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Perinatal Oxygen in the Developing Lung

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Abstract

Lung diseases, such as bronchopulmonary dysplasia (BPD), wheezing, and asthma, remain significant causes of morbidity and mortality in the pediatric population, particularly in the setting of premature birth. Pulmonary outcomes in these infants are highly influenced by perinatal exposures including prenatal inflammation, postnatal intensive care unit interventions, and environmental agents. Here, there is strong evidence that perinatal supplemental oxygen administration has significant effects on pulmonary development and health. This is of particular importance in the preterm lung, where premature exposure to room air represents a hyperoxic insult that may cause harm to a lung primed to develop in a hypoxic environment. Preterm infants are also subject to increased episodes of hypoxia, which may also result in pulmonary damage and disease. Here, we summarize current understanding of the effects of oxygen on the developing lung and how low vs. high oxygen may predispose to pulmonary disease that may extend even into adulthood. Better understanding of the underlying mechanisms will help lead to improved care and outcomes in this vulnerable population.

Keywords

Neonatal; Bronchopulmonary Dysplasia; Asthma; Hypoxia; Hyperoxia

Introduction

Premature birth (defined as birth prior to 37 weeks gestational age) continues to be a significant source of morbidity and mortality in neonatal and pediatric populations. Neonatal lung diseases, including bronchopulmonary dysplasia (BPD), neonatal wheezing, and asthma are particularly prevalent in this population, in large part due to inciting factors to which premature infants are disproportionately exposed. Pathophysiology of these lung diseases is complex and may involve genetic predisposition, perinatal inflammation,

environmental toxin exposure, maternal diet, and frequent requirement of respiratory support, including supplemental oxygen in the perinatal period (Britt et al. 2013; Jobe and Kallapur 2010; Martin et al. 2013).

Due to lung immaturity, preterm infants suffer from an inability to adequately oxygenate and ventilate, and will commonly require prolonged oxygen supplementation often with positive pressure ventilation. The issue of the dose and duration of supplemental oxygen in the perinatal period continues to be an issue of debate, with many ongoing studies seeking to develop guidelines for when and to what extent supplemental oxygen should be provided to neonates in order to balance alleviation of hypoxic effects against the detrimental effects of hyperoxia. This issue is further complicated by the fact that transition from the hypoxic *in utero* environment to the 21% oxygen of the postnatal environment fundamentally represents a transition from fetal “normoxia” to a relatively “hyperoxic” environment for the neonatal lung: an effect accelerated by premature birth.

Conversely, infants in the neonatal ICU often experience increased episodes of hypoxia in the setting of apnea of prematurity, which likely represents immaturity of the brainstem and respiratory control centers. This leads to a scenario of fluctuating oxygen saturations, where desaturation is treated with iatrogenic supplemental oxygen leading to relative hyperoxia. The infant is then weaned to room air, where another episode of hypoxia may occur and the cycle repeats in an intermittent hypoxia-hyperoxia pattern. All of this occurs in an immature lung that is not yet ready to buffer these rapid shifts in oxygen tension. What is important to recognize is that infants in all of these situations are at higher risk for asthma, wheezing and other respiratory disorders. What is less clear is how oxygen plays a role in the pathogenesis of pediatric lung diseases. In this review, we summarize the current state of knowledge regarding the ramifications of hyperoxia and hypoxia in the immature and developing lung during this critical perinatal period (Figure 1). In addition to bronchopulmonary dysplasia and alveolar damage, we will also focus specifically on the ramifications of oxygenation for bronchial airway diseases as an emerging area of interest in pediatric diseases.

Hypoxia and the Developing Lung

The issue of hypoxia in the perinatal setting is an intriguing one, particularly in the context of prematurity, as the natural environment for the lungs of premature infants is hypoxic. Oxygen requirements are considerably lower during fetal life, and the fetal lung develops in a markedly hypoxic *in utero* environment (Joshi and Kotecha 2007; Smith et al. 2010). Fetal oxygen supply can be detrimentally influenced by multiple factors such as maternal oxygen supply (obstructive sleep apnea, high altitude) and uterine blood flow (uteroplacental insufficiency, maternal tobacco use) (Haworth and Hislop 2003). Postnatally, despite advances in medical care, exposure to chronic or intermittent hypoxia in the neonatal period as a result of prematurity is common, typically in the setting of apnea of prematurity or as a result of immature lungs and musculature unable to adequately support the neonate in an extrauterine environment (Adams et al. 1997; Di Fiore et al. 2010). These abnormal hypoxic perinatal exposures may compromise alveolar, airway, and pulmonary vascular development, significantly contributing to the pathogenesis of multiple pulmonary diseases.

