Inhaled Nitric Oxide in Treatment of Persistent Pulmonary Hypertension of Neonate: An Insight into Biochemical Pathways

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Authors' contributions

This work was carried out in collaboration between all authors. Author SB designed the study. Author AMH managed the literature searches and wrote the first draft of the manuscript. Author VNP managed the literature searches and completed the final draft. All authors read and approved the final manuscript.

Review Article

ABSTRACT

Persistent pulmonary hypertension of neonate (PPHN) is a syndrome of failed circulatory adaptation in the neonatal period and is defined by sustained elevation of PVR and is often associated with hypoxemia, severe cyanosis, and cardiac dysfunction and normal or low systemic vascular resistance. Stability of pulmonary vessels is achieved through the formation of vascular complexes and Nitric oxide (NO). The decline in the secretion of endothelial diastolic factors in the pulmonary vasculature aggravates the injury of endothelial cells, which induces shrinkage of the vasculature. Currently available agents in the treatment of PPHN include oxygen, inhaled nitric oxide (iNO), prostanoids, sildenafil, milrinone, and bosentan. NO the smallest signaling molecule known is synthesized from L-arginine in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) and oxygen by four major isoforms of NO synthase (NOS). The most important physiological signaling pathway stimulated by NO is the activation of soluble guanylyl

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cyclase and the generation of cyclic GMP. NO can also bind to the ferrous heme forming iron nitrosyl Hb, $Fe^{II}NO$ Hb and to ferric heme, but with a substantially lower affinity. Both of these reactions end up inactivating NO. There are several diffusional factors that contribute to the negative modulation of the reaction of NO with ferrous hemoglobin and include the erythrocyte membrane, or sub-membrane network of protein, an unstirred layer surrounding the erythrocyte and an erythrocyte-free zone within the lumen nearest to the endothelial layer that results from dynamic, flow-mediated, axial concentration of the red cells. Such an effect is believed to be the cause of pulmonary and systemic hypertension and many other diseases. Inhaled NO can disperse to vascular smooth muscle cells, and soluble guanylate cyclase can increase the levels of NO, which causes specific expansion of the pulmonary vasculature. NO has allosteric effects on hemoglobin, which increases the exchange of oxygen and carbon dioxide, and causes blood flow distribution from oxygenation tissue to hypoxic tissue, which improves systemic and pulmonary circulation. iNO has been approved by the U.S. Food and Drug Administration (FDA) for the treatment of PPHN.

1. INTRODUCTION

The lung must establish itself as the organ of gas exchange within the first few minutes of life. This amazing feat is accomplished via a rapid decrease in pulmonary vascular resistance (PVR) and increase in pulmonary blood flow. Subsequently, pulmonary artery pressure and vascular resistance progressively decrease through the first few weeks of life. Abnormalities of vascular development or function during this critical developmental window can result in persistent pulmonary hypertension of the newborn (PPHN). PPHN can be easily triggered by causes such as pneumonia, pulmonary vascular spasm, asphyxia, respiratory distress syndrome, meconium aspiration, maternal urinary tract infection, and drug use during pregnancy.

PPHN is a syndrome of failed circulatory adaptation in the neonatal period and is defined by sustained elevation of PVR and is often associated with hypoxemia, severe cyanosis, and cardiac dysfunction and normal or low systemic vascular resistance. The incidence of PPHN is about 0.2% in term and near term births and 2% in pre-term infants presenting with early respiratory distress [1,2].

Neonatal pulmonary hypertension results in the inhibition of the production of NO. Stability of pulmonary vessels is achieved through the formation of vascular complexes and NO. The decline in the secretion of endothelial diastolic factors in the pulmonary vasculature aggravates the injury of endothelial cells, which induces shrinkage of the vasculature. Furthermore, this increases interaction between endothelial cells, macrophages, and neutrophils, which promotes thrombosis, and in turn aggravates endothelial cell injury, usually with the characteristics of resistance of the pulmonary circulation and increasing pressure.

The principle of pharmacotherapy for PPHN is vasodilation of the pulmonary vasculature. Ideally, the agents used for PPHN dilate the pulmonary vasculature with minimal effects on systemic vasculature. Currently available agents in the treatment of PPHN include oxygen, iNO, prostanoids, sildenafil, milrinone, and bosentan [3].

This review article discusses the biochemical processes involved in NO metabolism and the clinical importance of its derivatives in the management of neonatal pulmonary hypertension.

