Hydrogen Sulfide Induced Erythropoietin Synthesis is Regulated by HIF Proteins

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Purpose: Anemia of end stage renal disease affects 90% of patients on hemodialysis and it is a tremendous concern of patients and health care providers. Renal disease creates a state of renal hypoxia, which may contribute to a lack of erythropoietin production from the kidney when low oxygen levels are sensed. This necessitates the use of exogenous erythropoietin preparations.

Materials and Methods: Recent evidence suggests that endogenously derived hydrogen sulfide may mediate oxygen sensing in tissues. Given the known involvement of other small molecules such as nitric oxide in erythropoietin production and the observation of decreased urinary H_2S levels in patients with renal failure, we postulated that H_2S may be the primary mediator of erythropoietin production during hypoxia. PK1, 786-O and Hep3B cells were incubated in hypoxia (1% O_2) for 24 hours. Hypoxic cells were treated with the H_2S donor GYY 4137 and the H_2S inhibitor hydroxylamine. Following hypoxia erythropoietin, HIF-1α, HIF-2α and CBS expression was measured by quantitative real-time polymerase chain reaction and Western blot.

Results: Hydroxylamine administration led to a significant decrease in erythropoietin, HIF-1α, HIF-2α and CBS protein levels during hypoxia. This was rescued by administration of GYY 4137 for erythropoietin, CBS and HIF-2α. Additionally, CSE −/− mice placed in hypoxia for 72 hours showed decreased renal erythropoietin production compared to wild-type mice.

Conclusions: These data suggest previously undocumented interplay of the production and action of H_2S during hypoxia with subsequent erythropoietin production. The use of novel hydrogen sulfide donors could represent an alternative to standard therapies of anemia of renal failure.

Key Words: kidney failure, chronic; anemia; anoxia; erythropoietin; hydrogen sulfide

Abbreviations and Acronyms

CBS = cystathionine-β-synthase
CSE = cystathionine-γ-lyase
EPO = erythropoietin
ESRD = end stage renal disease
HA = hydroxylamine
qRT-PCR = quantitative real-time polymerase chain reaction
SDS-PAGE = sodium dodecyl sulfate-polyacrylamide gel electrophoresis

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End stage renal disease is considered the final stage of chronic kidney disease in which patients experience a number of complications in addition to kidney failure.[1] One such complication is anemia, which can be attributable to a variety of factors with inadequate EPO production being the primary cause.[1,2] EPO, a 34 kDa glycoprotein that promotes erythrogenesis, is produced mainly by the interstitial cells of the peritubular

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capillaries in response to hypoxia.[2,3] Due to inadequate EPO production patients with ESRD who have anemia are often prescribed EPO stimulating agents.[4,5] While EPO stimulating agents are highly beneficial in the early stages of treatment, a number of associated adverse effects often develop in such patients as well as drug resistance.[5] Evidently there exists a need to develop novel and alternative ways to treat the anemia of ESRD.

Patients with ESRD commonly have regions of tubular hypoxia due to progressive interstitial fibrosis, which develops around the peritubular capillaries, thus, decreasing the efficiency of oxygen diffusion and resulting in hypoxia induced cellular damage.[6] To combat this damage cells turn on the HIF pathway, which up-regulates the transcription of various genes, including EPO.[7–11] The master regulator believed to initiate this hypoxic response is the gene HIF-1α, although recent evidence also suggests a compelling role for the HIF-2α gene.[12] When low oxygen levels develop, the HIF proteins stabilize and dimerize to form HIF transcription factor with vast downstream signaling effects.[7]

For cells to initiate the hypoxic response and, thus, trigger the HIF pathway they must first sense low oxygen levels. Currently there are 3 known gasotransmitters, including NO, CO and H_2S, which are believed to aid in oxygen sensing.[13] Endogenous H_2S production occurs in the cellular cytoplasm and mitochondria of mammalian cells from the substrate L-cysteine.[13–15] This is done using the biosynthetic enzymes CBS, CSE and 3-MST (3-mercaptopyruvate sulfurtransferase).[16] Levels of H_2S are relatively low during normoxia but they quickly increase during hypoxia. Additionally, a high level of H_2S biosynthetic enzymes in the renal medulla corresponds to the high levels of HIF found there.[17] Taken together it is believed that H_2S may act as the primary renal oxygen sensor and restore oxygen levels through interaction with the HIF pathway (fig. 1).

The objective of this study was to elucidate the interaction between H_2S and the HIF pathway, and determine its effects on EPO production. Because EPO is produced endogenously by hepatic and renal tissue, we used the renal cell lines PK1 and 786-O, and the hepatic cell line Hep3B to perform our study. Additionally, we chose a CSE −/− mouse model in which to examine the effects of H_2S supplementation at the in vivo level.

It was hypothesized that inhibiting endogenous H_2S production would lead to a decrease in the expression of EPO, HIF-1α and HIF-2α, and other HIF regulated genes, which would then be rescued upon the addition of an exogenous H_2S donor. Overall our results indicate that EPO production is H_2S dependent and this effect is moderated by the HIF pathway.

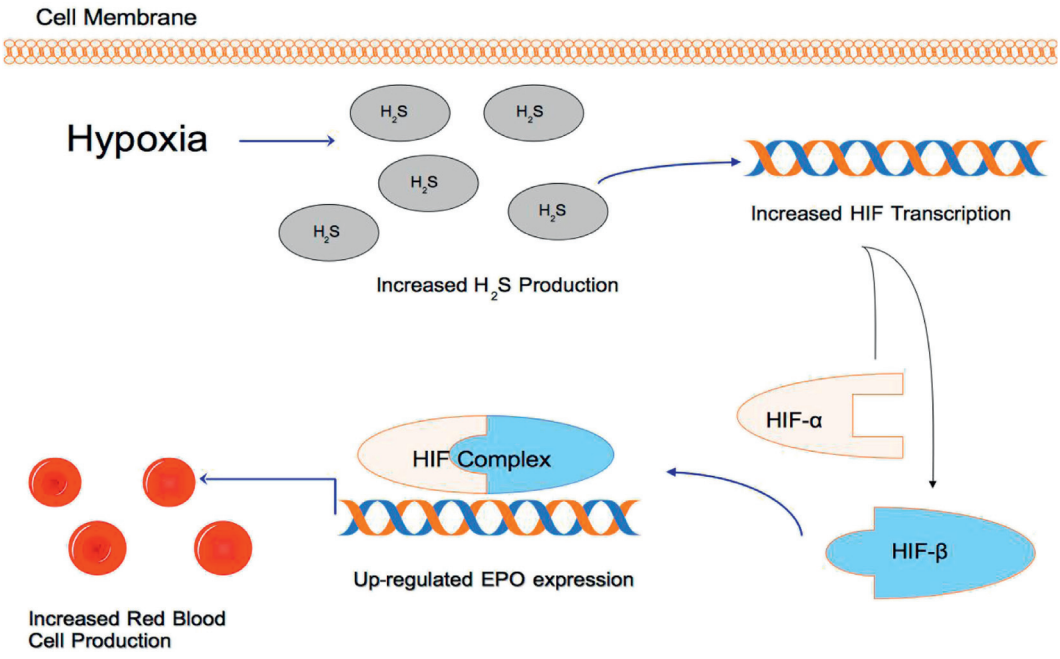

Figure 1. Proposed model of how H_2S stimulates production of EPO and other HIF regulated genes. During hypoxia H_2S levels increase, leading to increased HIF-α subunit transcription. Increased HIF production leads to increased transcription of EPO and other HIF regulated genes.