Fetal hypoxia (fetal pO₂ of 20-30 mm Hg) is critical for lung branching morphogenesis, angiogenesis and extracellular matrix deposition during the pseudoglandular and canalicular stages of lung development (Gebb and Jones 2003). *In vitro* studies using rat lung explants demonstrated an increase in epithelial branching and cellular proliferation when the explants were cultured at 3% oxygen as compared to explants cultured at 21% oxygen (Gebb and Jones 2003). Similar studies in mice demonstrated that explants cultured at 3% oxygen compared with 21% oxygen showed an increase in both epithelial and vascular branching morphogenesis (van Tuyl et al. 2005). These studies highlight the importance of maintaining an appropriately hypoxic environment during prenatal lung development and demonstrate the harm that may occur if the developing lung is exposed to higher levels of oxygen too early, as occurs in premature birth.

Hypoxia Inducible Factors and Vascular endothelial growth factor in prenatal development

The cellular responses to low oxygen tension are mediated, in part, by the highly conserved transcription factors hypoxia-inducible factors (HIFs), which are known to control more than 100 genes involved in cellular metabolism, proliferation, angiogenesis, extracellular matrix formation, and regulation of apoptosis (Hu et al. 2003; Wenger et al. 2005). The HIF transcriptional complex is a heterodimer composed of one of the three oxygen-sensitive subunits (HIF-1 α , HIF-2 α , or HIF-3 α) and the constitutive HIF- β subunit (Hirota and Semenza 2006; Maxwell 2005). The regulation of HIF by oxygen occurs through modifications of the HIF α -subunit while the β -subunit is a constitutively expressed in the nucleus and does not respond to changes in oxygen tension.

Under normoxic conditions, the α -subunit is rapidly degraded by ubiquitination and proteasomal degradation. Oxygen is rapidly utilized as a substrate by prolyl hydroxylases (PHD 1–3) to hydroxylate proline residues on the HIF α -subunit (Maxwell et al. 1999; Ohh et al. 2000). Once hydroxylated, the α -subunit is targeted for ubiquitination and subsequent proteasomal degradation (Yu et al. 2001). Under hypoxic conditions, on the other hand, the degradation of PHDs by E3-ubiquitin ligases is enhanced, allowing stabilization of the α -subunit (Nakayama and Ronai 2004). The α -subunit then accumulates in the nucleus and subsequently binds to the β -subunit, allowing it to recognize hypoxia response elements (HRE) within the promoter regions of hypoxia-responsive target genes. HIF regulates multiple genes that drive vascular development, most notably vascular endothelial growth factor (VEGF). VEGF expression is regulated by HIF-1 α which encodes a transcription factor that is expressed in most, if not all, cells in response to hypoxia (Semenza 2000). VEGF expression is increased under hypoxic conditions and is important for pulmonary vascular and airway development. In the lung, VEGF is expressed by airway epithelial cells while its receptors are expressed in the mesenchymal cells immediately underlying the alveolar epithelium and vasculature (Acarregui et al. 1999; Groenman et al. 2007). Midtrimester human fetal lung explants cultured at fetal oxygen tension had increased expression of VEGF compared with explants cultured at ambient oxygen cultures (Acarregui et al. 1999). Disruption of pulmonary vascular development, via inhibition of HIF-1 α and VEGF, led to interruption of epithelial branching morphogenesis (van Tuyl et al. 2005). Postnatally, disruption of VEGF-mediated signaling results in diminished early alveolar growth (McGrath-Morrow et al. 2005). These studies suggest that the interplay of HIF-1 α

and VEGF-A under hypoxic conditions may have significant consequences for alveolar, airway, and vascular development.

Hypoxia and alveolar development

Human alveolar development primarily occurs postnatally, beginning at 36 weeks gestational age and extending through early childhood (Davis and Mychaliska 2013; Joshi and Kotecha 2007; Smith et al. 2010). Thus, most alveolar development takes place in the setting of ambient, 21% oxygen air. It has been demonstrated that the effects of hypoxia on alveolar formation depend largely on when hypoxia is experienced (Massaro and Massaro 2002). Mouse pups born to pregnant dams kept in a hypoxic environment during pregnancy developed impaired alveolar development which was associated with reduced HIF-1 α , HIF-2 α , VEGF expression (Tsao and Wei 2013). These data suggest that prenatal maternal hypoxia can have a long term impact on alveolar development. Similarly, postnatal hypoxia exposure to newborn pups also impairs alveolarization (Truong et al. 2008). These effects are mediated through transforming growth factor- β (TGF β) (Ambalavanan et al. 2008), while activation of peroxisome proliferation-activated receptor- γ (PPAR γ) agonists can attenuate these effects (Nicola et al. 2011). Collectively, these studies suggest that pre- and postnatal hypoxia exposure is detrimental to alveolar development, which is due to impaired vascular maturation within alveolar walls. Targeting HIF and VEGF (Vadivel et al. 2014) or antagonizing the effects of pro-fibrotic growth factors such as, TGF β , may be a useful strategy for enhancing postnatal angiogenesis and alveolarization during hypoxia exposure (Nicola et al. 2011).

