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Bronchopulmonary Dysplasia

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Bronchopulmonary Dysplasia

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Dedicated to the babies who constantly remind us physicians and scientists to continue to strive to help them breathe easier.

Preface

While I have edited two issues (*BPD: State of the Art—Seminars in Perinatology* August 2006;30:163–232 and *Progress in Experimental and Clinical BPD—Seminars in Perinatology* April 2013;37:59–138) dedicated to various aspects of *Bronchopulmonary Dysplasia (BPD)* previously, putting this book on BPD together was a unique opportunity that was offered to me. Since this is a topic near and dear to many neonatologists given the fact that babies in various phases of this disease can be found in most neonatal intensive care units (NICUs), compiling this book presented some interesting challenges. I wanted an international representation of authors for two reasons. One, BPD is a major problem (or becoming one, in some countries) as neonatologists are getting better at saving premature lives around the world, and two, I wanted to tap into the reservoir of international talent of physicians and scientists who have been tackling this problem and making some significant inroads in understanding the pathogenesis and/or providing clinical care and/or evaluating therapeutic options for this condition. My goal was to share the knowledge of the latest state-of-the-art information provided by this international collaborative exercise with those interested in this disease.

With the above in mind, I first divided the different areas of BPD into four categories highlighting *Basic Research*, *Translational Aspects*, *Clinical Aspects*, and *Novel Therapeutic Options*. Next, I distributed the chapters as noted in the table of contents and went about inviting authors who I felt would provide the latest information, as they were actively involved in that area of work. It was such a wonderful feeling when I was able to secure all my first choices of senior authors, in a fairly short time frame. I am extremely grateful to all of them—I consider them more friends, rather than colleagues—for their acceptance of the respective chapters allotted to them and have them delivered to me by the deadline. I am also grateful to the coauthors for working with their mentors and to all of them for their patience as we went through the editing process. The authors hail from the USA, Germany, France, Canada, Spain, Australia, Finland, and South Korea. I hope all of them will enjoy reading the completed product and appreciate the complementary nature of their contributions.

Putting this book together would have been impossible without the support of the Springer team. I would like to express my heartfelt gratitude to Michael Griffin for all his hard work in dotting the "i's" and crossing the "t's" (or more precisely, checking on affiliations, permissions, as well as locating "missing" figures and tables).

I sincerely hope that this book will provide an excellent reference to an international audience for those wishing to stay up to date with BPD. This should (I hope) spur more research into preventing, curing, or at the very least, ameliorating this devastating disease of immaturity. This would not only improve the short-term outcomes for the babies we care for, but in the long run, also decrease the respiratory and neurodevelopmental consequences that impact their health and impose such a significant financial burden on these infants (and their parents) during childhood, adolescence, and even reaching up to adulthood.

Philadelphia, PA Vineet Bhandari

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Part I
BPD: Basic Research

Chapter 1
Hyperoxia in the Pathogenesis of Bronchopulmonary Dysplasia

Anantha K. Harijith and Vineet Bhandari

Introduction

Bronchopulmonary dysplasia (BPD) is a disease that is multifactorial in origin secondary to genetic and environmental factors including exposure to invasive mechanical ventilation, ante- and post-natal infections, and hyperoxia [1]. Among the aforementioned environmental factors, the contribution of hyperoxia to the pathogenesis of BPD is well established [2–5]. Compulsive evidence of the role of hyperoxia in the causation of BPD was demonstrated in a clinical study by Deulofeut et al. who found decreased incidence of BPD in infants born at <1250 g when they 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 % [6]. A major clinical study that studied the efficacy and safety of supplemental therapeutic oxygen in infants was the Supplemental Therapeutic Oxygen for Prethreshold Retinopathy of Prematurity (STOP-ROP) trial. The STOP-ROP trial randomized infants to two target groups—one with a lower saturation of 89–94 % and the other with a higher level of 96–99 % [7]. The trial found that use of supplemental oxygen at saturations of 96–99 % did not cause additional progression of prethreshold ROP but higher rates of pneumonia and BPD. Those infants with underlying lung disease targeted

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to the higher oxygen saturation range were more likely to be hospitalized and in need of supplemental oxygen and diuretic therapy at 3 months of age. Other clinical studies have also found that chronic exposure to high oxygen saturations can injure the lungs of preterm infants [6, 8].

