Mini Review

*Corresponding author
Qiwei Yang, PhD
Research Associate Professor
Department of Pediatrics
College of Medicine
University of Illinois at Chicago
840 S. Wood Street, M/C 856
Chicago, IL 60612, USA
Tel. 312 413-8502
Fax: 312-355-0748
E-mail: qiwei@uic.edu

Neonatal Hyperoxia and Pulmonary Hypertension

Miranda Sun and Qiwei Yang*

Department of Pediatrics, College of Medicine, University of Illinois at Chicago, 840 S. Wood Street, M/C 856, Chicago, IL 60612, USA

ABBREVIATIONS: ACh: Acetylcholine; ALK: Activin-like Kinase; BMP: Bone Morphogenetic Protein; BMPR2: BMP Receptor type II; BPD: Bronchopulmonary Dysplasia; cGMP: Cyclic Guanosine Monophosphate; ETA-R: Endothelin Receptor type A; ETB-R: Endothelin Receptor type B; LRP: Low-density lipoprotein Receptor-related Protein; MYPT1: Myosin-associated Phosphatase Type I; NO: Nitric Oxide; PASMC: Pulmonary Artery Smooth Muscle Cell; PDE: Phosphodiesterase; PH: Pulmonary Hypertension; PPHN: Persistent Pulmonary Hypertension of the Newborn; RVH: Right Ventricular Hypertrophy; sGC: Soluble Guanylate Cyclase; TGF-β: Transforming Growth Factor-β; WISP: Wnt-induced Secreted Protein.

INTRODUCTION

Oxygen therapy and mechanical ventilation with hyperoxia are necessary to treat patients with respiratory distress and failure.[1] However, premature infants requiring oxygen supplementation and ventilation often develop Bronchopulmonary Dysplasia (BPD) as a chronic lung disease, and Pulmonary Hypertension (PH) occurs in 25% to 35% of premature infants with significant BPD. Recent reports indicate that the morbidity and mortality from pulmonary hypertension due to BPD is high, with up to 48% mortality 2 years after the diagnosis of PH.[2] PH is a disease of the pulmonary vasculature defined by an elevated pulmonary vascular resistance leading to right ventricular failure and ultimately death. The effects of adverse environmental factors on a newborn's lungs lead to the failure of the pulmonary circulation to fully adapt to postnatal life. This, in turn, contributes to the pathogenesis of pulmonary vascular dysfunction later in life.[3-5] There is increasing evidence in humans and experimental animal models that exposure to neonatal hyperoxia results in factors that may be linked to the development of pulmonary vascular disease and hypertension. The focus of this review is to elaborate on hyperoxia-activated key sensing molecules and signaling pathways, summarized in Figure 1 and Table 1, in neonatal hyperoxia-induced PH.
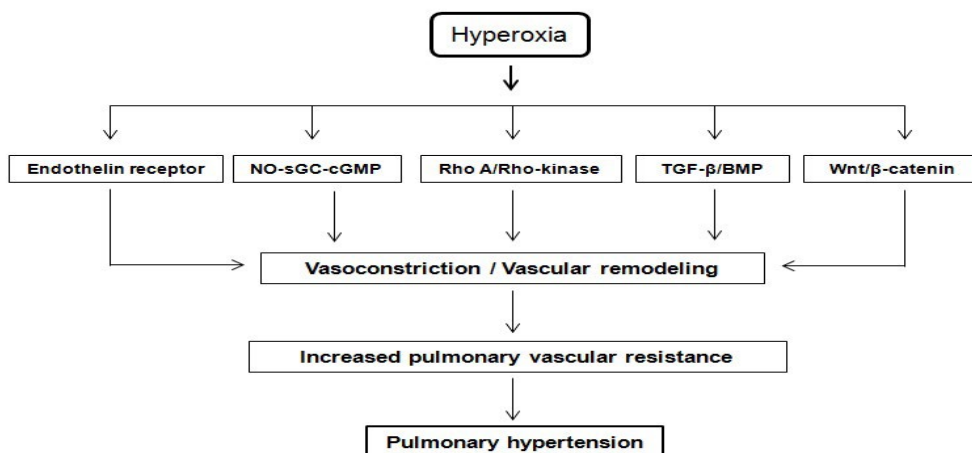

Figure 1: Neonatal hyperoxia modulates signaling pathways leading to development of pulmonary hypertension.

