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Therapeutic potential of soluble guanylate cyclase modulators in neonatal chronic lung disease

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Wagenaar GT, Hiemstra PS, Gosens R. Therapeutic potential of soluble guanylate cyclase modulators in neonatal chronic lung disease. *Am J Physiol Lung Cell Mol Physiol* 309: L1037–L1040, 2015. First published October 2, 2015; doi:10.1152/ajplung.00333.2015.— Supplemental oxygen after premature birth results in aberrant airway, alveolar, and pulmonary vascular development with an increased risk for bronchopulmonary dysplasia, and development of wheeze and asthma, pulmonary hypertension, and chronic obstructive pulmonary disease in survivors. Although stimulation of the nitric oxide (NO)-soluble guanylate cyclase (sGC)-cGMP signal transduction pathway has significant beneficial effects on disease development in animal models, so far this could not be translated to the clinic. Oxidative stress reduces the NO-sGC-cGMP pathway by oxidizing heme-bound sGC, resulting in inactivation or degradation of sGC. Reduced sGC activity and/or expression is associated with pathology due to premature birth, oxidative stress-induced lung injury, including impaired alveolar maturation, smooth muscle cell (SMC) proliferation and contraction, impaired airway relaxation and vasodilation, inflammation, pulmonary hypertension, right ventricular hypertrophy, and an aggravated response toward hyperoxia-induced neonatal lung injury. Recently, Britt et al. (10) demonstrated that histamine-induced Ca^{2+} responses were significantly elevated in hyperoxia-exposed fetal human airway SMCs compared with normoxic controls and that this hyperoxia-induced increase in the response was strongly reduced by NO-independent stimulation and activation of sGC. These recent studies highlight the therapeutic potential of sGC modulators in the treatment of preterm infants for respiratory distress with supplemental oxygen. Such treatment is aimed at improving aberrant alveolar and vascular development of the neonatal lung and preventing the development of wheezing and asthma in survivors of premature birth. In addition, these studies highlight the suitability of fetal human airway SMCs as a translational model for pathological airway changes in the neonate.

airway remodeling, calcium response; fetal human airway smooth muscle cells; hyperoxia; oxidative stress; nitric oxide; soluble guanylate cyclase; guanosine 3′, 5′-cyclic monophosphate

TREATMENT OF RESPIRATORY DISTRESS after premature birth with supplemental oxygen may interfere with the development of the immature lung, resulting in aberrant airway, alveolar, and vascular development. Survivors of premature birth are at risk for developing pulmonary arterial hypertension, recurrent wheeze and asthma, and chronic obstructive pulmonary disease (COPD) at relatively young ages (3, 6, 17, 21, 34). The underlying pathology has a multifactorial etiology, and the NO-soluble guanylate cyclase (sGC)-cGMP pathway is involved in many factors that contribute to it. Nitric oxide (NO) is a key signaling molecule in many biological and physiological processes, regulating cell growth, differentiation, proliferation, smooth muscle relaxation, vascular and airway tone, neuronal transmission, and fetal lung liquid production (33). NO is a gas that is produced by nitric oxide synthase (NOS) after conversion of arginine into citrulline and exerts its biological activity via activation of sGC, which converts GTP into the second messenger molecule cGMP (Fig. 1). The intracellular biological effects of cGMP are mediated via activation of cGMP-dependent protein kinases, cGMP-gated ion channels, and cGMP-regulated phosphodiesterases, which degrade cGMP (16). Oxidative stress, either induced by hypoxia or hyperoxia, reduces the NO-sGC-cGMP pathway by oxidizing heme-bound sGC, which leads to sGC inactivation or degradation. Reduced sGC expression or activity is associated with pathology due to premature birth and oxidative stress, including impaired alveolar maturation, smooth muscle cell proliferation and contraction, impaired airway relaxation and vasodilation, inflammation, pulmonary hypertension, right ventricular hypertrophy, and an aggravated response toward hyperoxia-induced neonatal lung injury. This ultimately culminates in bronchopulmonary dysplasia (BPD), which is the most common complication of premature birth in very preterm infants born at $<$30 wk of gestational age (1, 6, 20, 26, 34).

