Impaired Pulmonary Vascular Development in Bronchopulmonary Dysplasia

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Key Words

Bronchopulmonary dysplasia · Premature birth · Vascular development · Chronic lung disease of infancy

Abstract

Bronchopulmonary dysplasia (BPD), the chronic lung disease associated with preterm birth, results from the disruption of normal pulmonary vascular and alveolar growth. Though BPD was once described as primarily due to postnatal injury from mechanical ventilation and oxygen therapy after preterm birth, it is increasingly appreciated that BPD results from antenatal and perinatal factors that interrupt lung development in infants born at the extremes of prematurity. The lung in BPD consists of a simplified parenchymal architecture that limits gas exchange and leads to increased cardiopulmonary morbidity and mortality. This review outlines recent advances in the understanding of pulmonary vascular development and describes how the disruption of these mechanisms results in BPD. We point to future therapies that may augment postnatal vascular growth to prevent and treat this severe chronic lung disease.

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Introduction

Bronchopulmonary dysplasia (BPD) was initially described almost 50 years ago as the chronic lung disease that developed when positive pressure ventilation and oxygen therapy were used to treat ‘hyaline membrane disease’ or neonatal respiratory distress syndrome in preterm infants [1]. This form of postnatal lung injury was characterized by diffuse inflammation, heterogeneous parenchymal lung injury with areas of marked hyperinflation alternating with fibrosis and atelectasis, airway smooth muscle hypertrophy, edema, and hypertensive vascular remodeling [1–3]. However, advances in both technology and medical management, including routine use of antenatal corticosteroids, artificial surfactant and protective or non-invasive strategies for mechanical ventilation, have led to a dramatic increase in survival of extremely preterm neonates born much earlier in gestation than those originally described by Northway et al. [1] and Jobe [4]. For these infants with ‘new BPD’, birth occurs during the late canalicular stage of lung development at a time when sufficient thinning of the epithelium has occurred in terminal bronchioles to permit gas exchange and sustain life, well before the terminal airspaces have formed [5–7]. Survival is thus conceivably possible before this stage of maturation only in a setting where extracorporeal oxygenation is provided

and the period has been described as ‘the margin of viability’ [8]. Recently, the NICHD Neonatal Research Network reported a decline in mortality and pulmonary-related mortality among preterm infants born at 22–28 weeks of gestation [9]. However, the incidence of BPD in this population has been reported to be as high as 68% [10]. Importantly, in the setting of increased survival, the prevalence of BPD has increased [11, 121].

Preterm birth results not only in postnatal respiratory distress but also in arrested development of the distal lung [4, 12]. Lungs from children with severe BPD have a histological appearance of alveolar simplification, reduced secondary septation and markedly abnormal microvascular growth [13–15]. In this setting of vascular hypoplasia and impaired gas exchange, hypoxia-induced vasoconstriction causes an elevation of pulmonary vascular resistance that leads to vessel remodeling, pulmonary hypertension (PH) and lasting pulmonary vascular disease [16]. Vascular endothelial growth factor (VEGF), a potent angiogenic factor and mediator of nitric oxide (NO) synthesis in endothelial cells, is decreased in the lungs of preterm infants with fatal BPD [17]. In neonatal rodents, inhibition of angiogenesis results in alveolar simplification similar to that seen in preterm infants with BPD [18, 19]. More recently, angiogenic signaling has been shown to promote epithelial differentiation and alveolarization after pneumonectomy in a mouse model [20]. These observations suggest that preterm birth disrupts angiogenesis to cause BPD. A greater understanding of the mechanisms of impaired pulmonary vascular growth will lead to novel therapies for this high-risk population.

Pulmonary Vascular Development: Angiogenesis and Vasculogenesis

The arrest of growth in the preterm lung results in a decreased capillary density throughout the pulmonary circulation and a smaller cross-sectional area for blood flow with decreased surface area for gas exchange [21]. However, the underlying mechanisms driving pulmonary vascular growth remain the subject of ongoing investigation. In the developing lung, vessel growth occurs by two distinct processes: the direct extension of existing vessels (angiogenesis) and the differentiation of primitive angioblasts and hemangioblasts into *de novo* vascular structures (vasculogenesis) [22, 23]. During branching morphogenesis of the airways, pulmonary vascular structures form in close proximity, suggesting that the airways may form a template for early vascular development [5].

