HIF stabilizing agents: shotgun or scalpel?

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THE FETUS DEVELOPS IN HYPOXIA (3–5% O2), which is essential for the vasculature. Vascular growth, in turn, is required for lung development (11). For these processes, the essential role of the transcription factor hypoxia-inducible factor (HIF) is increasingly apparent. Genetic inactivation of either HIF-1α or -2α in mice leads to fetal or neonatal lethality because of major developmental defects including those involving the vasculature.

Preterm delivery occurs in ~12% of births and causes >85% of perinatal illness and death. The premature newborn leaves the hypoxic fetal environment to confront relative hypoxia ex utero. Many of the babies that survive being born before 28 wk of gestation (late canalicular to saccular stages) are left with bronchopulmonary dysplasia (BPD), a chronic lung disease of prematurity. Two hallmarks of this disease are pulmonary vascular and alveolar hypoplasia (11). Hypoxia and HIFs may promote pulmonary vascular and alveolar development, and presence of stable HIF-α subunits may rate-limit HIF function in gene expression. Since HIF-1α and -2α normally are highly expressed in developing lungs during the third trimester and decline promptly in neonatal respiratory distress syndrome (RDS) and evolving BPD (6), prolonging half-lives of HIFs by inhibiting their degradation could be an effective strategy for correcting pulmonary vascular and alveolar defects in BPD (1).

Besides discovery of the HIFs themselves, discovery of their control by the prolyl hydroxylase domain-containing proteins (PHDs), which regulate the α-subunit stability, and by the asparaginyl hydroxylase called factor-inhibiting HIF (FIH), which modulates HIF transcriptional activity, have been recent major boons to our understanding of hypoxic signaling. Upon hydroxylation, HIF α-subunits are targeted for proteasomal degradation. Functional HIF-α expression is therefore regulated at the posttranslational level by the PHDs and FIH. The three known HIF-PHDs and FIH belong to the family of 2-oxoglutarate dioxygenases, as do the collagen prolyl hydroxylases associated with mature collagen synthesis (7).

PHDs have several essential cofactors including molecular oxygen, iron, 2-oxoglutarate, and ascorbate. Indeed, iron chelators like desferrioxamine (DFO) and oxoglutarate analogs like dimethylxaloylglycine (DMOG) are potent inhibitors of PHDs. Both types of PHD inhibitors could, however, be associated with untoward effects in a setting involving preterm neonates. First, DFO, although appearing promising in alleviating alveolar hypoplasia caused by hyperoxic exposure in term newborn rodents, caused cardiovascular collapse and lethality when given to preterm primates to treat RDS (3). The mechanism(s) responsible for the demise of these animals are unknown but could include iron withdrawal from mitochondrial iron sulfur-containing proteins by DFO (4), suppression of mitochondrial respiration because of HIF-1α activation (8, 9), or both. Second, DMOG is a nonspecific PHD inhibitor and therefore might be associated with inhibition of collagen synthesis. Because of the essential role of matrix proteins in developing lung, such less specific PHD inhibitors also could impair lung growth. On the other hand, it is also conceivable that they could limit fibrosis in evolving BPD. PHD inhibition might have more global untoward effects as well, even when using more specific HIF-PHD inhibitors. Very recently, HIFs have been found to have broad immunomodulatory effects (10). Such effects, if not controlled for, could understandably be detrimental for preterm infants in intensive care as these babies commonly suffer from infection, bacteremia, or sepsis.

In this issue of *AJP-Lung*, Groenman et al. (5) have pursued the important topic of effects of PHD inhibition on lung development. Specifically, they investigated the effect of nonspecific PHD inhibitors on lung development during embryonic period (E11.5) in mouse with the principal endpoint of branching morphogenesis. Previously, the same group of investigators elegantly showed in embryonic lung buds starting at E11.5 that branching morphogenesis of fetal lungs was dependent on pulmonary vascular development and could be disrupted by an antisense approach directed against HIF-1α or vascular endothelial growth factor (VEGF) (12). Furthermore, hypoxia was shown to enhance branching of distal lung epithelial and pulmonary vascular tissue (12). In this issue, Groenman et al. (5) show that fetal lung development can be impaired or stimulated in different ways by different PHD inhibition. For example, they demonstrated that DFO caused toxicity to fetal lung explants and that this could be reversed with supplemental iron (5). The investigators also found a toxic effect of cobalt chloride, a chemical hypoxia mimic, on the lung explants (5). The authors performed more extensive studies using DMOG. There they found that higher concentrations of DMOG (500 μM to 1 mM) caused disintegration of explants in experiments lasting for ~48 h (5). Interestingly, these higher DMOG concentrations were inhibitory to epithelial branching, whereas they stimulated vascularization along with changes in VEGF and VEGF receptors Flt-1 and KDR mRNAs (5). By comparison, lower doses of DMOG (25–100 μM) had no effect on lung growth and branching (5), although the impact of these concentrations on HIF signaling were not specifically evaluated in these tissues, and diffusion/penetration of inhibitors into the explants could have been limited or variable. This could imply an opposing effect of HIF stabilization on epithelial branching and vascularization or, since DMOG can inhibit collagen synthesis, a divergent dependence of these processes on collagen production at this stage of development. In any case, given that the explants were destined to begin disintegration after 48 h, one must assume a potential toxic process could be active in them at earlier times.
Since human preterm birth associated with frequent BPD routinely occurs at the late canalicular/saccular stages (2, 11), it would be of interest to extend the current studies of HIF enhancement through PHD inhibition beyond the embryonic period (when independent life of the fetus still is impossible). As noted above, pharmacological strategies to assess this hypothesis would greatly benefit by application of more specific PHD inhibitors that, unfortunately, are generally proprietary at present. Availability of the more specific PHD inhibitors to the research community could help better define their potential utility or harm in perinatal RDS, BPD, asphyxia, brain injury, and other diseases of the prematurely born. The most promising specific inhibitors of PHDs are in drug development and/or human testing (erythropoietin stimulation and ischemia-reperfusion injury). However, as discussed above, the potential for global untoward effects suggest that even more specific approaches are needed to define roles of individual HIFs during development. Such strategies could include, for example, gene silencing of one or more PHDs and/or FIH. For cell or in vitro systems, these may also include viral systems using forced expression of HIF-1α and/or HIF-2α or, better yet, stable mutants of these proteins that avoid PHD and/or FIH interaction. Most valuable will be in vivo studies using transgenic mice with inducible overexpression of such mutants or knockdown of the native protein(s).

PHD inhibition to stabilize HIF(s) holds tremendous future potential for a spectrum of disease conditions involving patients of all ages. However, basic and clinical investigators must remain vigilant. Untoward effects of PHD inhibitors could come in two forms: nonspecific inhibitor effects or those due to global interactions of the pathways themselves. If the two occur simultaneously, interpreting the results could be even more confusing.

GRANTS

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REFERENCES