Hypoxia and the developing airway

Low oxygen tension has multiple effects on structural and inflammatory airway cells which could contribute to development of airway disease in young children. Structural changes to the airway may begin during the perinatal period, particularly in preterm infants. Postnatal hypoxia is one factor that can adversely affect the airway and contribute to structural and functional changes in infants at risk of developing airway disease. In human airway smooth muscle (ASM), hypoxia stimulates proliferation and secretion of angiogenic mediators, such as VEGF and IL-8, which contribute to remodeling mechanisms of human adult and fetal ASM (Hartman et al. 2012; Keglrich et al. 2014). Hypoxia also reduces transepithelial Na⁺ transport and promotes mucus production in airway epithelial cells (Tan et al. 2012).

Preterm infants can also be bombarded by pro-inflammatory events which are influenced by hypoxia. Recent studies observed that pro-inflammatory cytokines, such as TNF, can increase HIF mRNA and protein expression but suppress hypoxia response element (HRE) activity in normoxic and hypoxic conditions in human ASM (Tsapournioti et al. 2013), while treatment with TNF and IL-4 increase HIF transcription and VEGF expression in BEAS-2B cells (Jiang et al. 2010). Hypoxia exposure exacerbates responses to allergen challenge leading to increased inflammation and heightened levels of TGF β (Baek et al. 2013). Meanwhile, disruption of HIF-1 α in airway epithelial cells of newborn pups results in increased susceptibility to ovalbumin-induced allergic airway inflammation (Greenwood et al. 2012). These studies highlight the complexity of hypoxia/HIF and inflammation in the context of airway disease. Although studies suggest that hypoxia can regulate airway

inflammation and remodeling, the extent to which hypoxic exposure affects airway function in newborn infants remains an open question for future investigations.

Hyperoxia and the Developing Lung

At birth, the lung is subject to a major change in oxygen tension when the fluid which fills the lung *in utero* is replaced with ambient air during the first few breaths (Davis and Mychaliska 2013; Smith et al. 2010). This change is critical, especially in premature birth, where crucial lung morphogenesis may be interrupted or impaired. As previously noted, the transition from the hypoxic intrauterine environment to room air at the time of birth is fundamentally a transition to hyperoxia for the neonate. This is particularly true for premature infants who have underdeveloped lungs not yet ready to cope with the additional redox stress of a 21% oxygen environment. To further compound this hyperoxic insult, supplemental oxygen is frequently used in the resuscitation, care, and support of premature infants. While this respiratory support is necessary and beneficial to the neonate in the short term, it is not without risk to long term pulmonary health and is associated with significant morbidities (Bhandari 2010; Deuber and Terhaar 2011; Jobe and Kallapur 2010; Sola and Deulofeut 2006).

Exposure to hyperoxia leads to alterations in pulmonary structure involving both the parenchyma and the airways. Bronchopulmonary dysplasia (BPD) has been the figurehead of hyperoxia-induced neonatal lung disease since first described by Northway et al. in 1967 (Northway et al. 1967). However, even infants who do not develop BPD have been shown to have long-term morbidities associated with preterm birth including increased susceptibility to respiratory infections, childhood wheezing, and childhood and adult asthma (Been et al. 2014; Deuber and Terhaar 2011; Martin et al. 2013; Pramana et al. 2011; Vrijlandt et al. 2013). Multiple animal and *in vitro* studies have demonstrated a possible contributing role for hyperoxic exposure in these airway pathologies (Belik et al. 2003; Hartman et al. 2012; O'Reilly et al. 2014a; O'Reilly et al. 2014b; Wang et al. 2014).