During the later stages of lung growth, sprouting angiogenesis results in further branching of vascular networks that then coalesce to permit blood flow [5]. As alveolarization continues, double capillary layers fuse to become an endothelial monolayer joined in close approximation to the alveolar epithelium [7, 24]. Further division and septation of alveoli into complex acinar units was once thought to occur postnatally throughout infancy but has recently been shown to continue into adolescence [25].

Vascular Disease in Animal Models of Experimental BPD

Experimental animal models continue to contribute to our understanding of how vascular growth is impaired in BPD [26–29]. One of the most commonly studied models of BPD involves the exposure of newborn rodent pups to oxidative stress [27, 29–31]. Hyperoxia causes a simplification of lung structure similar to that seen in BPD, with both alveolar simplification and decreased vessel density. The severity of lung injury, including both structural and functional changes, depends on the concentration of inspired oxygen in a dose-dependent manner [32]. After exposure to 7 days of hyperoxia at birth, adult mice (postnatal age 10 months) were recently shown to have sustained airway hypertrophy as well as a significant, albeit mild, reduction in alveolar complexity long after the exposure impaired vascular development and alveolarization [33]. Neonatal hyperoxia-induced lung injury serves as an excellent model for mechanistic studies to better understand how hyperoxia disrupts lung development and for pre-clinical testing of potential therapies for BPD. The following ‘two-hit’ models combine postnatal hyperoxia with other antenatal stressors to further study the pathogenesis of BPD: (1) hyperoxia and lipopolysaccharide (LPS) to model perinatal inflammation, such as chorioamnionitis [34, 35], (2) hyperoxia and maternal nicotine administration [36] and (3) hyperoxia with intermittent hypoxia, to represent a combined oxidative injury [37–39].

Impaired Angiogenic Signaling in BPD

The association between VEGF signaling and pulmonary vascular growth has been extensively studied in both large and small animal models [40–42]. Disruption of VEGF signaling impairs angiogenesis and decreases alveolarization to cause experimental BPD [18, 43, 44], whereas treatment with recombinant human VEGF as

well as VEGF gene therapy promote angiogenesis to prevent BPD in newborn rats [19, 45]. Antenatal intra-amniotic treatment with soluble fms-like tyrosine kinase-1 (sFlt-1), an inhibitor of VEGF signaling, results in a BPD phenotype with PH in newborn rats [46, 47]. sFlt-1 is elevated in the amniotic fluid of human mothers with preeclampsia – a strong risk factor for the development of BPD in preterm infants [48–50]. Increased sFlt-1 in the tracheal aspirates of preterm newborns may be predictive of BPD [51]. In a recent study of preterm infants by Voller et al. [52], the ratio of VEGF to sFlt-1 was decreased in infants with poor postnatal growth but not directly associated with BPD. Lambs with experimental intrauterine growth restriction demonstrate impaired VEGF signaling and develop a BPD phenotype [53]. This finding is consistent with recent clinical observations that the risks of both BPD and death are greater in growth-restricted preterm infants born at <32 weeks of gestation compared to extremely preterm infants (<28 weeks) with age-appropriate birth weights [54].

Many other pro- and antiangiogenic mediators also contribute to the pathogenesis of BPD [55]. The potent vasoconstrictor ET-1 (endothelin-1) impairs angiogenesis via activation of intracellular Rho-kinase and decreased PPAR- γ signaling [56, 57]. Nebulized rosiglitazone, a PPAR- γ agonist, reduces the severity of hyperoxia-induced lung injury in rat pups [58]. The antiangiogenic mediator endostatin and the ratio of endostatin to angiopoietin-1, a proangiogenic factor, were recently shown to be increased in the serum of infants with severe BPD and PH compared to those with severe BPD without PH and those with no or mild BPD [59]. Mice deficient in endothelial NO synthase demonstrate increased susceptibility to experimental BPD, suggesting that VEGF-NO signaling is a key part of the protective response [60].