2. NITRIC OXIDE SYNTHESIS AND FUNCTIONS

The discovery of nitric oxide (NO) represented a critical advance in the understanding of cell signaling mechanisms and subsequently into major new advancements in many clinical areas. It was first identified as the endothelium-derived relaxing factor in 1987 [4,5].

NO the smallest signaling molecule known is synthesized from L-arginine in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) and oxygen by four major isoforms of NO synthase (NOS): neuronal NOS, endothelial NOS (eNOS), inducible NOS (iNOS), and, more recently, mitochondrial NOS [6].

The NO formed by NOS can act on a number of target enzymes and proteins. The most important physiological signaling pathway stimulated by NO is the activation of soluble guanylyl cyclase and the generation of cyclic GMP [7]. Other functions are listed in Table 1 [7,8,9].

Table 1. Miscellaneous functions of NO and their respective mechanisms [7,8,9]

Function	Mechanism
Regulates gene transcription	Binding to iron-responsive elements [7].
MRNA translation	Binding to iron-responsive elements [8].
Post-translational modification of proteins	ADP ribosylation [9].

On the other hand, excessive and unregulated NO synthesis has been implicated as causal or contributing to pathophysiological conditions including Neonatal pulmonary hypertension. An important mode of inactivation of NO is its reaction with superoxide anion (O2 2†) to form the potent oxidant peroxynitrite (ONOO2). This compound can cause oxidative damage, nitration, and S-nitrosylation of biomolecules including proteins, lipids, and DNA [10].

3. HEMOGLOBIN AND NO

Hemoglobin is critical for normal oxygen delivery to tissues; it is also present in erythrocytes in such high concentrations that it can alter red cell shape, deformability, and viscosity. Different hemoglobins are produced during embryonic, fetal, and adult life. Each consists of a tetramer of globin polypeptide chains. Each of the four subunits has an iron-containing heme prosethic group that is attached to the protein via a histidine bond. The heme iron is the primary site for ligand binding so that Hb can carry out its main function; transporting oxygen from the lungs to the tissues.

Hemoglobin only binds oxygen when the iron heme is ferrous (+2), forming oxyhemoglobin (oxyHb). When iron is oxidized to the ferric form (+3), oxygen no longer binds. The ferric form of is called methemoglobin (metHb).

The efficiency of oxygen transport by Hb is through to its allosteric mechanism. There are is two states models compromised of the R-state (relaxed) and the T-state (tight). The relaxed state shows an increased affinity for oxygen in contrast to the tight form which shows a decreased affinity. When hemoglobin is fully deoxygenated, it is in the T-state. Oxygen binding is cooperative so that binding of one oxygen at one heme sub-unit increases the affinity of binding of next heme to next subunit. Having these two states allow Hb to be fully saturated in the lungs where the oxygen pressure is high, while it can easily release oxygen when the oxygen pressure is low [11].

NO also binds to the ferrous heme forming iron nitrosyl HB, $Fe^{II}NO$ Hb (in this abbreviation the superscript represents the iron (Fe) oxidation state. Nitric oxide can also bind to the ferric heme, but with a substantially lower affinity ($Kd \frac{1}{4} 2.5 10-4$ M) [12]. Both of these reaction end up inactivating NO and thus one can imagine many ways Hb can mediate NO signaling.

4. CELL-FREE HEMOGLOBIN AND THE INACTIVATION OF NO

The inhibition of NO signaling is mainly due to its reaction with oxyHb and deoxyHb to produce nitrates plus methemoglobin and iron nitrosylhemoglobin [13]. This reaction is known as the deoxygenation reaction.

NO can be both scavenged by intra-erythrocytic and cell-free hemoglobin, importantly the rate of NO scavenging is reduced 1,000-fold by sequestering hemoglobin within the red cell membrane [14].

There are several diffusional factors that contribute to the negative modulation of the reaction of NO with ferrous hemoglobin and include the erythrocyte membrane, or sub-membrane network of protein, an unstirred layer surrounding the erythrocyte and an erythrocyte-free zone within the lumen nearest to the endothelial layer that results from dynamic, flow-mediated, axial concentration of the red cells. Decompartmentalized, cell-free hemoglobin is not constrained by these physical factors, and as little as 6 µM cell-free oxyhemoglobin eliminates NO mediated basal vasodilation [15].

Such an effect is believed to be the cause of pulmonary and systemic hypertension and many other disease especially if there is decompartmentalization of hemoglobin seen in chronic RBCs lyses that increases the concentration of cell-free hemoglobin which would limit NO bioavailability as a direct result of cell-free hemoglobin mediated NO dioxygenation and nitrosylation.