Hyperoxia and Alveolar Formation

The detrimental effects of hyperoxia have been most commonly associated with its effects on alveolar formation. Bronchopulmonary dysplasia (BPD) was initially described as disease due to barotrauma and lack of surfactant in the setting of respiratory support resulting in severe inflammation and fibrosis (Northway et al. 1967). With improvements in neonatal care, BPD is now characterized by mild inflammation, abnormal alveolarization and interstitial fibrosis in the context of prematurity and a combination of other insults (Jobe and Kallapur 2010; Schulzke and Pillow 2010; Velten et al. 2010). The direct effects of oxygen on development of BPD are not clear cut due to the concomitant presence of other risk factors such as infection, mechanical ventilation, maternal and neonatal nutrition, and the risk of preterm birth. While it is difficult to tease apart the contribution of each of these factors to the overall clinical outcome, it has been well documented that hyperoxia is contributory to alveolar simplification and decreased gas exchange (Bhandari 2010; Buczynski et al. 2013; Jobe and Kallapur 2010; Velten et al. 2010). Air spaces gradually develop and expand during the saccular phase (24-36 weeks gestational age) while secondary septation and true alveolar development begins at approximately 36 weeks

gestational age and extends through about the age of 2 (Davis and Mychaliska 2013; Joshi and Kotecha 2007; Smith et al. 2010). Premature infants are born during the saccular phase resulting in disruption of appropriate alveolar formation.

The additional insult of hyperoxia on alveolar formation has been particularly demonstrated in animal models of bronchopulmonary dysplasia. Newborn mouse models of alveolar simplification similar to BPD have been developed by exposure of neonatal mice to 85-100% hyperoxia immediately following birth (Warner et al. 1998; Yee et al. 2009). Similarly, in a study of preterm ventilated baboons, it was demonstrated that the level of hyperoxic exposure may play a critical role in alveolar morphology and overall pulmonary outcome (Delemos et al. 1987). In this study, preterm baboons exposed to 100% oxygen for 11 days developed severe lung injury and BPD anatomy while those exposed to a lower level of 30% oxygen had minimal lung damage. This study and others also highlight the fact that extent and duration of hyperoxia are important. A clinical study by Deulofeut et al. found decreased incidence of chronic lung disease in infants born at <1250g who were treated with target oxygen saturation of 85-92% compared to a similar cohort of infants who had a target oxygen saturation of 92-100% (Deulofeut et al. 2006).

HIF and VEGF in Hyperoxia

As discussed above, stability of HIF-1 α is directly related to oxygen tension: hypoxia stabilizes HIF-1 α while normoxia and hyperoxia result in degradation of this subunit (Hirota and Semenza 2006; Maxwell 2005; Nakayama and Ronai 2004). In the hypoxic intrauterine environment, HIF-1 α increases VEGF and other factors involved in angiogenesis, resulting in appropriate formation of the pulmonary and alveolar vasculature (Groenman et al. 2007). Premature exposure to the relative hyperoxia of the extrauterine environment following premature birth further compounded by supplemental oxygen in the NIUC results in degradation of HIF-1 α , decreased levels of VEGF, and interruption of postnatal pulmonary vascular formation. A number of recent studies have explored the impact of disrupted vascularization on alveolar formation. These studies have demonstrated that vascular development does not simply follow that of the alveoli, but rather that the pulmonary vasculature drives alveolar development (Thebaud et al. 2005). Interruption in vascular formation through inhibition of VEGF results in blunted alveolar formation and may be a key mechanism underlying BPD and decreased alveolarization in premature infants (Vadivel et al. 2014).

A number of studies have examined the use of exogenous VEGF to promote continued postnatal pulmonary vascularization (Kunig et al. 2006; Thebaud et al. 2005). Kunig et al. found that intramuscular administration of recombinant VEGF to neonatal hyperoxia-exposed rats improved alveolarization, although it also acutely increased pulmonary edema (Kunig et al. 2006). A second study also using a rat model of hyperoxia-induced lung injury found improved alveolarization, pulmonary capillary formation, and survival following intratracheal administration of adenovirus-mediated VEGF gene therapy, compared to room air controls (Thebaud et al. 2005). The latter study found that concurrent administration of angiopoietin-1 gene therapy improved vessel maturation and reduced the vascular leakiness noted with VEGF therapy alone.

In light of the issues with increased vascular permeability with just VEGF therapy, focus has shifted to HIF-1 α as a target since it has the potential to mediate a number of factors involved in angiogenesis and alveolarization. Vadival et al. recently demonstrated that intratracheal administration of an adenovirus encoding for a stable form of HIF-1 α increased HIF-1 α and VEGF, and further improved alveolar growth and capillary formation in a neonatal rat model of hyperoxia-induced alveolar arrest (Vadivel et al. 2014). Another recent study by Tibboel et al. utilized mice expressing a stable form of HIF-1 α (Tibboel et al. 2014). They found that HIF-1 α and VEGF levels in the HIF-1 α stable mice were increased under hyperoxic conditions compared to control mice, and these mice demonstrated increased alveolar formation during the early prenatal period. However, the improvement in alveolar formation was transient and ultimately the stable HIF-1 α mice demonstrated worse lung function compared to controls. These studies further highlight the importance of the HIF-1 α -VEGF axis for alveolar formation, particularly in the context of hyperoxia. Modulation of these factors may be a potential treatment for hyperoxic lung injury although, as these studies demonstrate, there is still much to be learned in terms of timing of therapy and modulation of the many factors involved.