Intrapulmonary Shunt Vessels in BPD

Disrupted angiogenesis in BPD not only results in fewer vessels, but some fully developed vessels demonstrate dysmorphic anastomoses. Specifically, intrapulmonary arteriovenous anastomotic vessels have been identified in the lung parenchyma of infants with severe BPD [61]. These shunt vessels, not unlike those seen in alveolar capillary dysplasia, prevent gas exchange from occurring at the alveolar capillary interface [62]. This impairment of gas exchange could result in persistent hypoxemia, hypoxic vasoconstriction of the pulmonary arteries and progression to PH in the preterm infant [55].

Genetic and Epigenetic Regulation of Impaired Angiogenesis

Twin studies have suggested that genetic predisposition accounts for a large portion of BPD risk [63, 64]. However, genome-wide association studies have been variably successful [65, 66] and unsuccessful [67] in identifying candidate single nucleotide polymorphisms associated with BPD. Polymorphisms in the vitamin D receptor gene were recently shown to be associated with BPD in preterm newborns, supporting preclinical studies in which vitamin D augments angiogenesis both in vitro and in lipopolysaccharide-induced experimental BPD [68, 69].

Mediated by antenatal gene-environment interactions, epigenetic mechanisms may directly affect vascular development in infants at risk for BPD. Upregulation of the proangiogenic IGF-1 in the lungs of infants with fatal BPD through histone modification after postnatal mechanical ventilation may reflect a response to injury in the pulmonary vasculature [70–72]. Pulmonary histone deacetylase activity is decreased in experimental BPD with resultant upregulation of p21, a cyclin-dependent kinase that may be proangiogenic [73, 74]. In both mice and human lung tissues, DNA methylation, another form of epigenetic regulation, has been shown to alter the expression of genes associated with lung development and alveolar septation, including a number of genes in angiogenic pathways [75]. Several studies have also shown that epigenetic regulation by microRNA expression is aberrant in BPD [76, 77]. Transgenic miR-150 knockout mice demonstrate increased angiogenesis when exposed to neonatal hyperoxia, which may be due to the upregulation of glycoprotein nonmetastatic melanoma protein B [78]. The differential regulation of gene expression by these and other mechanisms may explain previous observations that mechanical ventilation and oxygen therapy increase antiangiogenic gene expression (thrombospondin-1, endoglin) and decrease the expression of angiogenic genes (VEGF-B, VEGFR-2, Tie-2) [79, 80].

Endothelial Progenitor Cells and Mesenchymal Stromal Cells in BPD

In addition to branching angiogenesis, circulating and resident endothelial progenitor cells (EPCs) appear to contribute to postnatal vasculogenesis [81, 82]. It has been hypothesized that cord blood EPC levels could serve as important clinical biomarkers of BPD risk and indeed

Fig. 1. Paracrine mechanisms mediate the proangiogenic effects of ECFCs and MSCs. PAEC = Pulmonary artery endothelial cell; AT2 = alveolar epithelial type 2 cell; RVH = right ventricular hypertrophy; BM = bone marrow; LPS = lipopolysaccharide; RLM-VEC = rat lung microvascular endothelial cell. CM is collected af-

ter 24 h in culture with either cord blood-derived ECFCs (**a**) or cord blood/marrow-derived MSCs (**b**). ECFC-CM and MSC-CM as well as the cells themselves promote angiogenesis both in vitro and in experimental BPD.

the umbilical cord blood of infants who later develop moderate or severe BPD is deficient in two differing types of EPCs – late-outgrowth endothelial colony-forming cells (ECFCs) and angiogenic circulating progenitor cells [83–85]. However, other progenitor cell populations, including those first described by flow cytometry analysis as ‘triple-positive’ EPCs for their expression of CD34, AC133 and VEGFR-2, do not correlate with BPD outcome and cannot be reliably quantified in human cord blood using currently available antibodies [85–87]. CD34+AC133+VEGFR-2+ EPCs are likely to be angiogenic macrophages, which might explain their correlation with adult cardiopulmonary disease [88].