Transition from in utero life from a relative hypoxic environment into room air at birth in itself is a relatively hyperoxic event, even for a term infant with mature lungs. In case of a preterm infant with less mature lungs, the transition to ex utero environment takes a toll on lung development. The structurally and functionally immature lungs of preterm infants are inadequately prepared to oxygenate the body in room air. This necessitates administration of elevated levels of O_2 to prevent hypoxia, exacerbating the magnitude of the injury from hyperoxic exposure. Clinical evidence of the harmful effects of hyperoxia to the newborn developing lung has emerged recently, as detailed below.

Pathology of New BPD

Advances in medicine along with the use of newer therapies, such as prenatal steroids, exogenous surfactant, and "gentle" ventilation strategies, have increased the survival of infants of younger gestational age. This increase in survival rates of extreme preterm newborns has resulted in an increase in the number of children with BPD and associated respiratory pathologies. It has been noted that along with the improvement in survival the morphology of BPD has changed to what is described as the "new" BPD. "New" BPD occurring in very preterm infants (mean gestational age of less than 28 weeks) is characterized by alveolar hypoplasia and abnormal vascular organization [9, 10]. There is minimal to no fibrosis of lung tissue. This represents an impairment in the saccular stage of lung development due to interference, or an interruption, in the stage of rapid alveolar and distal vascular development of the lung [9, 11]. It has also been argued that BPD represents altered developmental programming of the lung due to external factors. The exact mechanisms leading to the impaired or altered programing of lung development are unclear. Hence, there is an urgent need to understand how prematurity and related exposure to O_2 contribute to the pathogenesis of BPD and morbidity later in life.

Hyperoxia-Induced Animal Models of BPD

Newborn animal models have helped us to understand the effects of exposure to hyperoxia in the developing lung, resulting in histopathologic findings similar to those observed in BPD of the human neonate [2, 12–14]. Newborn animal models of BPD survive at least twice as long as adults in hyperoxia and are noted to have a significantly later onset of inflammation [15, 16]. Along with monocytes/macrophages and lymphocytes, stromal, epithelial, and endothelial cells can release

significant amount of chemokines. Experiments in adult animal species showed that exposure to high levels of O_2 leads to pulmonary endothelial and epithelial cell injury, followed by pulmonary edema and hemorrhage impairing lung function [17–19]. However, some interesting observations have been made regarding the differences in response to hyperoxia in newborn and adult animals [15–17, 20]. Upon exposure to hyperoxia, 100 % of the adult mice die within 3–7 days, whereas most newborn pups survive beyond 1 week [12, 21]. This difference is probably due to the developmental regulation of the response to hyperoxia. This sensitivity to hyperoxia in adult animals has been attributed, in part, to rapid death of microvascular endothelial and alveolar type I epithelial cells [22, 23] leading to respiratory insufficiency.

Newborn mouse models of BPD with alveolar simplification have been developed by exposure of neonatal mice to 85–100 % O_2 following birth [14, 24, 25]. Similarly, in another model of preterm ventilated baboons, it was demonstrated that the percentage of O_2 exposure plays a role in alveolar simplification and the overall outcome [26]. Preterm baboons exposed to 100 % O_2 for 11 days developed severe lung injury and BPD-like lung morphology, whereas animals exposed to a lower level of 30 % O_2 had minimal lung damage.