Hyperoxia and the developing airway

While alveolar changes with hyperoxia exposure have been a predominant focus over the past decades, an emerging area of interest is the long term impact of premature birth and hyperoxic exposure on the developing bronchial airway. As the pathophysiology of BPD has changed with surfactant therapy and greater awareness of preventing barotrauma, increased airway reactivity, wheezing, and asthma have emerged as significant long term morbidities associated with premature birth (Been et al. 2014; Britt et al. 2013; Holditch-Davis et al. 2008; Martin et al. 2013; Vrijlandt et al. 2013). A recent systemic meta-analysis looking at incidence of childhood wheezing disorders found a 30-90% increased risk of wheezing disorders in children born preterm compared to term infants (Been et al. 2014). Children born very preterm were at the highest risk with a three times greater incidence of wheezing disorders compared to term infants (Been et al. 2014). This increased risk of wheezing and airway disease extends through childhood, with another recent study by Vrijlandt et al. finding increased respiratory symptoms including wheezing and cough as late as five years of age in former premature infants (Vrijlandt et al. 2013). This last study examined respiratory symptoms in moderately premature infants (gestational age 32-37 weeks), indicating that even these relatively healthier premature infants are impacted by respiratory morbidities.

From a mechanistic standpoint, the increased incidence of wheezing and asthma in premature infants is likely multifactorial and may involve airway remodeling, characterized by ASM cell hyperplasia and hypertrophy, increase in ASM reactivity and calcium responses, and increased extracellular matrix deposition, similar to adult asthma. These remodeling changes result in a thicker, more narrow, and hypercontractile airway resulting in obstructive airflow. There is evidence from a number of *in vitro* and *in vivo* papers that hyperoxic exposure may contribute to airway remodeling and may therefore be a significant contributor to development of wheezing and asthma in preterm infants.

Bronchial rings from neonatal rats exposed to 60% oxygen for 14 days demonstrate increased ASM contraction compared to room air controls (Belik et al. 2003). Human fetal ASM show increased proliferation with exposure to moderate hyperoxia (<60%) while higher levels of hyperoxia resulted in increased expression of apoptotic markers and likely toxicity (Hartman et al. 2012). Further evidence of the dose dependent nature of airway remodeling is presented in a recent paper by Wang et al. in which mice exposed to moderate hyperoxia (40%) for 7 days demonstrated increased airway reactivity and increased ASM compared to room air and 70% hyperoxia groups (Wang et al. 2014). Hyperoxia exposure may contribute to neonatal lung injury and airway hyperreactivity through disruption of nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) signaling and increased arginase activity (Ali et al. 2012). In rat pups, hyperoxia exposure has also been shown to increase BDNF leading to increased contraction and impaired relaxation of airway smooth muscle (Prakash and Martin 2014; Sopi et al. 2007). Intriguingly, there is evidence that these remodeling changes may persist beyond the neonatal and childhood years into adulthood. A recent study by O'Reilly et al. examined the bronchiolar airways of 10 month old mice that had been exposed to a week of 65% oxygen at the time of birth. They found that even in adulthood, the airways of hyperoxia exposed mice demonstrated increased ASM and decreased alveolar-bronchiolar attachments compared to room air controls (O'Reilly et al. 2014b). Overall, these studies highlight that early hyperoxic exposures may detrimentally affect the developing airway, predisposing to airway disease both acutely and much later in life.

Hyperoxia influences not only the airway smooth muscle, but may also exert a detrimental effect on the airway epithelium. The airway epithelium, exposed to the external environment, serves as a protective barrier for the airway and plays key roles in the initiation of immune and inflammatory responses that contribute to airway inflammation and remodeling. During the course of hyperoxic lung injury, disruption of the barrier may lead to pulmonary permeability and infiltration of inflammatory mediators and fluid accumulation (Bhandari 2008). Adult human airway epithelial cells have been noted to initiate a pro-inflammatory response and rhinovirus exposed epithelial cells were found to induce IL-8 and RANTES release (Chun et al. 2013). Hyperoxia exposure has been shown to augment these responses by enhancing production of pro-inflammatory cytokines and chemokines induced by TLR4 agonists (Aggarwal et al. 2010).