Protein	Signaling pathway	Major functions	Response to hyperoxia	Drug (function)	Dose (route of administration)	Experimental models	Treatment effects	Ref.
PDE5	NO-sGC-cGMP	Hydrolyze and decrease the concentration of cGMP, causing vasoconstriction	Increased expression and activity leading to increased vasoconstriciton	Sildenafil (inhibitor)	50-150 mg/kg/day (subcutaneous injection)	Neonatal rats and mice exposed to hyperoxia	Prolonged survival and reduction of RVH	9-11
PDE4				Piclamilast (inhibitor)	5 mg/kg/day (subcutaneous injection)	Neonatal rats exposed to hyperoxia	Attenuation of RVH	8
sGC	NO-sGC-cGMP	Produces cGMP from GTP, playing an important role in vasodilation	Decreased activity	Cinaciguat (stimulator)	0.05 mg/min (intrapulmonary infusion)	Intrauterine PH in sheep	Significant drop in pulmonary vascular resistance after birth compared with other conditions	11-12
Rho-kinase	RhoA/Rho-kinase	Plays a role in vasoconstriction and maintaining vascular tone	Increased activity	Y-27632 (inhibitor)	10 mg/kg/day (intraperitoneal injection)	Neonatal rats exposed to hyperoxia	Attenuation of MYPT1 phosphorylation and RVH	13-14
ETA-R	Endothelin-1	Induces vasoconstriction and proliferation of smooth muscle cells	Decreased mRNA expression	Ambrisentan (antagonist)	1-20 mg/kg/day (subcutaneous injection)	Neonatal rats exposed to hyperoxia	Attenuation of RVH, pulmonary vascular remodeling, and right ventricular pressure	15
TGF-β superfamily receptors	TGF-β/BMP	Play an important role in cell growth, differentiation, and homeostasis	Down-regulation	Not tested	Not tested	Aged mice exposed to neonatal hyperoxia	Not tested	16-17
LRP5/6	Wnt/β-catenin	Plays a role in cell proliferation and differentiation	Increased activity	Mesd (inhibitor)	(intraperitoneal injection)	Neonatal rats exposed to hyperoxia	Attenuation of RVH, right ventricular systolic pressure, and pulmonary vascular remodeling	18
β-catenin	Wnt/β-catenin	Plays a role in tissue development and remodeling	Increased expression	ICG001 (inhibitor)	10 mg/kg/day (intraperitoneal injection)	Neonatal rats exposed to hyperoxia	Reduction in pulmonary vascular remodeling and PH	19

Table 1: Proteins involved in hyperoxia and pulmonary vascular dysfunction.

NO-sGC-cGMP SIGNALING

Nitric oxide-soluble guanylate cyclase-cGMP (NO-sGC-cGMP) signaling dysregulation has been described in pulmonary hypertensive disease and is a current target of therapeutic agents in humans.[6] Hyperoxic ventilation in the management of Persistent Pulmonary Hypertension of the Newborn (PPHN) results in the formation of reactive oxygen species, such as superoxide anions, which can inactivate Nitric Oxide (NO) and cause vasoconstriction and oxidation. NO is able to act as an antioxidant and has recently been found to improve the oxidative balance in preterm infants.[7]

In the pulmonary vasculature, cGMP concentrations are regulated in part by cGMP-dependent phosphodiesterases (PDEs). PDEs hydrolyze the cyclic nucleotide second messengers of important pulmonary vasodilator agents, including prostacyclin and NO.[8] *In vitro* data have shown that hyperoxia increases PDE5 expression and activity in ovine fetal Pulmonary Artery Smooth Muscle Cells (PASMCs). Exposure of fetal PASMCs to high levels of oxygen leads to decreased responsiveness to exogenous NO. Inhibition of PDE5 activity with sildenafil partially rescues cGMP responsiveness to exogenous NO.[9] In addition, abnormal PDE5 activity has been reported in neonatal animal models of hyperoxia-induced PH. In a rat model, de Visser et al. reported that sildenafil treatment, started simultaneously with exposure to hyperoxia after birth, prolongs survival, increases pulmonary cGMP levels, and reduces Right Ventricular Hypertrophy (RVH).[10] A separate experiment in a neonatal mouse PH model also demonstrated the beneficial effects of sildenafil treatment during chronic hyperoxia or acute hyperoxia with recovery.[11] Untreated animals exhibited increased RVH and disrupted pulmonary artery cGMP signaling. Sildenafil reduced RVH and restored vascular cGMP signaling. As such, PDE5 inhibition with sildenafil has been used to treat severe PH and BPD in humans. Administration of sildenafil is associated with a significant increase in oxygenation as well as a reduction in mortality with no clinically important side effects.