Stimulation of the NO-sGC-cGMP signal transduction pathway has enormous therapeutic potential in pediatric cardiovascular and pulmonary disease by improving aberrant lung development of the immature lung and reducing neonatal lung damage. This is supported by data obtained from animal models of neonatal chronic lung disease or BPD, caused by hypoxia- or hyperoxia-induced oxidative stress (8, 31): *1*) sGC-α1-deficient mice show reduced alveolar development (2) and similar to eNOS-deficient mice show an aggravated response toward hypoxic neonatal lung injury (4); *2*) treatment with inhaled NO improved aberrant hyperoxia- or bleomycin-induced lung development and reduced lung injury in neonatal rats, and in neonatal eNOS-deficient mice with hypoxia-induced lung injury (5, 25, 35, 39); *3*) treatment of hyperoxia-exposed neonatal rats with growth factors that activate eNOS, including vascular endothelial growth factor and apelin, improves aberrant alveolar development and attenuates lung injury (14, 23, 36); and *4*) treatment of hyperoxia- or hypoxia-exposed neonatal rats with the cGMP-specific phosphodiesterase 5 inhibitor sildenafil improves aberrant alveolar development and reduces cardiopulmonary disease (13, 15, 24), but combined inhaled NO and sildenafil did not have a synergistic effect on hypoxia-induced

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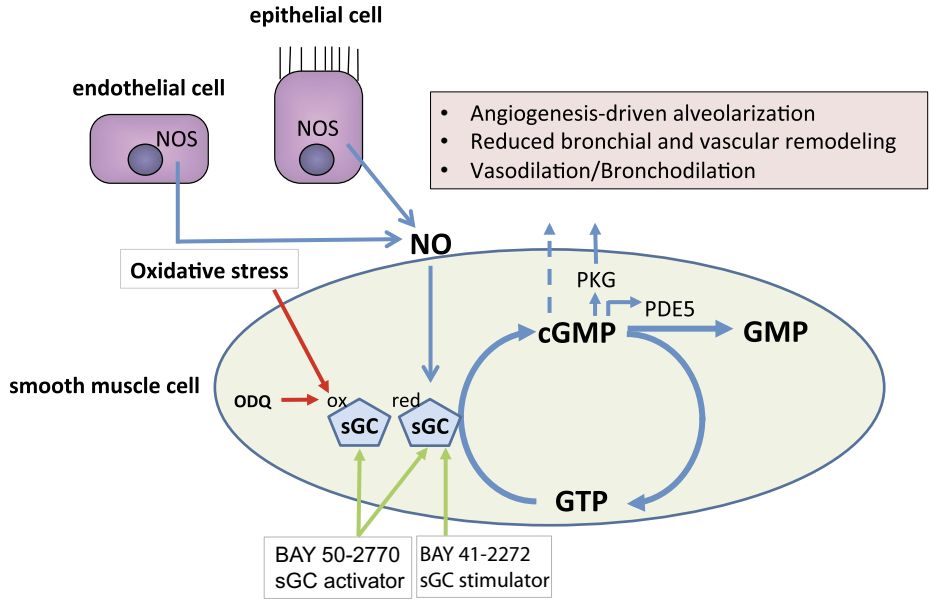

Fig. 1. Regulation of cGMP production by nitric oxide (NO). NO produced by nitric oxide synthase (NOS) enzymes in airway epithelial or endothelial cells activates soluble guanylate cyclase (sGC) in airway or vascular smooth muscle cells. Human endothelial cells express endothelial NOS (eNOS), whereas lung epithelial cells express eNOS, neuronal NOS, and inducible NOS. sGC activation results in the production of cGMP, which is a central regulator in a variety of processes involved in complications following supplemental oxygen treatment in preterm infants. The activity of sGC is inhibited under conditions of oxidative stress (induced by either hyperoxia or hypoxia) or pharmacologically via the sGC oxidizer ODQ, and can be stimulated by pharmacological compounds such as BAY 41–2272 and BAY 60–2770. The sGC stimulator BAY 41–2272 can only activate reduced sGC, whereas the sGC activator BAY 60–2770 can activate both reduced and oxidized heme-bound sGC, resulting in cGMP formation and blunted Ca^{2+} responses in airway smooth muscle cells in a protein kinase G (PKG)-dependent way. The biological effects of cGMP are mediated via protein kinases, including PKG, phosphodiesterases (PDEs) which degrade cGMP and cAMP (in the lung the cGMP-specific PDE5 is the most abundantly expressed PDE), and cGMP-gated ion channels or other mechanisms as indicated by the dashed arrow. Increased intracellular cGMP levels are associated with bronchodilation, vasodilation, reduced vascular and bronchial remodeling, and angiogenesis.

