Angiogenic Therapy for Bronchopulmonary Dysplasia
Rationale and Promise
Kurt R. Stenmark, MD; Vivek Balasubramaniam, MD

Bronchopulmonary dysplasia (BPD) is one of the most common and significant medical complications associated with preterm birth, affecting 5000 to 10 000 babies annually. It was originally defined as a disorder occurring in infants who were ventilated for neonatal respiratory distress with the primary lung features being mucosal metaplasia of the airways, emphysema, widespread interstitial fibrosis, and remodeling of the small pulmonary arteries. Improvements in neonatal care, including antenatal steroids, surfactant therapy, better ventilator strategies, and improved nutritional support, although not reducing the incidence of the disease (the overall numbers of patients with BPD has increased), have led to the development of what is now called “the new BPD.” This so-called new BPD is characterized histopathologically primarily by an impairment of alveolar formation, which often leads to long-term global reductions in alveolar number and gas exchange surface area. This impairment of alveolar and vascular development is believed to contribute increased morbidity including the development of late cardiopulmonary disease, exercise intolerance, increased risk of asthma, pulmonary hypertension, and the development of chronic obstructive pulmonary disease in these infants as they grow into adulthood. Thus, therapies aimed at promoting lung alveolar and vascular growth in these infants may be highly beneficial in the prevention of these sequelae of premature birth.

Article p 2477

BPD is a multifactorial disease resulting from the impact of injury (including oxygen toxicity, barotrauma, volutrauma, and infection) on the immature lung. Infants most susceptible to the development of BPD (≤750 to 1000 g and 28 weeks’ gestation) are in an early stage of lung development, probably late canicular or early saccular. At this time, the lung is just beginning to develop the distal airspaces and microcirculation that will ultimately allow it to effectively serve its postnatal role in gas exchange. In the normal fetus, hypoxia and hypoxia-inducible transcription factors appear to play a key role in these processes. With premature birth, the normal sequence of lung vascular development is disrupted, probably as a consequence of alterations in the normally complex signaling between the developing alveolar epithelium and the adjacent mesenchyme and pulmonary capillaries. As such, hypoxic injury to the epithelium could disrupt these critical signaling pathways and disrupt angiogenesis. In addition, direct injury to the developing vasculature could disrupt angiogenesis, which may further impair the alveolarization process. Defining critical factors involved in lung angiogenesis and alveolarization is critical for the design of therapeutic interventions.

Vascular endothelial growth factor (VEGF) is a potent angiogenic growth factor whose importance in the developing lung is evidenced by its presence at the distal tips of the developing lung buds and its receptor, VEGFR-2 (KDR/flk-1) on the endothelium surrounding these developing airways. Exposure of the developing lung to hyperoxia in a number of animal models, including baboons, rats, and mice, decreases the expression of VEGF and/or its receptors and inhibits alveolar development. In addition, it has been shown that antagonism of angiogenesis and, in particular, VEGF, leads to vascular and alveolar simplification in the developing lung. Diminished expression of VEGFR-2 has also been identified as pivotal in the alveolarization process. Interestingly, overexpression of VEGF also leads to loss of integrity of the alveolar capillary membrane and alveolar simplification. The heparin sulfate–binding isoform of VEGF, VEGF188, an isoform whose expression increases significantly shortly before birth, appears especially important in the alveolarization process. Mouse expressing only the diffusible VEGF isoform presented at birth with reduced peripheral air spaces and microvasculature with fewer air blood barriers. That VEGF is important not only in alveolar development but also in the maintenance of alveolar structure is supported by the occurrence of emphysema in adult mice with lung-targeted VEGF inactivation. Collectively, these observations provide strong support for the hypothesis that VEGF-driven angiogenesis is crucial for normal alveolarization and that restoration of VEGF in situations characterized by decreased lung VEGF expression, if achieved in the right balance in the proper location, will enhance lung angiogenesis and thereby promote alveolarization.

In this issue of Circulation, Thebaud et al tested these hypotheses and present convincing evidence that lung-specific adenoviral (Ad) -mediated overexpression of VEGF promotes lung angiogenesis and stimulates alveolarization in a hyperoxia-induced neonatal rat model of BPD. Administration of Ad-VEGF by intratracheal injection allowed deliv-
ery of the gene of interest to the target site (ie, the distal airways and small pulmonary arteries) while avoiding widespread dissemination of VEGF and thus induction of angiogenesis in extrapulmonary sites. Of interest is that VEGF gene transfer also increased alveolar eNOS expression raising the possibility that some of the beneficial effect of VEGF on alveolarization may be NO mediated. This observation is consistent with other recent studies demonstrating concomitant decreases in eNOS protein expression with VEGF and VEGFR-2 in hypoxic neonatal models of hypoaalveolarization.11,15 Lin et al showed that inhaled nitric oxide (iNO) treatment after hypoxic exposure of neonatal rats not only increased overall growth but also improved distal lung growth despite persistent decreases in lung VEGF expression.15 McCurnin et al also demonstrated that iNO treatment preserves alveolar architecture in a primate model of BPD.20 A human clinical trial has suggested that iNO could be beneficial in decreasing the incidence of BPD in premature infants with respiratory failure, although other more recent studies may suggest otherwise.21,22

