clinical application.

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References

1. Travadi JN, Patole SK. Phosphodiesterase inhibitors for persistent pulmonary hypertension of the newborn: a review. *Pediatr Pulmonol* 2003;**36**:529—35.
2. Nelin LD, Thomas CJ, Dawson CA. Effect of hypoxia on nitric oxide production in neonatal pig lung. *Am J Physiol Heart Circ Physiol* 1996;**271**:H8—14.
3. Noguchi Y, Hislop AA, Haworth SG. Influence of hypoxia on endothelin-1 binding sites in neonatal porcine pulmonary vasculature. *Am J Physiol Heart Circ Physiol* 1997;**272**:H669—78.
4. Dargaville PA, Mills JF, Headley BM, Chan Y, Coleman L, Loughnan PM, et al. Therapeutic lung lavage in the piglet model of meconium aspiration syndrome. *Am J Respir Crit Care Med* 2003;**68**:456—63.
5. Reddy VM, Meyrick B, Wong J, Khoor A, Liddicoat JR, Hanley FL, et al. In utero placement of aortopulmonary shunts: a model of postnatal pulmonary hypertension with increased pulmonary blood flow in lambs. *Circulation* 1995;**92**:606—13.
6. Steinhorn RH, Morin 3rd FC, Fineman JR. Models of persistent pulmonary hypertension of the newborn (PPHN) and the role of cyclic guanosine monophosphate (GMP) in pulmonary vasorelaxation. *Semin Perinatol* 1997;**21**:393—408.
7. Steinhorn RH, Kinsella JP, Pierce C, Butrous G, Dilleen M, Oakes M, et al. Intravenous sildenafil in the treatment of neonates with persistent pulmonary hypertension. *J Pediatr* 2009;**15**:841—7.
8. Walther FJ, Benders MJ, Leighton JO. Persistent pulmonary hypertension in premature neonates with severe respiratory distress syndrome. *Pediatrics* 1992;**90**:899—909.
9. Gao Y, Raj JU. Regulation of the pulmonary circulation in the fetus and newborn. *Physiol Rev* 2010;**90**:1291—335.
10. Konduri GG, Mital S. Adenosine and ATP cause nitric oxide-dependent pulmonary vasodilation in fetal lambs. *Biol Neonate* 2000;**78**:220—9.
11. Konduri GG, Solimano A, Sokol GM, Singer J, Ehrenkranz RA, Singhal N, et al. A randomized trial of early versus standard inhaled nitric oxide therapy in term and near-term newborn infants with hypoxic respiratory failure. *Pediatrics* 2004;**113**:559—64.
12. Garcia-Cardena G, Oh P, Liu J, Schnitzer JE, Sessa WC. Targeting of nitric oxide synthase to endothelial cell caveolae via palmitoylation: implications for nitric oxide signaling. *Proc Natl Acad Sci USA* 1996;**93**:6448—53.
13. Pearson DL, Dawling S, Walsh WF, Haines JL, Christman BW, Bazyk A, et al. Neonatal pulmonary hypertension. Urea-cycle intermediates, nitric oxide production, and carbamoyl-phosphate synthetase function. *N Engl J Med* 2001;**344**:1832—8.
14. Hernández-Díaz S, Van Marter LJ, Werler MM, Louik C, Mitchell AA. Risk factors for persistent pulmonary hypertension of the newborn. *Pediatrics* 2007;**120**:e272—82.
15. Murphy JD, Rabinovitch M, Goldstein JD, Reid LM. The structural basis of persistent pulmonary hypertension of the newborn infant. *J Pediatr* 1981;**98**:962—7.
16. Dargaville PA, Copnell B, Mills JF, Haron I, Lee JK, Tingay DG, et al. Randomized controlled trial of lung lavage with dilute surfactant for meconium aspiration syndrome. *J Pediatr* 2011;**158**:383—9.
17. Chambers CD, Hernandez-Diaz S, Van Marter LJ, Werler MM, Louik C, Jones KL, et al. Selective serotonin-reuptake inhibitors and risk of persistent pulmonary hypertension of the newborn. *N Engl J Med* 2006;**354**:579—87.
18. Fornaro E, Li D, Pan J, Belik J. Prenatal exposure to fluoxetine induces fetal pulmonary hypertension in the rat. *Am J Respir Crit Care Med* 2007;**176**:1035—40.
