A Breath of Fresh Air for Diabetic Nephropathy

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doi: 10.1681/ASN.2014080754

Diabetic nephropathy (DN) is a leading cause of CKD and a common complication in patients with type 1 or type 2 diabetes mellitus. The disease, as it progresses through defined morphologic and clinical stages, frequently leads to ESRD. Despite certain therapeutic interventions that slow its progression (such as blockade of the renin-angiotensin axis and strict BP, lipid, and glycemic control), the risk of advancing to ESRD remains high. Innovative therapeutic interventions are urgently needed to halt the development and progression of this devastating disease, which is responsible for approximately 40% of all new ESRD cases in the United States.1

In this issue of JASN, Nordquist and colleagues report that activation of the hypoxia-inducible factor (HIF) pathway with cobalt chloride (CoCl2) protects kidneys from DN in a rat model of type 1 diabetes.2 The investigators used streptozotocin to induce diabetes mellitus and began to treat animals with CoCl2 at the time of streptozotocin administration for 4 weeks. Their study demonstrates that CoCl2 treatment has strong beneficial effects on a functional and morphologic level. By the end of the study, diabetic rats had normal GFR and showed significant improvements in proteinuria and morphologic injury scores. Renoprotection was further associated with the reversal of hyperglycemia-induced mitochondrial dysfunction and a marked improvement in cortical and medullary oxygenation, suggesting that CoCl2 maintained mitochondrial health and protected renal O2 metabolism from the adverse effects of a high-glucose environment. To assess the latter, the authors used their expertise in high-resolution respirometry and mitochondrial function analysis, as well as techniques to measure tubular sodium transport, renal tissue pO2, and renal blood flow.

Cobalt, like other transition metals, such as nickel, has been used experimentally as a hypoxia mimic to activate HIF signaling. In the kidney, the heterodimeric transcription factors HIF-1 and HIF-2 are expressed in a cell type–specific manner and regulate a wide spectrum of biologic hypoxia responses that protect renal cells from hypoxic damage and oxidative stress. These include, among others, anaerobic glycolysis, mitochondrial metabolism and biogenesis, angiogenesis, proliferation, antioxidant defenses, and erythropoietin production.3 CoCl2 inhibits Fe (II)–dependent and 2-oxoglutarate (2OG)–dependent prolyl-hydroxylase domain proteins (PHDs), which function as O2 sensors and control hypoxic/HIF signaling by catalyzing the hydroxylation of specific proline residues within the oxygen–dependent degradation domain of HIF-α.4 These O2 sensors belong to a larger family of 2OG-dependent dioxygenases (approximately 60 family members in mammals), which use molecular O2 for catalysis and regulate multiple biologic processes.5 Under normoxia, hydroxylated HIF-α is targeted for proteasomal degradation by the von Hippel-Lindau–E3 ubiquitin ligase complex, whereas in the absence of molecular O2 hydroxylation is inhibited and HIF signaling is activated. Cobalt also interferes with the binding of the von Hippel-Lindau protein to hydroxylated HIF-α.6

HIF’s role in renoprotection is strongly dependent on cell type and context. While pharmacologic HIF activation ameliorates acute ischemic injuries and holds great promise as a novel therapeutic strategy for the prevention of AKI,7,8 its role in the pathogenesis and progression of CKD remains controversial.9 This may be partly due to differences in the timing of HIF activation (i.e., early versus late-stage disease) and in the CKD models used.10 While the authors provide indirect evidence for cobalt-induced renal HIF activation by measuring the expression levels of classic HIF target genes, such as erythropoietin (Epo), vascular endothelial growth factor, and heme oxygenase-1, HIF-independent signaling events have also probably contributed to renoprotection afforded by CoCl2 treatment. Cobalt is a rather nonspecific PHD inhibitor and is likely to inhibit other 2OG-dependent dioxygenases, which act on non-HIF substrates. Indeed, several studies have compared the effects of hypoxia with the effects of multiple hypoxia mimics on gene expression in different cell types and found only partial overlaps between groups (28% overlap between hypoxia-treated and CoCl2-treated hepatoma cells).11,12 To address the issue of HIF dependence versus non–HIF-mediated effects, future studies must use genetic models or pharmacologic approaches that target the PHD/HIF system specifically, such as PHD-specific structural analogues of 2OG.