Premature baboons delivered at equivalent to approximately 30 weeks of gestation (75 % of gestation) in humans and ventilated with the minimum required O_2 to maintain normal arterial oxygen concentrations had significantly less damage than those ventilated with 100 % O_2 [26]. However, baboons delivered at a stage comparable to 26 weeks of gestation (67 % of gestation) in humans and managed with the minimum necessary mechanical ventilation and supplemental O_2 still developed BPD-like lung morphology characterized by alveolar hypoplasia and variable saccular wall fibrosis [13]. Hyperoxia appears to worsen the severity of lung injury in animal models of BPD. Lung injury is likely to occur with lower amounts of supplemental O_2 at earlier stages of lung development.

Reports of BPD in infants <1000 g birth weight with minimal initial O_2 supplementation and ventilatory support suggest that high level of O_2 supplementation is not an absolute requirement for the development of BPD [9, 27–29]. It is a combination of the immature stage of lung development and some degree of (relative) hyperoxia that induces the pathology of BPD. However, supplemental O_2 exposure remains as one of the main inciting agents for the development of BPD.

Mechanism of Lung Injury Secondary to Hyperoxia

Causation of BPD is primarily attributed to the premature exposure to high concentration of O_2 and the production of cytotoxic reactive oxygen species (ROS) and reactive nitrogen species (RNS) that injure developing lungs [30, 31]. Studies in animal models have demonstrated that hyperoxia initially induces focal endothelial cell injury and, with continued exposure, necrosis of epithelial cells [32, 33]. Pulmonary microvascular endothelial cells undergo rapid necrosis, leaving areas of

denuded capillary basement membrane. Disruption of the alveolar–capillary basement membrane leads to exudation of fluid into the alveoli, adversely affecting gas exchange as well as the pulmonary mechanics [34]. Premature infants born during the stage of primary septation have disruption of rapid alveolar formation following exposure to hyperoxia. Primary septation of early air spaces occurs during the saccular phase (24–36 weeks gestational age) and the secondary septation and true alveolar development in the alveolar stage at approximately 36 weeks of gestation extending through 2 to perhaps, 8 years of age [14, 35–37].

In the normal intrauterine development, hypoxia-inducible factor 1 alpha (HIF-1α) produced in the hypoxic environment increases production of vascular endothelial growth factor (VEGF), which is involved in facilitating appropriate formation of the pulmonary and alveolar vasculature [38]. Premature exposure to hyperoxia results in the degradation of HIF-1α followed by decreased VEGF levels and interruption of pulmonary vascular development. Studies have shown that the disrupted vascularization adversely impacts alveolar formation. Thebaud et al. have demonstrated that development of the pulmonary vasculature drives alveolar development [39]. Vadivel et al. showed that inhibition of VEGF interrupted vascular development and blunted alveolar formation [40]. The same authors demonstrated that intratracheal administration of an adenovirus encoding for a stable form of HIF-1α increased HIF-1α and VEGF in the lung tissue. This improved alveolar growth and capillary formation in a neonatal rat model of hyperoxia-induced alveolar arrest. Another study utilized mice expressing a stable form of HIF-1α demonstrated increased alveolar formation during the early prenatal period [41].

Oxygen-induced injury is primarily mediated through both ROS and RNS [42–44]. It is well established that ROS, when produced in moderation, plays a physiological role in mediating intracellular signaling pathways involved in normal cell growth and differentiation as well as cytotoxic responses during host defense [45]. ROS function physiologically as signaling molecules mediating various growth-related responses including angiogenesis. ROS-driven angiogenesis can be regulated by endogenous antioxidant enzymes such as superoxide dismutase (SOD) and thioredoxin. NADPH oxidase (Nox) is the major source of ROS in endothelial cells (ECs). Nox is composed of proteins Nox1, Nox2, Nox4, Nox5, p22phox, p47phox, and the small G-protein Rac1 [46]. In addition to hypoxia and ischemia, NADPH oxidase is activated by various growth factors including VEGF and angiopoietin-1. VEGF stimulates EC proliferation and migration primarily through the VEGF receptor type 2 (VEGFR2, Flk1/KDR) [47]. ROS derived from Nox is involved in VEGFR2 autophosphorylation, leading to induction of transcription factors and genes involved in angiogenesis. Another angiogenic agent known to enhance hyperoxia-induced lung injury is angiopoietin-2 [48, 49]. Increased levels of angiopoietin-2 have been associated with increased BPD and/death in human preterm neonates [50].