5. USE OF NO IN MANAGEMENT OF PPHN

The morbidity and mortality associated with persistent pulmonary hypertension of the newborn are related to the severity and duration of systemic hypoxemia. Treatment with high fractions of inspired oxygen (FiO2) and mechanical ventilation improves oxygenation in some infants with pulmonary-artery hypertension, but in many others it does not. Although intravenous therapy with vasodilator drugs has been used for pulmonary hypertension, it often causes dilation of the systemic circulation and severe hypotension. Extracorporeal membrane oxygenation can save the lives of some infants with severe pulmonary hypertension, but it requires anticoagulation and cannulation of the great vessels, causes important morbidity, and is unavailable at many intensive care nurseries [16].

In 1999, inhaled nitric oxide (iNO) was approved by the FDA for use in near-term and term infants with PPHN. It has been the mainstay of PPHN treatment. Inhaled nitric oxide achieves potent and selective pulmonary vasodilation without decreasing systemic vascular tone. It diffuses into vascular smooth-muscle cells in the lungs, where it increases concentrations of cyclic guanosine monophosphate, causing pulmonary vasodilation. Inhaled nitric oxide does not cause systemic hypotension when it diffuses into the intravascular space, because it is inactivated by avid binding to hemoglobin. Further, iNO reduces V/Q mismatch by entering only ventilated alveoli and redirecting pulmonary blood by dilating adjacent pulmonary arterioles [Fig. 1].

Large multi-center trials have demonstrated that iNO reduces the need for ECMO. A meta-analysis of seven randomized trials of iNO use in newborns with PPHN also revealed that 58% of hypoxic near-term and term infants responded to iNO within 30 to 60 minutes. While use of iNO did not reduce mortality in any study analyzed, but the need for rescue ECMO therapy was significantly decreased [17].

There has been significant debate concerning the optimal starting dose as well as time of initiation of iNO therapy. Inhaled NO has several potential side effects including platelet dysfunction (prolong bleeding time by inhibiting collagen-induced platelet aggregation and adenosine diphosphate expression), pulmonary edema, methemoglobinemia and production of toxic byproducts such as nitrates [17, 18, 19]. In combination with superoxide, it further potentiates oxidative injury by forming peroxynitrites [17]. iNO has a short half-life (2 s to 6 s) and has been used in newborns at doses of 1 ppm to 80 ppm, which is then titrated to achieve the desired effect. Doses greater than 40 ppm have the potential to increase toxicity without additional benefits. The recommended starting dose for term infants is 20 ppm. The expected response is rapid, occurring in less than 30 min with a PaO_2 increase ≥20 mmHg. If there is no response, the iNO dose may be increased up to 40 ppm. The dose used in studies involving premature infants was 10 ppm and was increased up to 20 ppm in non-responders [20].

Controversy exists over the appropriate timing of initiation of iNO in hypoxic respiratory failure. In the presence of an indwelling arterial line, severity of PPHN is assessed by calculation of oxygenation index (OI).

OI = Mean airway pressure in cm H2O × FiO2 × 100 ÷ PaO2 in mmHg

An OI of 25 is associated with a 50% risk of requiring ECMO or mortality, while an OI of 40 is generally accepted as an indication for ECMO. Based on current available evidence, an acceptable indication for treatment with iNO would be an OI >15-25 with echocardiographic evidence of PPHN or a higher OI with or without evidence of right-to-left shunt [17].

Due to rebound vasoconstriction and resultant pulmonary hypertension on abrupt withdrawal, iNO needs to be weaned gradually [17]. Following improvement in oxygenation and after a 4 h to 6 h period of stability, during which the inspired oxygen concentration is decreased to 60% to 80%, or the OI falls to ≤10, the dose of iNO should be weaned. An accepted method of weaning is to decrease the dose by 50% at 4 h to 6 h intervals as long as the OI remains at ≤10. Once a dose of 5 ppm has been attained, the dose should be decreased more gradually, by 1 ppm every 4 h and discontinued at 1 ppm if the infant remains well oxygenated in <60% oxygen with PaO_2 consistently >50 mmHg. If deterioration occurs during weaning or after treatment has been discontinued, the dose should be increased to the previous level or iNO therapy should be restarted. Once the infant has improved, weaning should be slower, taking place over a 24 h to 48 h period [20].