In addition to PDE5, PDE4 has also been observed to play a role in neonatal hyperoxia-induced PH.[8] Although PDE4 inhibition by piclamilast does not advance alveolar development in neonatal rats with hyperoxic lung injury, piclamilast attenuates neonatal hyperoxia-induced RVH when administered either concurrently with hyperoxia exposure or only during the late injury and recovery period.

Experimental animal models have demonstrated

that NO-responsive soluble Guanylate Cyclase (sGC) activity is decreased in chronic hyperoxia-induced PH.[11] Due to its importance in this signaling pathway, sGC has been the target of recent drug discovery efforts in pulmonary disease. For example, riociguat, a sGC stimulator recently approved by the U.S. Food and Drug Administration to treat adult patients with PH, accelerates the production of cGMP by both synergizing with endogenous NO and directly stimulating sGC independent of NO availability, which can be used to restore NO-sGC-cGMP signaling. Chester et al. have reported that cinaciguat (BAY 58-2667), another sGC activator, augments cGMP levels after oxidative stress and causes pulmonary vasodilation in sheep exposed to chronic intrauterine PH.[12] In contrast to the impaired vasodilator response to Acetylcholine (ACh), an endothelium-dependent relaxing agent, cinaciguat-induced pulmonary vasodilation was significantly increased. After birth, cinaciguat also caused a significantly greater fall in pulmonary vascular resistance compared to 100% oxygen, inhaled NO, or ACh. These data suggest that increased NO-insensitive sGC and sGC stimulators may provide favorable treatment strategies for neonatal hyperoxia-induced PH.

Rho A/Rho-KINASE

Several lines of evidence indicate that the Rho A/Rho-kinase pathway plays an important role in the progression of PH. Rho-kinase is one of several factors that contribute to the normally elevated pulmonary vascular resistance of the fetus and newborn.[13] Chou et al. have reported that neonatal hyperoxia increases Rho-kinase activity in rats, and administration of the Rho-kinase inhibitor Y-27632 effectively blocks the hyperoxia-induced Rho-kinase activity in lungs, attenuating the hyperoxia-induced phosphorylation of myosin-associated phosphatase type I (p-MYPT1) and RVH.[14] This study provides evidence that there are beneficial effects from Rho-kinase inhibitors such as Y-27632 in experimental models of neonatal hyperoxia-induced PH, which will hopefully lead to future clinical trials with new potent compounds selectively targeting this pathway.

ENDOTHELIN RECEPTOR

The development of PH is believed to arise in part from disturbances in the balance between endogenous vasoconstrictors and vasodilators. The endothelin signaling pathway is one of the known vasodilatory pathways involved in the pathogenesis and development of PH. Endothelin-1, a potent vasoconstrictor, binds to Endothelin receptor type A (ETA-R) and B (ETB-R) on PASMCs, which leads to vasoconstriction, proliferation, and migration of the PASMCs. On the other hand, vasodilation occurs due to increased production of prostacyclin and NO when endothelin-1 binds to ETB-R on endothelial cells. Therefore, specific targeting of ETA-R while preserving the potential beneficial effects of ETB-R is an important therapeutic strategy for PH patients. For example, strategies for targeting ETA-R have been used in the treatment of adult PH using ambrisentan, an ETA-R antagonist. In addition, Wagenaar et al. have demonstrated that early treatment with ambrisentan improves survival in neonatal rats exposed to hyperoxia and reduces lung fibrin and collagen III deposition, arterial medial wall thickness, and RVH.[15] When neonatal rats were treated during the late hyperoxic and recovery period, ambrisentan did not improve alveolarization and vascularization, but treatment moderated PH, RVH, and right ventricular peak pressure, demonstrating that ambrisentan prolongs survival and attenuates PH but does not affect inflammation or alveolar and vascular development. This study demonstrates that specific ETA-R antagonists like ambrisentan can diminish the adverse effects of endothelin-1, and that targeting the endothelin-mediated vasoconstriction pathway may be beneficial in treating neonatal hyperoxia-induced PH.