In vivo animal studies have previously demonstrated that hyperoxia exposure increases airway epithelial layer thickness determined by morphometric analysis (Hershenson et al. 1992). Conversely, a more recent study in neonatal rats revealed that exposure to moderate levels of hyperoxia, FiO₂, 50%, resulted in increased airway smooth muscle area, without an effect seen in the epithelium, connective tissue, or submucosa (Denis et al. 2001). Another study examined more long-term effects of neonatal exposure to hyperoxia in mice and found that at 10 months post-natal age neonatal mice exposed to 65% O₂ from birth until post-natal day 21 demonstrated increased airway smooth muscle, however no differences were seen in the bronchiolar epithelium or collagen deposition (O'Reilly et al. 2014b). This same study points towards a potential multifactorial etiology for alterations in the airway from hyperoxia exposure as neonatal growth restriction also had an effect on airway collagen

deposition (O'Reilly et al. 2014b). Interestingly, the study showed that different from females, males exposed to neonatal hyperoxia regardless of presence/absence of growth restriction demonstrated bronchiolar epithelial thickening. This raises the possibility of gender differences with respect to hyperoxia exposure and its subsequent effects on the airway. There is currently minimal data on the molecular mechanisms involved in how hyperoxia contributes to changes in the airway epithelium. While there is evidence that the detrimental effects of hyperoxia on the airway may extend into adulthood, the underlying mechanisms and intricacies of these effects remain to be elucidated.

Hyperoxia-induced inflammatory response

Hyperoxia is associated with a robust inflammatory response in the acute phase of exposure in the immature lung (Bhandari 2008, 2010; Konsavage et al. 2012). Initial exposure to hyperoxia results in a significant macrophage and polymorphonuclear cell (PMN) infiltration into the immature lung, which results in interstitial edema as well as injury to endothelial and epithelial cells (Bhandari 2008). Immune cell infiltrates, particularly neutrophils, produce significant reactive oxygen species (ROS) that may result in increased mitochondrial stress and activation of apoptotic pathways, resulting in significant cell death and instigation of pulmonary and airway remodeling. This inflammatory response has been well documented in premature infants at risk of developing BPD. Tracheal aspirates and bronchoalveolar lavage fluid from preterm infants has been shown to have elevated levels of pro-inflammatory mediators (Ambalavanan et al. 2009). Increased mast cell and eosinophil levels have also been noted in infants with BPD (Bhattacharya et al. 2012; Brostrom et al. 2007).

Mechanisms of Hyperoxia and Hypoxia Induced Lung Injury

Generation of reactive oxygen species is the mechanism most immediately associated with hypoxia and hyperoxia induced lung injury. Oxygen is necessarily present at the inner mitochondrial membrane as the final acceptor for electrons as part of cellular respiration. However, excess levels of oxygen, as may occur in the setting of supplemental oxygen administration, result in increased generation reactive oxygen species (ROS) that accumulate proportionally to the level of oxygen present in the mitochondria (Bhandari 2008). While somewhat counterintuitive, hypoxia also results in generation of ROS via a superoxide burst that occurs rapidly with hypoxic exposure (Guzy and Schumacker 2006; Hamanaka and Chandel 2009; Hernansanz-Agustin et al. 2014). This burst occurs in the mitochondria and while the exact mechanism of ROS generation is unknown, these bursts have been shown to be necessary for stabilization of HIF-1 α and activation of the HIF system (Guzy and Schumacker 2006; Hamanaka and Chandel 2009). The ROS generated by hyperoxia and hypoxia may be involved in upregulation of transcription factors that can result in alterations in cellular proliferation and apoptosis. Key transcription factors regulated by ROS include HIF, nuclear factor E2 related factor 2 (Nrf2), and AP-1 (Bhandari 2008; Cho et al. 2012; Lee and Choi 2003). Additionally, lipid peroxides can form as a result of hyperoxia. These peroxides result in damage to the inner mitochondrial membrane and may lead to release of cytochrome C and cellular apoptosis. For preterm infants, activation of these transcription factors and pathways can contribute to disruption of postnatal alveolar development,

inflammation, and potentially fibrosis (Figure 2)(Hilgendorff et al. 2014; Madurga et al. 2013).