Oxidative stress occurs when the production of ROS exceeds the antioxidant capacity of the cell resulting in cellular and tissue injury via lipid peroxidation, DNA damage, and protein oxidation [43]. ROS is generated both by the mitochondrial

system and by the NADPH oxidase systems in lung tissue upon exposure to hyperoxia. Lung ECs generate ROS initially followed by inflammatory cells. It has been shown that both necrosis and apoptosis occur simultaneously in the same cell types upon hyperoxia exposure. It is assumed that both apoptotic and necrotic pathways of cell death might be induced in hyperoxia [51–55].

Studies in human neonates have shown a decrease in levels of antioxidants and an increase in oxidative damage to pulmonary proteins in the epithelial lining fluid or plasma of ventilated oxygen-dependent preterm infants in comparison to those infants who were not oxygen dependent [56–58]. The nutritional state of the newborn and exposure to antenatal corticosteroids also play a role in early-life sensitivity to oxygen [59–64].

ROS generated in excess such as the superoxide anion (O_2^-) reacts with nitric oxide (NO) generated in the vascular endothelium forming peroxynitrite. Peroxynitrite reacts to form additional RNS including nitrogen dioxide ($NO_2^\bullet$), dinitrogen trioxide (N_2O_3), nitrosoperoxycarbonate ($ONOOCO_2^-$), and carbonate radical ($O{=}C(O^\bullet)O^-$). RNS reacts with lipids, thiols, amino acid residues, DNA bases, and low-molecular weight antioxidants while depleting the activity of NO [65]. NO is a key mediator of vascular smooth muscle tone and cell signaling [66].

It is known that alveolar type II epithelial cells are more resistant to hyperoxic injury and participate in repair of the injured lung [67]. Hyperoxia not only triggers increased production of ROS and RNS activating cytotoxic pathways but also protective pathways such as that of the transcription factor nuclear factor-erythroid 2-related factor 2 (Nrf2). Nrf2 is known to regulate the inducible gene expression of antioxidant enzymes, critical in detoxifying oxygen-mediated generation of ROS [68, 69]. Nrf2 knockout mice exhibit aggravated lung injury and the absence of upregulation of antioxidant enzymes in response to hyperoxia [70]. Assessment of the role of Nrf2 activation in the neonatal lung has improved our understanding of endogenous antioxidant responses. Under normal conditions, Nrf2 is sequestered by Keap1, and oxidative stress leads to activation and degradation of Keap1 [71–73]. Nrf2 released from Keap1 translocates to the nucleus and activates antioxidant response elements which modulate antioxidant genes. Nrf2 hence plays a key role in activating antioxidant mechanisms necessary for cell survival [68, 72]. Newborn $Nrf2^{-/-}$ mice have more lung injury and impaired alveolarization due to hyperoxia exposure [74, 75]. In fact, in human subjects, a single nucleotide polymorphism in the Nrf2 promoter region in humans has been identified to increase the risk of acute lung injury [76]. Newborn mice overexpressing mitochondrial manganese SOD (MnSOD) or extracellular SOD (ecSOD) in alveolar type II epithelial cells, and exposed to hyperoxia, had preservation of postnatal lung development [77, 78]. Impaired mitochondrial function promotes alveolar simplification in newborn mice, mimicking the impact of exposure to hyperoxia [79]. P53, a known tumor suppressor protein, protects cells from damage by modulating the transcription of genes that inhibit cell proliferation, promote DNA repair, and facilitate apoptosis of damaged cells. Studies have revealed that p53 can affect the maintenance of