pulmonary hypertension (15). Collectively, these studies indicate that NO-dependent signaling not only modulates lung development but that stimulation of the NO-sGC-cGMP pathway also improves many factors that contribute to aberrant airway, alveolar, and vascular development leading to persistent alveolar simplification, lung inflammation, airway and vascular remodeling, pulmonary hypertension, and fibrosis. Unfortunately, these very promising data could not yet be translated to the clinic, because *1*) inhaled NO had no beneficial effects on BPD development (12, 28) and *2*) the United States Food and Drug Administration posted a Drug Safety Communication recommending against the use of sildenafil in children after unexpected increased mortality in children treated for pulmonary hypertension at high concentrations (7, 27).

Recent studies in animal models provided new clues for the pharmacological treatment of BPD using compounds affecting multiple regulatory pathways (31), involved in endothelin receptor type B inhibition (42), stimulation of the beneficial arm of the renin-angiotensin pathway with agonists of the MAS oncogene receptor and the angiotensin type 2 receptor (43, 44), and stimulation of AMP-activated protein kinase with metformin (11). These recent interventions significantly reduced severe BPD pathology by attenuating septal thickness, vascular remodeling and pulmonary hypertension, lung inflammation, and right ventricular hypertrophy but did not improve alveolar simplification. In contrast, stimulation of the NO-sGC-cGMP pathway was shown to reduce severe BPD pathology and improve aberrant alveolar growth in experimental BPD (13, 14, 35), probably by stimulation of arrested angiogenesis-driven alveolarization (37).

sGC stimulators or activators are NO-independent stimulators of the NO-sGC-cGMP pathway with important therapeutic potential in cardiopulmonary disease (16). Their therapeutic potential is superior to NO or NO donors because they lack uncontrolled NO release, development of tolerance after prolonged administration, and nonspecific interactions of NO with other biological molecules (16). Exposure of the neonatal lung to oxidative stress, either by hyperoxia or hypoxia, reduces the NO-sGC-cGMP pathway by oxidizing heme-bound sGC, which leads to sGC inactivation or degradation. Because sGC stimulators only stimulate cGMP production when sGCβ is bound to reduced heme, whereas sGC activators maintain cGMP production when heme-bound sGCβ is oxidized, sGC activators are preferred under conditions of oxidative stress to maintain the beneficial effects mediated by increased intracellular cGMP levels.

Impact of Neonatal Lung Disease and Oxygen Treatment on Asthma Development

Airway hyperresponsiveness and asthma are serious complicating factors in survivors of premature birth (3, 6, 17, 21). Smooth muscle cells play an important role in asthma by regulating airway tone and play an important role in remodeling, a process that is triggered by oxidative stress. NO is produced by three different isoforms of nitric oxide synthase