19. Alano MA, Ngougmna E, Ostrea Jr EM, Konduri GG. Analysis of nonsteroidal antiinflammatory drugs in meconium and its relation to persistent pulmonary hypertension of the newborn. *Pediatrics* 2001;**107**:519—23.
20. Walsh-Sukys MC, Tyson JE, Wright LL, Bauer CR, Korones SB, Stevenson DK, et al. Persistent pulmonary hypertension of the newborn in the era before nitric oxide: practice variation and outcomes. *Pediatrics* 2000;**105**:14—20.
21. Hendricks-Muñoz KD, Walton JP. Hearing loss in infants with persistent fetal circulation. *Pediatrics* 1988;**81**:650—6.
22. Cheung PY, Barrington KJ. The effects of dopamine and epinephrine on hemodynamics and oxygen metabolism in hypoxic anesthetized piglets. *Crit Care* 2001;**5**:158—66.
23. Bouissou A, Rakza T, Klosowski S, Tourneux P, Vanderborght M, Storme L. Hypotension in preterm infants with significant patent ductus arteriosus: effects of dopamine. *J Pediatr* 2008;**153**:790—4.
24. Clark RH, Huckaby JL, Kueser TJ, Walker MW, Southgate WM, Perez JA, et al. Low-dose nitric oxide therapy for persistent pulmonary hypertension: 1-year follow-up. *J Perinatol* 2003;**23**:300—3.
25. Inhaled nitric oxide in term and near-term infants: neurodevelopmental follow-up of the neonatal inhaled nitric oxide study group (NINOS). *J Pediatr* 2000;**136**:611—7.
26. Ananthakrishnan M, Barr FE, Summar ML, Smith HA, Kaplowitz M, Cunningham G, et al. L-Citrulline ameliorates chronic hypoxia-induced pulmonary hypertension in newborn piglets. *Am J Physiol Lung Cell Mol Physiol* 2009;**297**:L506—11.
27. Wu TJ, Teng RJ, Yau KIT. Persistent pulmonary hypertension of the newborn treated with magnesium sulfate in premature neonates. *Pediatrics* 1995;**96**:472—4.
28. Lapointe A, Barrington KJ. Pulmonary hypertension and the asphyxiated newborn. *J Pediatr* 2011;**158**:e19—24.
29. Baquero H, Soliz A, Neira F, Venegas ME, Sola A. Oral sildenafil in infants with persistent pulmonary hypertension of the newborn: a pilot randomized blinded study. *Pediatrics* 2006;**117**:1077—83.
30. Mohamed WA, Ismail M. A randomized, double-blind, placebo-controlled, prospective study of bosentan for the treatment of persistent pulmonary hypertension of the newborn. *J Perinatol* 2012;**32**:608—13.
31. Chinese Collaborative Study Group for Neonatal Respiratory Diseases. Treatment of severe meconium aspiration syndrome with porcine surfactant: a multicentre, randomized, controlled trial. *Acta Paediatr* 2005;**94**:896—902.
32. Farrow KN, Lakshminrusimha S, Reda WJ, Wedgwood S, Czech L, Gugino SF, et al. Superoxide dismutase restores eNOS expression and function in resistance pulmonary arteries from neonatal lambs with persistent pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol* 2008;**295**:L979—87.
33. Wedgwood S, Black SM. Role of reactive oxygen species in vascular remodeling associated with pulmonary hypertension. *Antioxid Redox Signal* 2003;**5**:759—69.
34. Wedgwood S, Black SM. Molecular mechanisms of nitric oxide-induced growth arrest and apoptosis in fetal pulmonary arterial smooth muscle cells. *Nitric Oxide* 2003;**9**:201—10.
35. Wedgwood S, Black SM. Induction of apoptosis in fetal pulmonary arterial smooth muscle cells by a combined superoxide dismutase/catalase mimetic. *Am J Physiol Lung Cell Mol Physiol* 2003;**285**:L305—12.
36. Teng RJ, Du J, Xu H, Bakhutashvili I, Eis A, Shi Y, et al. Sepiapterin improves angiogenesis of pulmonary artery endothelial cells with in utero pulmonary hypertension by recoupling endothelial nitric oxide synthase. *Am J Physiol Lung Cell Mol Physiol* 2011;**301**:L334—45.