ECFCs are highly proliferative, capable of self-renewal in clonogenic assays and form *de novo* vessels *in vivo* [89, 90]. VEGF-NO signaling is impaired in circulating ECFCs isolated from the cord blood of preterm infants [91]. Recently, ECFCs were shown to be decreased in the cord blood of mothers with preeclampsia, suggesting that impaired progenitor cell-mediated vasculogenesis is a mechanism through which the infant is affected by this maternal complication of pregnancy [92, 93]. ECFCs may also contribute to the development of the placental vasculature and maternal vascular underperfusion in the placenta is associated with BPD risk [94]. Mothers with placental maternal vascular underperfusion were much more likely to

have preeclampsia, further strengthening the association between preeclampsia, impaired angiogenesis and BPD in the preterm infant. Highly proliferative endothelial subpopulations reside in vessel walls [82] and may serve as a type of resident vascular progenitor that also contributes to the development of the microvasculature. The paradigm that a circulating EPC homes to a vascular bed to engraft and populate the expanding endothelium, either during development or after vessel injury, has been challenged by studies that fail to show sustained EPC engraftment in the pulmonary vasculature [95] and conditioned media (CM) studies that suggest EPCs may act via paracrine mechanisms to induce local angiogenesis [96, 116].

More recently, mesenchymal stromal (stem) cells (MSCs) derived from bone marrow [97, 98], umbilical cord blood [99] or the umbilical cord itself [100, 101] have been considered as potential contributors to BPD pathogenesis and as a promising therapeutic modality [102]. Popova et al. [103] showed that MSCs are increased in tracheal aspirates of preterm infants who go on to develop BPD. Furthermore, airway MSCs isolated from these infants demonstrate autocrine production of TGF- β_1 that promotes their differentiation into a myofibroblast lineage and subsequent fibrotic lung injury [104]. However, bone marrow-derived MSCs do not differentiate into myofibroblasts in the presence of TGF- β_1 activation

[104], which points to how airway MSCs can be indicative of disease while treatment with MSCs from bone marrow is beneficial [102]. Tracheal MSCs are also deficient in the expression of the proangiogenic platelet-derived growth factor receptor, but this gene expression has not been studied in bone marrow-derived MSCs [105].

Augmentation of Angiogenesis to Treat or Prevent BPD

A number of prospective clinical trials have studied whether iNO (inhaled NO) therapy can prevent BPD in human preterm infants, with variable [106] to no success [107–109]. However, a recent retrospective analysis of one of these trials suggested that combining iNO with enteral vitamin A supplementation may improve outcomes in certain preterm infants [110]. A prospective randomized clinical trial is now underway to determine whether 28 days of high-dose vitamin A supplementation reduces BPD and death in preterm infants [111].

ECFC and MSC therapy to promote pulmonary vascular growth continues to be studied in both animal models (fig. 1) and early clinical trials. In neonatal mice, MSCs prevent experimental BPD when administered intravenously [112] and intratracheally [113–115]. MSC-CM prevents experimental BPD by these routes as well as when injected into the peritoneal space [112–114]. ECFC and ECFC-CM therapy prevent hyperoxia-induced experimental BPD in newborn rats [116]. ECFC-CM also augments endothelial cell growth and in vitro angiogenesis [96]. A phase 1 clinical trial demonstrated the safety and feasibility of delivering a single intratracheal dose of MSCs to extremely preterm infants at risk for BPD [117].

A phase 2 trial to determine efficacy is in progress. However, questions persist pertaining to the ideal source of stem cell therapy, the ideal timing and route of delivery, the potential for side effects, and whether perinatal complications of pregnancy affect the utility of autologous progenitor cells from the cord or cord blood for therapeutic use [118, 119].

Conclusion

Preterm birth causes impaired vascular and alveolar growth that leads to BPD, the chronic lung disease of infancy. Maternal complications of pregnancy contribute to the risk of preterm birth and BPD by unclear mechanisms, but associations between these complications and impaired angiogenesis have been identified. As noted in a recent workshop of the National Heart Lung and Blood Institute, the primary prevention of BPD in preterm infants has proven elusive [120]. Nevertheless, further studies such as those described in this review will lead to improved outcomes for this devastating neonatal lung disease.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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