The findings by Nordquist et al. advance two important and underappreciated concepts that are relevant for the
pathogenesis of DN: (1) hyperglycemic injury inducing renal hypoxia, which results from mitochondrial dysfunction and increased O2 consumption (QO2) rather than from structural changes (such as extracellular matrix expansion impairing O2 diffusion)\textsuperscript{13} and (2) HIF activation in diabetic kidneys that is submaximal for the observed degree of hypoxia.\textsuperscript{14} The initial conceptual advance of hyperglycemia inhibiting hypoxic HIF-α stabilization was made in the setting of wound healing.\textsuperscript{15,16}

While the molecular mechanisms that underlie mitochondrial dysfunction in DN are complex and only incompletely understood, oxidative stress is a major contributing factor.\textsuperscript{17} The effects of CoCl\textsubscript{2} in this study were, at least with regard to renal oxygenation, mimicked by the acute administration of the antioxidant tempol, suggesting that CoCl\textsubscript{2} may have exerted its renoprotective effects by counteracting hyperglycemia-induced oxidative stress. This notion is consistent with the substantially increased expression of Cu/Zn superoxide dismutase in CoCl\textsubscript{2}-treated rats. Along the same lines, it would be important to determine to what degree the effects of cobalt on hyperglycemia-induced alterations in renal O2 metabolism, O2\textsubscript{2} and tubular sodium transport/O2\textsubscript{2} can be phenocopied by treatment with antioxidants alone, and to what degree cobalt treatment engages mechanisms that are independent of oxidative stress responses. HIF, for example, directly regulates the protein composition of mitochondrial complex 4, thereby optimizing O2 utilization and reducing the generation of reactive oxygen species.\textsuperscript{18}

Previous studies have suggested that HIF activation in response to hypoxia is submaximal or suppressed in diabetic kidneys and can be enhanced by the administration of tempol.\textsuperscript{14} This is in line with the suppression of renal Epo mRNA observed by Nordquist and colleagues; under normal conditions the level of hypoxia measured in the diabetic kidney cortex would have substantially stimulated renal Epo transcription. The molecular mechanisms that underlie this observation are not clearly understood but may involve reactive oxygen species, hyperosmolarity responses in the renal microenvironment, chemical modifications of HIF transcriptional complexes, and various epigenetic changes.\textsuperscript{15,17,19}

In addition to its beneficial effects on renal injury, administration of CoCl\textsubscript{2} decreased blood glucose levels in streptozotocin-treated rats. While the authors suggested that this may be potentially related to increased glucose uptake (a well established feature of HIF activation in many cells types), it also plausible that systemic cobalt administration protected islet cells from streptozotocin-induced injury or had beneficial effects on insulin secretion. Several studies have indicated a protective role for HIF signaling in islet function and survival.\textsuperscript{20,21}

An obvious question raised by the authors’ study pertains to what degree their findings can be translated into clinical medicine. Structural analogues of 2OG, which specifically target PHDs, have been successfully used in clinical trials of correction of renal anemia and could be further investigated for other therapeutic indications. Aside from undesirable off-target effects, such as increased erythropoietin production and raised red blood cell numbers (also observed by the authors), major issues that will have to be addressed include the need for biomarkers that reliably identify patients who would benefit from treatment with HIF activators, the time point at which treatment would need to be started, the duration of treatment, potential adverse effects associated with long-term treatment, and the efficacy of treatment in patients with advanced disease.

While many questions remain unanswered, the studies by Nordquist and colleagues provide strong rationale for targeting O2 metabolism and renal hypoxia for the prevention and treatment of hyperglycemic renal injury. Their findings will certainly prompt additional investigations into how the PHD/HIF pathway can be further exploited for therapeutic intervention in DN.

ACKNOWLEDGMENTS
V.H.H. is supported by the Krick-Brooks Chair in Nephrology, by National Institutes of Health grants R01-DK081646 and R01-DK080821, and by a Department of Veterans Affairs Merit Award (1I01BX002348).

DISCLOSURES
None.

REFERENCES