Perhaps even more important, the present study demonstrates the ability of lung-specific VEGF gene transfer to reverse established hypoxia-induced hypoaalveolarization of the lung. Rescue experiments performed by administering recombinant Ad-VEGF beginning at day 21 of hypoxic exposure restored lung growth, which is perhaps surprising because it has been suggested that hypoxic exposure of neonatal rats produces permanent changes in lung structure. Recent studies with systemic VEGF administration also showed an improvement in alveolar structure during recovery from neonatal hypoxia.23 These observations further support the possibility that interventions aimed at increasing lung growth are possible in established lung disease (ie, BPD).

It has been noted in other organ systems that treatment with VEGF alone can lead to the development of capillaries that are immature and are inherently leaky, probably because they lack a pericyte coating. Le Cras et al have described vessels of this type in the lungs of VEGF–overexpressing mice.16 Thus, the investigators in the present study provided angiopoietin-1 (Ang-1), a protein known to act in stabilizing nascent blood vessels, with VEGF. Using this strategy, they not only observed enhanced angiogenesis but also provided evidence by electron microscopy that this combined gene therapy approach resulted in more mature capillaries that were functionally less permeable. These studies suggest that combined therapies likely will be necessary to re-establish normal capillary development and function in the setting of neonatal lung injuries.

Given the fact that hypoxia and hypoxia-inducible transcription factors are necessary for normal fetal lung growth, other investigators have recently explored the possibility that stabilizing hypoxia-inducible factor-1α and 2α (HIF-1α, HIF-2α), which have important downstream targets including VEGF and NO, may lead to similar improvements in situations of interrupted postnatal lung development. In the vasculature, HIF regulates >500 genes, which are necessary for normal vascular growth and function.24 Studies in the fetal baboon model of bronchopulmonary dysplasia have demonstrated reductions in the lung expression of HIF-1α expression. Thus, it seems possible that preventing HIF degradation under hypoxic conditions could be beneficial. Prolyl-hydroxylase activity is necessary for HIF degradation under normoxic conditions. A variety of prolyl-hydroxylase inhibitors have been described recently. Asikainen et al examined the effect of a number of prolyl-hydroxylase inhibitors in lung tissue and cells and found that they could potentially stabilize HIF even under hypoxic conditions.24 In addition, they found that these prolyl-hydroxylase inhibitors caused the release of VEGF into the culture media of human lung microvascular endothelial cells as well as A459 cells and showed that these changes were mediated, at least in part, through changes in VEGF-2 receptor binding. Preliminary experiments in the premature baboon model of BPD with prolyl-hydroxylase inhibitors have been performed and have shown improvement in overall lung development and lung function. In addition, treatment of hypoxic neonatal rats with the well-known HIF target erythropoietin was recently shown to improve alveolar structure, enhance vascularity, and decrease fibrosis.25

In conclusion, it is becoming increasingly clear that the processes of alveolarization and lung capillary development are tightly linked. These processes are interrupted in the infant born prematurely and the consequent abnormalities on lung growth can have long-lasting effects on lung function. The studies reported by Thebaud and associates raise new possibilities for the treatment of infants with severe chronic lung disease. It seems possible that by augmenting or restoring vascular growth, overall lung growth and ultimately lung function can be restored. Extreme caution must be exercised, however, before extending findings from preclinical studies in rodent models to the treatment of human disease. The hypoxia-exposed neonatal rodent lung displays only the alveolar simplification but not the interstitial fibrosis and severe inflammatory response seen in human infants dying from BPD. Interestingly, a recent study in infants with BPD suggests that the changes in lung architecture are not simply a result of decreased angiogenesis. In fact, there is evidence in some patients that overall lung capillary density is increased.26 Thus, attention must be given to the timing of angiogenic therapy to avoid potentially deleterious effects, such as stimulating abnormal vascular growth. It is possible that stimulating angiogenesis at the wrong time or in the wrong compartment could contribute to rather than ameliorate lung injury. Large systemic (bronchial) collaterals have been observed in infants with BPD.27 Angiogenic therapy could stimulate the growth of these vessels, which have been considered to contribute to the morbidity of the disease. Furthermore, abnormal vascularization could provide a conduit for the continued delivery of inflammatory cells or progenitor cells, which may act in maladaptive ways, causing further fibrosis. The present findings, however, do suggest that we must continue to investigate the possibility that someday we will be able to restore normal lung growth and thus ameliorate one of the major problems of premature birth.

Acknowledgment
Supported by grants NIH SCOR HL-57144-09, NIH PPG HL-14985-33, and NIH K08 HL-073893.
Angiogenic Therapy for Bronchopulmonary Dysplasia: Rationale and Promise
Kurt R. Stenmark and Vivek Balasubramaniam

_Circulation_. 2005;112:2383-2385
doi: 10.1161/CIRCULATIONAHA.105.574061
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/112/16/2383

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/