Mitogen activated pathway kinases (MAPK) p38 and JNK are activated upon oxidative stress and regulate a variety of cellular functions. Jun and Fos family transcription factors are downstream targets of MAPKs and are involved in formation of AP-1 transcriptional complexes. These AP-1 complexes regulate multiple cellular functions including cytokine expression, stress response and cell death (Lee and Choi 2003). In addition to AP-1 activation, hyperoxia can also induce ER stress responses, which are important for determination of cell survival and death. Recent studies show that hyperoxia exposure activates the integrated stress response in newborn rodent lungs and cells isolated from the human lung (Konsavage et al. 2012). Treatment of newborn mice with cyclooxygenase-2 inhibitors reduced hyperoxia-induced lung injury, which was attributed to reduction in the ER stress response and improved alveolarization (Choo-Wing et al. 2013). MAPKs contribute to activation of ER stress responses but may themselves also be activated by mediators of ER stress (Darling and Cook 2014). Thus MAPKs serve as a link between oxidant injury and cellular stress responses. Further identification and understanding of the stress pathways activated in pulmonary cells could provide a novel therapeutic target for infants at risk of developing BPD.

Targeted antioxidant therapies have been a logical field of interest for treatment of preterm infants exposed to hyperoxia, however they have largely been a disappointment in their ability to prevent development of BPD (Ahola et al. 2003; Davis et al. 2003). In recent years, studies related to Nrf2 activation in the neonatal lung have improved our understanding of endogenous antioxidant responses. Under homeostatic redox conditions, Nrf2 is bound to and sequestered by Keap1, and increases in intracellular oxidation leads to activation and degradation of Keap1 (Cho and Kleeberger 2007, 2010; Zhang 2006). Release from Keap1 allows Nrf2 to translocate to the nucleus and activate antioxidants response elements which regulate many antioxidant genes. During hyperoxic lung injury, Nrf2 is important for activating antioxidant mechanisms and preserves cell survival (Cho and Kleeberger 2010; Cho et al. 2006). Newborn mice Nrf2^{-/-} have more lung injury and more pronounced alveolar deficits due to hyperoxia exposure (Cho et al. 2012; McGrath-Morrow et al. 2009). Enhancement of Nrf2 activity may be a useful therapeutic strategy for alleviating the adverse developmental effects of hypoxia and hyperoxia on the developing lung.

Intersection of hypoxia and hyperoxia with systemic inflammation

In addition to hyperoxia and hypoxia, neonates are often exposed to infectious etiologies of inflammation. The intersection of changes in oxygen tension with these infectious inflammatory processes may result in synergistic effects leading to further pulmonary injury and remodeling. Maternal infections or inflammatory states from either intra-uterine or extra-uterine sources are commonly associated with preterm birth (Britt et al. 2013; Genc and Onderdonk 2011; Murthy and Kennea 2007). Perinatal exposure to inflammation and post-natal hyperoxia and/or hypoxia exposure can be proinflammatory and deleterious not only to alveolar development, but also to airway structure and function. The precise

mechanisms of intersection between perinatal inflammation and oxygen tension remain an ongoing area of investigation.

A mouse model exposing pups to systemic maternal inflammation *in utero* and subsequent postnatal hyperoxia demonstrated increased TGF β and phosphorylation of Smad2 corresponding to a heightened pro-fibrotic response (Velten et al. 2012; Velten et al. 2010). This pro-fibrotic response is supported by notable increases in Collagen-I, Collagen-III mRNA expression in the neonatal lungs. Functional data in rats indicate that combined exposure to intra-amniotic LPS and hyperoxia increases airway hyper-reactivity (Choi et al. 2013). These data suggest that the combination of maternal inflammation and hyperoxia exposure can uniquely impact neonatal pulmonary development and function.

Increased free radical production resulting from hypoxia may initiate inflammatory cascades compounding infectious etiologies of inflammation that depress respiratory activity (Martin et al. 2011). A study in rat pups demonstrated that systemic exposure to IL-1 β decreased respiratory activity in response to hypoxia while another study showed similar hypoxic respiratory depression in the context of intra-tracheal administration of LPS (Balan et al. 2011; Olsson et al. 2003). Maternal and fetal pro-inflammatory stimuli can also initiate signaling cascades through HIF-1 α . *In vitro* studies using alveolar type II epithelial cells treated with IL-1 β under normoxic conditions identified increased HIF-1 α activity (Haddad 2002). In spite of the current paucity of data regarding these insults, these studies suggest that examination of the intersection between perinatal infection and hypoxia would be a fruitful area for future investigation.

Discussion and Conclusions

Exposure to hyperoxia and hypoxia remains a critical perinatal issue with significant ramifications for lung development and long term pulmonary health. Premature infants, with their underdeveloped pulmonary structure and immature antioxidant system are at particular risk for harm. Both hyperoxia and hypoxia may result in altered alveolar structure, leading to decreased number of alveoli as well as thickened alveolar walls and septa predisposing to less efficient gas exchange (Figure 1). Airway structure and function may also be detrimentally affected by alterations in oxygen tension leading to increased incidence of reactive airway disease such as asthma, even in adulthood.