TGF-β/BMP SIGNALING

The importance of TGF-β/BMPR2 in PH has been supported by a number of studies in both animal models and clinical research.[16] Moreover, several studies have indicated that neonatal hyperoxia modulates TGF-β/BMP signaling, causes pulmonary vascular disease, and shortens life span in aging mice.[17] Growth and respiratory compliance are significantly impaired in mouse pups exposed to hyperoxia, and these pups also exhibit a pronounced arrest of alveolarization accompanied by dysregulated expression and localization of both receptor (ALK-1, ALK-3, ALK-6, and the TGF-β type II receptor) and Smad (Smads 1, 3, and 4) proteins. Yee et al. reported that mice exposed to hyperoxia between postnatal days 1 to 4 showed a significantly shortened life span compared to siblings exposed to room air by 67 weeks of age. Mice exposed to neonatal hyperoxia exhibited increased RVH and pulmonary wall thickness. BMP receptors and downstream p-Smad1/5/8 were reduced in the lungs of aging mice exposed to neonatal hyperoxia.[17] These data suggest that loss of BMP signaling in aged mice exposed to hyperoxia as neonates is correlated with a shortened life span, pulmonary vascular disease, and associated cardiac failure. However, there is currently a lack of studies focused on the rescuing of BMP signaling in neonatal hypoxia-induced PH.

Wnt/β-CATENIN SIGNALING

Wnt/β-catenin signaling is a key regulator of multiple aspects of embryonic development and tissue homeostasis. Elevated expression of Wnt signaling molecules is observed in the remodeled vessels of patients with idiopathic pulmonary arterial hypertension. The importance of Wnt/β-catenin signaling was also identified in neonatal hyperoxia-induced lung injury and PH. Alapati et al. have reported that inhibition of LRP5/6-mediated Wnt/β-catenin signaling by Mesd, a LRP5/6 inhibitor, attenuates hyperoxia-induced PH in neonatal rats.[18] Hyperoxia exposure markedly induced p-LRP5/6, cyclin D1, and WISP-1 expression in the lungs of animals. Administration of Mesd significantly attenuated hyperoxia-induced RVH, right ventricular systolic pressure, and pulmonary vascular remodeling. However, there was no effect on alveolarization or vascularization after Mesd administration. A further experiment by Alapati et al. demonstrated that treatment with ICG001, a pharmacological inhibitor of β-catenin, significantly increased alveolarization and reduced pulmonary vascular remodeling and pulmonary hypertension during hyperoxia.[19] Administration of ICG001 decreased PASMC proliferation and expression of extracellular matrix

remodeling molecules in vitro in hyperoxia. Finally, these structural, cellular, and molecular effects of ICG001 were associated with the down-regulation of multiple β-catenin target genes. These data indicate that Wnt/β-catenin signaling mediates hyperoxia-induced alveolar impairment and PH development in neonatal animals, thereby suggesting a potential therapeutic target to alleviate PH in neonates with severe BPD.

CONCLUDING REMARKS

Neonatal hyperoxia elicits a distinct phenotype of compromised alveolar and vascular development. Exposure of neonates, both humans and in animal models, to high concentrations of inspired oxygen modulates signaling molecules such as transcriptional factors, protein kinases, receptors, and pro- and anti-apoptotic factors leading to the development of PH and chronic lung disease. However, it is difficult to predict which infants are at increased risk for developing PH. In addition, studies on hyperoxia-induced PH and its treatment are limited, and it is not known why some infants with moderate to severe BPD develop PH while others do not. Furthermore, it has not yet been determined whether neonatal hyperoxia alters epigenetic gene regulation through microRNAs, histone modifications, or DNA methylation leading to PH. So far, oxidative stress due to the generation of reactive oxygen species from hyperoxia has been identified as an important pathological feature in patients with BPD. However, the role and mechanism of oxidative stress in experimental models of neonatal hyperoxia-induced PH have not been well characterized. Moreover, unlike in adult PH, neonatal hyperoxia-induced PH is also associated with impaired lung growth and alveolar development, which continue to have adverse effects later in life. Multiple approaches or combination therapy should be considered for treatment of BPD with PH. It should be emphasized that these studies were performed using preclinical animal models. Therefore, the results are dependent on the animal model chosen and may not perfectly correlate with human diseases. As such, comprehensive studies are needed to fully explore the therapeutic potential of targeting vasodilatory pathways, reversal of vascular remodeling, and regenerative strategies and further our understanding of the mechanisms and pathology of neonatal pulmonary dysfunction that lead to adult diseases.

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