

Review Paper**Inhaled Nitric oxide in treatment of Persistent pulmonary hypertension of neonate: An insight into biochemical pathways****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), prostanooids, 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 cyclase and the generation of cyclic GMP. NO can also bind to the ferrous heme forming iron nitrosyl Hb, $\text{Fe}^{\text{II}}\text{NO}$ 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.

Keywords: Nitric oxide, Persistent pulmonary hypertension of neonate, Hemoglobin, Biochemical pathway of NO

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.

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 figure 1 (7, 8, 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 ($O_2^{2\cdot-}$) to form the potent oxidant peroxynitrite ($ONOO_2$). This compound can cause oxidative damage, nitration, and S-nitrosylation of biomolecules including proteins, lipids, and DNA. (10)

Figure 1

Function	Mechanism
Regulates gene transcription	Binding to iron-responsive elements
MRNA translation	Binding to iron-responsive elements
Post-translational modifications of proteins	ADP ribosylation

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 prosthetic 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 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 subunit 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, $\text{Fe}^{\text{II}}\text{NO Hb}$ (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 ($K_d \approx 2.5 \times 10^{-4}$ M). (12) Both of these reaction end up inactivating NO and thus one can imagine many ways Hb can mediate NO signaling.

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 μ M 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.

USE OF NO IN MANAGEMENT OF PPHN

Reducing pulmonary vascular resistance is the main objective of therapy in PPHN, this is done through the administration of vasodilator drugs combined with mechanical ventilation and the inhalation of high concentrations of oxygen. (16)

Inhaled Nitric Oxide is produced by the endothelial cells, low availability of NO is the pathological basis of vascular pulmonary hypertension.(17) iNO has been approved by the U.S. Food and Drug Administration (FDA) for the treatment of PPHN.

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.

It has minimal effects on the systemic vasculature and selectively dilates the pulmonary vasculature. Once NO disseminates to the pulmonary vasculature, it binds to hemoglobin forming methemoglobin, excessive amounts of methemoglobin results in cyanosis. (18) Fortunately the dose used in therapy rarely produces clinically significant methemoglobinemia. (19, 20)

NO can also prolong bleeding time by inhibiting collagen-induced platelet aggregation and adenosine diphosphate expression, although clinically significant bleeding has not been observed in randomized controlled trials.(21,22) Rebound pulmonary hypertension can occur as a complication of abrupt discontinuation of iNO, thus it should be weaned slowly.

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.

BIBLIOGRAPHY

1. Robin H. Steinhorn. Advances in Neonatal Pulmonary Hypertension. Neonatology. 2016; 109: 334-344.
2. Wu H. W, Li Z. G, Liu G, Lu G. Z, Liang H. Y. Effect of nitric oxide inhalation for the treatment of neonatal pulmonary hypertension. Eur Rev Med Pharmacol Sci. 2016 Nov;20(21):4607-4611.
3. Caitlyn L, McPherson C. Treatment of Persistent Pulmonary Hypertension of the Newborn: Use of Pulmonary Vasodilators in Term Neonates. Neonatal Network. Vol. 36, No. 3, May/June 2017
4. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci USA 1987, 84:9265–9269
5. Thomson LL, Lawton FG, Knowles RG, Basley JE, Riversomoreno V, Moncada S. NO synthase activity in human gynecological cancer. Cancer Res. 1994;54:1352–1354
6. Ying L, Hofseth LJ; An emerging role for endothelial nitric oxide synthase in chronic inflammation and cancer. Cancer Res 2007, 67:1407–1410.
7. Khan BV, Harrison DG, Olbrych MT, Alexander RW, Medford RM; Nitric oxide regulates vascular cell adhesion molecule 1 gene expression and redox-sensitive transcriptional events in human vascular endothelial cells; Proc Natl Acad Sci USA 1996; 93:9114–9119.
8. Gudi T, Hong GK, Vaandrager AB, Lohmann SM, Pilz RB; Nitric oxide and cGMP regulate gene expression in neuronal and glial cells by activating type II cGMP dependent protein kinase. FASEB J 1999;13:2143–2152.
9. Pantopoulos K, Hentze MW; Nitric oxide signaling to iron-regulatory protein: direct control of ferritin mRNA translation and transferrin receptor mRNA stability in transfected fibroblasts. Proc Natl Acad Sci USA 1995; 92: 1267–1271.
10. Mikkelsen RB, Wardman P; Biological chemistry of reactive oxygen and nitrogen and radiation-induced signal transduction mechanisms; Oncogene 2003; 22:5734–5754.
11. Dennis Kasper, et al. Disorders of Hemoglobin. Harrison's Principles of Internal Medicine, 19e Eds. New York, NY: McGraw-Hill, 2014
12. Cooper, CE. Nitric oxide and iron proteins. Biochim. Biophys. Acta-Bioenerg 1411:290–309; 1999

13. Herold, S., Exner, M. & Nauser, T. Kinetic and mechanistic studies of the NO-mediated oxidation of oxymyoglobin and oxyhemoglobin. *Biochemistry* 40, 3385–3395 (2001)
14. Liu X, Miller M. J. S, Joshi M. S, Krowicka H. S, Clark D. A, Lancaster J. R. Diffusion-limited reaction of free nitric oxide with erythrocytes. *J. Biol. Chem.* 273, 18709–18713 (1998).
15. Reiter CD, Wang X, Tanus-Santos JE, Hogg N, Cannon RO, Schechter AN, Gladwin MT. Cell-free hemoglobin limits nitric oxide bioavailability in sickle-cell disease. *Nat Med.* 2002 Dec;8(12):1383-9. Epub 2002 Nov 11.
16. Vargas-origel A, Gómez-Rodríguez G, Aldana-Valenzuela C, Vela-Huerta MM, Alarcón-Santos SB, Amador-Licona N. The use of sildenafil in persistent pulmonary hypertension of the newborn. *Am J Perinatol.* 2010 Mar;27(3):225-30. doi: 10.1055/s-0029-1239496. Epub 2009 Oct 28.
17. Cavallaro G, Agazzani E, Andaloro L, Bottura C, Cristofori G, Mussini P, Sacco F, Compagnoni G. Sildenafil and nitric oxide inhalation in neonatal pulmonary hypertension. Three case reports. *Pediatr Med Chir* 2008; 30: 149-155.
18. Oung JD, Sear JW, Valvini EM. Kinetics of methaemoglobin and serum nitrogen oxide production during inhalation of nitric oxide in volunteers. *Br J Anaesth.* 1996;76:652-656.
19. Neonatal Inhaled Nitric Oxide Study Group. Inhaled nitric oxide in fullterm and nearly full-term infants with hypoxic respiratory failure. *N Engl J Med.* 1997; 336:597-604.
20. Davidson D, Barefield ES, Kattwinkel J, et al. The I-NO/PPHN Study Group. Inhaled nitric oxide for the early treatment of persistent pulmonary hypertension of the term newborn: a randomized, double-masked, placebo-controlled, doseresponse, multicenter study. *Pediatrics.* 1998;101: 325-334.
21. Mersal A, Attili I, Alkhotani A. Severe neonatal pulmonary hypertension secondary to antenatal maternal diclofenac ingestion reversed by inhaled nitric oxide and oral sildenafil. *Ann Saudi Med* 2007; 27: 448-449.
22. George TN, Johnson KJ, Bates JN, Segar JL. The effect of inhaled nitric oxide therapy on bleeding time and platelet aggregation in neonates. *J Pediatr.* 1998; 132:731-734.