All of these detrimental effects have been characterized to varying extents in animal or cell models of the developing lung. However, while many of the effects of hyperoxia and hypoxia are known, a critical gap remains in terms of development of effective clinical interventions to prevent these harms. Supplemental oxygen remains an important and necessary treatment, making it even more important to find ways to prevent the negative side effects it produces. Unfortunately, the effects of changing oxygen tension in the developing lung are quite broad, impacting everything from alveolar and airway structure to causing inflammatory cell infiltration (Figure 2). With so many varying mechanisms and long term effects at play, the possibility of a “silver bullet” to prevent all of the deleterious effects simultaneously is unlikely. However, gaining a better understanding of some of the

key mechanisms, such as ROS generation and oxidant stress, may lead to improved therapy to prevent some of the main harms.

While this review has primarily focused on the issues of hyperoxia and hypoxia in the neonatal lung as isolated insults, the clinical reality is more complex. As noted previously, premature infants are exposed to an often rapidly changing environment with intermittent episodes of hyperoxia and hypoxia occurring throughout the early neonatal period. Currently, there is not an established model of newborn intermittent hyperoxia and hypoxia exposure. However, an intermittent hypoxia-hyperoxia newborn rat model was recently developed to examine retinopathy of prematurity (Winners-Mendizabal et al. 2014). Similar exposure protocols could be explored in newborn rodent models to examine the complex effects of intermittent hypoxia-hyperoxia exposure to improve our understanding of the pathogenesis of lung diseases impacting preterm infants. The effect of these rapid changes in oxygen tension is largely unknown and remains an interesting, albeit complicated, area for future examination.

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	Structure	Key Effects
Hypoxia	Alveoli	<ul style="list-style-type: none"> • ↓ Alveolarization • ↓ Gas exchange • Altered angiogenesis
	Airway Smooth Muscle	<ul style="list-style-type: none"> • ↑ ASM thickness • ↑ Airway inflammation
	Airway Epithelium	<ul style="list-style-type: none"> • ↓ Na⁺ transport • ↑ Mucous secretion
Hyperoxia	Alveoli	<ul style="list-style-type: none"> • ↓ Alveolarization • ↑ Interstitial fibrosis • ↓ Gas exchange • ↑ Macrophage and PMN infiltration • ↑ Interstitial edema • ↑ epithelial cell death
	Airway Smooth Muscle	<ul style="list-style-type: none"> • ↑ AHR • ↑ ASM thickness • ↑ ASM proliferation • ↑ ECM remodeling • ↓ Airway relaxation • ↓ Bronchial-alveolar attachment • ↑ immune cell infiltration
	Airway Epithelium	<ul style="list-style-type: none"> • ↑ Cytokine and chemokine expression • ↑ Epithelial thickness

Figure 1.

Effects of altered oxygen levels on the growing lung. Interestingly, both hypoxia and hyperoxia can induce opposing as well as similar, but overall detrimental changes, in different parts of the lung. In terms of the bronchial airways, increased airway smooth muscle (ASM) cell numbers and overall thickness, and increased contractility are common themes, as are increased inflammation and fibrosis, which likely contribute to an asthma-like phenotype in the growing airway. At the bronchial epithelial level, altered inflammation and mucus production are typical detrimental effects. At the alveolar level, characteristics of bronchopulmonary dysplasia including alveolar simplification and vascular dysmorphogenesis occur with both hypoxia and hyperoxia. The relevance of these contrasting as well as similar changes due to oxygen levels lies in the propensity for development of wheezing and asthma in children.

Figure 2.

Potential mechanisms by which hypoxia vs. hyperoxia affect different lung elements during perinatal development. As with the overall observations of similar and contrasting effects of low vs. high oxygen, there are both common and distinct mechanisms that have been identified as contributory to alterations in epithelium, ASM, extracellular matrix (ECM) and alveolar type I and II (ATI, ATII) cells. For example, the well-recognized hypoxia-induced HIF-1 α enhances vascular endothelial growth factor (VEGF) within bronchial epithelium which in turn can influence airway thickness and fibrosis. Alternatively, both hypoxia and hyperoxia can induce production of pro-inflammatory (e.g. IL-6, IL-8) and pro-fibrotic (e.g. TGF β) mediators. Reactive oxygen species (ROS) is a likely common mechanism that particularly affects the alveolar epithelium.