Almost 40% of infants with PPHN do not respond or sustain a response to iNO. Adequate lung expansion should be established by increasing Positive End Expiratory Pressure, surfactant therapy and use of High Frequency Ventilation prior to administration of iNO. If oxygenation remains low in spite of ventilator and hemodynamic optimization on iNO, ECMO is considered as a therapeutic option [17].

Inhaled NO is contraindicated in infants with congenital heart disease known to be dependent on right-to-left shunting of blood (such as hypoplastic left heart syndrome, interrupted aortic arch, etc.). There is also a high risk of pulmonary edema in patients with pre-existing left ventricular dysfunction who are placed on iNO [Fig. 2 – lower quadrant]. This underlines the importance of obtaining an echocardiogram prior to iNO therapy, not only to document PPHN and shunting, but also to rule out congenital heart disease [17].

The safe duration of iNO therapy is unknown. The mean duration of therapy in trials was 48 h to 96 h, and most randomized trials demonstrated that 90% of treated infants were off iNO therapy within one week of its initiation. Infants who cannot be weaned from iNO after seven days should be carefully evaluated for other forms of lung pathology and cardiac disease [20].
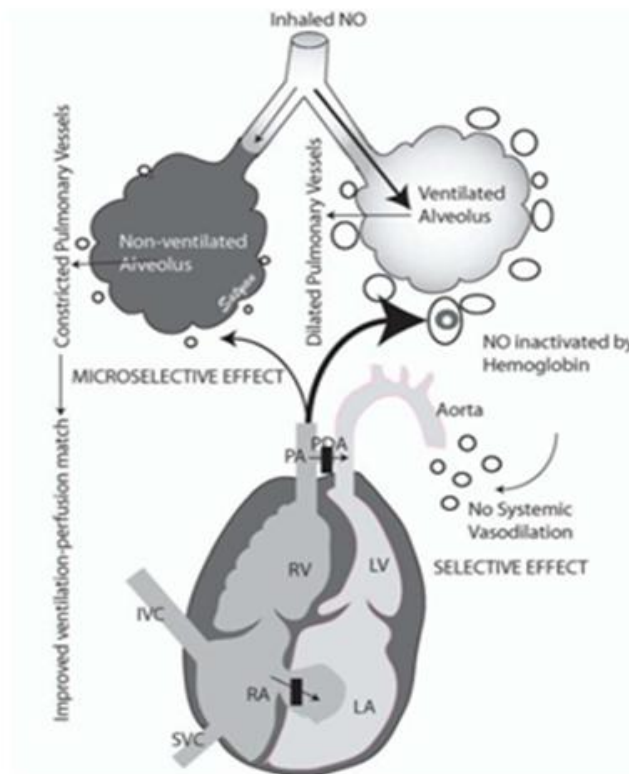
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Fig. 1. Inhaled NO is a selective dilator of the pulmonary circulation without any significant systemic vasodilation as it combines with hemoglobin to form methemoglobin. As it is an inhaled vasodilator, it selectively enters the well ventilated alveoli and improves blood flow to these alveoli and reduces V/Q mismatch [17]
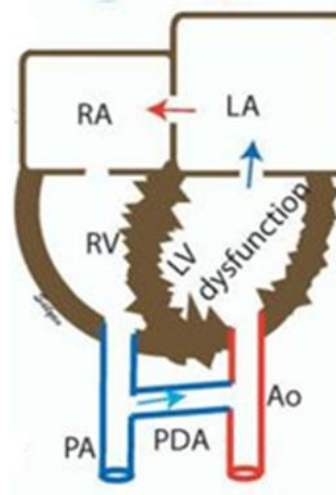
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Fig. 2. Echocardiographic evaluation of neonatal hypoxemia: The presence of right to left shunt at the ductal level and a left to right shunt at the atrial level is associated with left ventricular dysfunction (LVD), pulmonary venous hypertension (PVH) and ductal-dependent systemic circulation (DDSC) is a contraindication for inhaled pulmonary vasodilators such as iNO. PA – pulmonary artery; RV – right ventricle; LV – left ventricle; TR – tricuspid regurgitation; RA – right atrium; LA – left atrium; PDA – patent ductus arteriosus; Ao – aorta [17]

6. CONCLUSIONS

Inhaled NO along with oxygen makes up the first line therapy for PPHN. Inhaled NO is the only FDA approved vasodilator for term and near-term neonates with PPHN. Understanding of the molecular and biochemical processes involved in Nitric oxide synthesis, metabolism and function may provide future insights for the development of better treatment strategies.

CONSENT

Permission obtained from Dr. Satyan Lakshminrusimha for using the figures.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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