Specialized Center in Clinical Oriented Research (SCCOR) Update: Mechanisms and Treatment of Lung Vascular Disease in Infants and Children

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In contrast to lung branching morphogenesis, studies of the mechanisms that regulate lung vascular development and that link capillary growth with alveolarization are relatively recent and limited in scope. Lack of information regarding lung vascular growth and its connection with alveolar growth is unfortunate, because developmental abnormalities of the pulmonary circulation contribute to the pathogenesis of several important neonatal cardiopulmonary disorders including pulmonary hypertension (PH) in the newborn.

There is growing recognition that the importance of understanding basic mechanisms of lung vascular growth in the context of human disease may be best highlighted in the setting of bronchopulmonary dysplasia (BPD). BPD is a significant health care problem associated with acute and long-term pulmonary consequences.

Recent data from animal and clinical studies suggest that impaired vascular growth may contribute to abnormalities of lung architecture, especially decreased alveolarization, and thus play a critical role in the pathogenesis of BPD. However, little is known about the mechanisms of pulmonary vascular injury in the immature lung, the impact of this injury on growth and development of the lung, or its contribution to the pathogenesis of BPD and PH.

The overall goal of this SCCOR project is to generate clinical and basic information that will provide insight into the mechanisms contributing to pulmonary vascular abnormalities that characterize BPD, to evaluate currently available therapies aimed at reducing lung injury and restoring vascular and lung growth, and to examine in animal models new approaches to ameliorate perinatal lung injury and restore vascular and lung growth. Two clinical and 2 basic projects address these objectives. The clinical projects evaluate the impact of inhaled nitric oxide (iNO) on BPD and the development of improved techniques to assess the presence of PH and the responses to therapy in infants with PH. The 2 basic projects dissect the mechanisms that contribute to lung vascular remodeling in murine, rodent, ovine, and bovine models and evaluate the effects of novel pharmacological agents on lung vascular disease in these models. The long-term goal is to use information derived from these models to develop new and improved therapies for the infant with BPD and/or PH.

Noninvasive Inhaled NO in Premature Newborns

Project 1 is a randomized, placebo-controlled and masked pilot trial of low-dose, noninvasive iNO in premature newborns (500-1250 grams birth weight) that do not require intubation for respiratory failure in the first 36 hours of life. The rationale and background for this study have been recently summarized.[1,2] The aims of the study are to determine if iNO reduces BPD/mortality in premature newborns who do not require intubation in the first 24 hours of life and to determine if noninvasive iNO treatment decreases early and late pulmonary vascular abnormalities in this population.

Advanced Imaging and Diagnostics for Pediatric PH

The overall goal of Project 2 is to develop and evaluate more comprehensive measures of pulmonary arterial hypertension using a combination of advanced cardiovascular imaging and sophisticated computational modeling. The overall hypothesis for these studies is that pulmonary vascular input impedance provides a more comprehensive measure of pulmonary vascular function than pulmonary vascular resistance (PVR) alone since impedance includes both dynamic (stiffness or compliance) and steady state (resistance) components of the vascular circuit.

Measurement of PVR is the current standard for evaluating PH and pulmonary vascular reactivity in children with pulmonary arterial hypertension (PAH). However, PVR measures only the mean component of right ventricular afterload and neglects pulsatile or dynamic effects. Increased stiffness in the pulmonary vasculature is increasingly appreciated to affect right ventricular afterload and to perpetuate distal pulmonary vascular disease. The investigators in this project, therefore, recently developed and validated a method to measure pulmonary vascular input impedance (a parameter which evaluates dynamic [stiffness] and resistive components of the vasculature) and demonstrated excel-

lent correlation between impedance measurements and PVR as well as a correlation between impedance measurements and pulmonary vascular stiffness.[3,4]

The investigators have demonstrated that impedance can be measured routinely and easily in the cardiac catheterization laboratory. Most importantly, the investigators have demonstrated that impedance is a better predictor of disease outcome in pediatric patients with PAH than is simple measurement of PVR.[4] Similar observations have been made in adult studies of PH by the SCCOR program at Johns Hopkins. They have demonstrated that impedance is a better and more effective way of evaluating PH than measuring PVR alone. Work in this project may establish improved methods to evaluate and follow the impact of pharmacological interventions in patients with PAH.

Circulating Fibrocytes in Hyperoxic Lung Vascular Remodeling

The long-term goal of Project 3 is to determine the role of circulating fibrocytes (precursors of mesenchymal cells) in neonatal lung vascular remodeling. There is good evidence that mesenchymal progenitor cells are recruited to the injured lung in young animals (mice, rats, calves) and play important roles in the pulmonary hypertensive process.[5] There are few data that demonstrate recruitment of progenitor cells to the vasculature of humans with PAH. Therefore, in collaboration with one of the major groups investigating adult PH (Vanderbilt University), tissues from patients with severe PAH were evaluated to determine the presence of cells expressing progenitor cell markers (CD133).

A significant increase in the accumulation of CD133+ cells both in intimal lesions and in the perivascular regions of pulmonary arteries from patients with severe PH was observed. Because questions have arisen as to how these cells might affect vascular structure or function, we evaluated the possibility that they exerted their effects through a process of cell fusion and/or heterokaryon formation. This is one mechanism through which stem cells are often thought to exert their effects. Extensive analysis, however, did not demonstrate any evidence for fusion of these recruited cells to local vascular cells.[6] The recruited inflammatory/progenitor cells appear to exert effects on structure and function of blood vessels through processes other than cell fusion.

These are important findings because they demonstrate that human PAH is associated with progenitor cell recruitment just as has been shown in animal models. We are currently collecting tissues from human infants with PH to carry out similar studies.

In addition, we continue our efforts to determine the mechanisms through which inflammatory cells and progenitor cells are recruited to the lung. We are following up on our observations demonstrating that superoxide radical (O_2^-) plays a critical role in initiating and perpetuating the remodeling process in the injured lung.[7,8] Having shown that transgenic overexpression of EC-SOD attenuated superoxide-induced signaling and dramatically attenuated PH and remodeling, we have embarked on studies (in collaboration with the University of Colorado Denver) to evaluate the effects of EC-SOD mimetics in rodent models of PH.[7]

Hypoxia-Inducible Factors in Neonatal PH

The long-term goal of Project 4 is to develop agents that can specifically increase lung vascularization and thereby restore alveolarization to more normal levels. In important background studies the investigators established that hypoxia inducible factors (HIFs), important regulators of vascular endothelial growth factor (VEGF), are decreased in experimental acute lung injury.[9] The investigators are testing the hypothesis that prolylhydroxylase inhibitors (PHDI), agents that stabilize the transcription factor HIF, can decrease PH in the newborn by restoring the fetal VEGF/eNOS axis.[10]

Unfortunately, in preliminary experiments, PHDIs were found to cause increased lethality in premature baboons, apparently due to immunomodulation with an exuberant inflammatory response (unpublished observation). These effects were thought to be due to overexpression of HIF1α. Therefore, the investigators have worked to develop methods to selectively activate HIF2α in the hopes that activation of this pathway will selectively result in protective angiogenic effects. The investigators have developed targeted stabilization of HIF2α, and they have demonstrated that overexpression of HIF2α increases adenosine A_{2A} receptor expression. Importantly, the investigators show that overexpression of adenosine A_{2A} receptor in endothelial cells can increase endothelial cell proliferation and endothelial branching. Thus, significant progress is being made in determining the mechanisms through which transcription factors can be selectively manipulated to achieve the desired beneficial effects in the neonatal lung.

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