(NOS): neuronal NOS (nNOS or NOS-1), inducible NOS (iNOS or NOS-2), and eNOS (or NOS-3), which are all three expressed in the lung. NO, which is produced in large amounts by iNOS, compared with the constitutively expressed nNOS and eNOS, may exert both beneficial and harmful effects in the airways, which depends on the site of production and the amount of NO produced locally (33). NO produced in the lung epithelium by eNOS, nNOS, or iNOS diffuses in adjacent smooth muscle cells, stimulates sGC to synthesize cGMP, and induces bronchodilation. Reduced NO-dependent signaling is associated with airway remodeling and bronchoconstriction. Recently the beneficial effects of activators and stimulators of sGC on aberrant airway development of the immature lung and development of disease were demonstrated in animal models (19, 38), but the relevance of these findings for the human fetal lung were unclear. In this issue of *Am J Physiol Lung Cell Mol Physiol*, Britt et al. (10) investigated the interaction between hyperoxia and sGC on Ca^{2+} responses in human fetal airway smooth muscle cells. Fetal human airway smooth muscle cells are substantially different from adult human airway smooth muscle cells in their proliferative and Ca^{2+}-signaling characteristics and exhibit marked changes in the expression profile of contractile proteins, receptors, and Ca^{2+}-signaling effectors (18). They demonstrated that, upon exposure to moderate hyperoxia, fetal human airway smooth muscle cells show an increased histamine-induced Ca^{2+} response that could be blunted by sGC activators and stimulators in a protein kinase G-dependent way. The sGC stimulator BAY 41–2272 and in particular the sGC activator BAY 60–2770 promoted cGMP formation in fetal airway smooth muscle cells, and more so under conditions of hyperoxia (Fig. 1). BAY 41–2272 promotes cGMP production independent of NO, but only if sGC is bound to reduced heme, whereas BAY 60–2770 can promote cGMP formation by oxidized heme as well. In contrast to these compounds, sildenafil exhibited the opposite characteristics, since it increased cGMP levels under normoxia but blunted responses under hyperoxia, suggesting that sildenafil's actions are suboptimal under conditions of oxidative stress. These data suggest that sGC stimulators or activators may have beneficial effects on hyperoxia-induced dysfunctional Ca^{2+} regulation in the developing airway. Although both sGC stimulation (BAY 41–2272) and activation (BAY 60–2770) were effective, BAY 60–2770 increased cGMP generation even in the presence of the sGC oxidizer ODQ and increased the expression of $sGC\beta_1$, suggesting that sGC activation in the pro-oxidant environment that characterizes the oxygen-treated preterm infant lung is of particular relevance.

An interesting aspect of the work by Britt et al. (10) is the use of fetal human airway smooth muscle cells. Fetal human airway smooth muscle cells are not only different from adult human airway smooth muscle cells in their proliferative, Ca^{2+}-signaling, and contractile characteristics (18), but these cells also respond to cigarette smoke extract with increased proliferation and extracellular matrix protein expression (41). This is of clear relevance to the development of asthma, since fetal exposure to maternal smoking increases asthma susceptibility and the development of airway smooth muscle mass (9). These findings support the suitability of fetal human airway smooth muscle as a translational model and underscore the critical window of opportunity early in life during which asthmatic airway remodeling is established (32). In this respect, the impact of sGC modulators on Ca^{2+} signaling has additional implications, since Ca^{2+} signaling is coupled not only to contraction but also to cell proliferation and extracellular matrix protein production (22, 29, 40). The increased Ca^{2+} response in fetal human airway smooth muscle cells in hyperoxia-exposed cells is in line with previous data demonstrating a concentration-dependent increased proliferative response under mild to moderate hyperoxia (30–50%) and hypoxia, whereas proliferation was reduced under high oxygen levels in these cells (60–90%; see Ref. 18). These complimentary proliferative responses to moderate and high oxygen levels were recently confirmed in neonatal mice in which exposure to hyperoxia increased thickness of the airway smooth muscle layer, with a more severe response under moderate hyperoxia (40%) compared with high oxygen levels (70%; see Refs. 30 and 45).

Clearly, these findings are of relevance to the clinical observation that survivors of preterm birth are more prone to develop asthma and wheezing in later life. Recent findings indicate that the development of asthma in preschool wheezers is primarily related to increased airway smooth muscle mass in these children and that those who develop asthma can be differentiated from those who do not based on changes in the airway smooth muscle (32). These findings highlight the importance of the airway smooth muscle to the development of asthma in children and support the contention that the window of opportunity for the development of these airway smooth muscle changes is very early on in life. Clearly future studies in the development of asthma need to take into account this role of the airway smooth muscle and this specific window of opportunity, underscoring the translational value of the use of fetal human airway smooth muscle cells as a model for neonatal exposure of environmental triggers, including oxygen, in the development of asthma later in life.

DISCLOSURES

No conflicts of interest, financial or otherwise are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: G.T.W., P.S.H., and R.G. conception and design of research; G.T.W., P.S.H., and R.G. prepared figures; G.T.W., P.S.H., and R.G. drafted manuscript; G.T.W., P.S.H., and R.G. edited and revised manuscript; G.T.W., P.S.H., and R.G. approved final version of manuscript.

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