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EDITORIAL

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Get use to the -dustats: Roxadustat and molidustat, members of the hypoxia-inducible factor (HIF) prolyl hydroxylase (PHD) inhibitor drug class promote kidney function, perfusion and oxygenation in rats through nitric oxide

In the present issue of *Acta Physiologica*, Burmakin et al uncover a range of important acute effects on kidney function by a new class of drugs, the "-dustats" which are small molecule prolyl hydroxylase inhibitors.[1] Oxygen sensing is a fundamental process of most living organisms, and it can now be targeted pharmacologically. Burmakin et al show in a preclinical approach with rats that systemic administration of two different drugs, roxadustat and molidustat, lowers blood pressure and increases renal plasma flow and GFR. Treatment resulted in improved tissue oxygenation and induction of HIF-regulated erythropoietin (EPO). The effects on renal hemodynamics and GFR by the PHD inhibitors were mitigated by L-NAME treatment and associated with increased NO and NO-synthases suggesting a role for nitric oxide in the acute response to particularly roxadustat. The data add important novel information to our existing knowledge on the short-term effect on kidney hemodynamics of the prolyl hydroxylaste inhibitors, which will likely gain increased use in the years to come based on several ongoing large clinical trials.

It has been a vision since the 1960s to increase especially erythropoietin production pharmacologically. There is concentration-dependent and acute release of EPO from isolated kidneys that relates inversely to oxygen tension,[2] but for years the sensor was unknown. The continuously operating cellular oxygen sensing machinery was unravelled by the 2019 Nobel-laureated work of William Kaelin, Peter Ratcliffe, and Gregg Semenza during the 1990s. They discovered how cells sense and adapt to changing oxygen availability. Central is the hypoxia-inducible transcription factor (HIF). Under hypoxic conditions, nuclear HIF protein accumulates and supports transcription and synthesis of a broad range of signalling molecules and enzymes, for example, VEGF, EPO, adrenomedullin, glycolytic enzymes, glucose transporters and lactate dehydrogenase that counter the detrimental impact of tissue hypoxia and adapts the body to changes in oxygenation.

In kidneys, HIF-1α and 2α proteins are limited to the inner medulla under normoxic conditions and significantly increase by experimental hypoxia and appear in nuclei in distal nephron segments including the thick ascending limb of Henle's loop and collecting ducts.[3] The low level of HIF protein in kidneys with oxygen present is due to its rapid degradation. Residue(s) within the continuously synthesized α-chains of HIF are hydroxylated in the presence of oxygen by three HIF-prolyl hydroxylases, PHD1-3.[4] These enzymes are the real oxygen sensors since, only in the presence of oxygen, the hydroxylation targets HIF for proteasomal degradation through the ubiquitin E3 ligase, von Hippel Lindau factor.[4] With respect to EPO production, PHD2 and HIF-2α are of major importance. The prolyl hydroxylases show widespread localization in kidney tubular cells and vascular tissue, and their activity but not so much their abundance is sensitive to ambient oxygen.[5] What led to the pursuit of PHD inhibitors?

Targeted deletion of VHL in hypoxic injury-prone thick ascending limbs of Henle's loop, an approach not feasible in humans, yields significant protection after an ischemic insult in experimental animals.[6] In other words, priming a specific tissue or in this case, a nephron segment, with increased levels of HIF makes it more resistant to a later insult.

This and other observations combined with the unmet need to stimulate endogenous EPO production in patients with chronic kidney disease (CKD) prompted the development of small molecule drugs to elevate HIF and resulted in the PHD-inhibitor class. At least six PHD ligand antagonists are currently being tested in multiple ongoing clinical trials. Molidustat and roxadustat were used in the present study in *Acta Physiologica*.[1] Most current and published clinical trials as of spring 2021 focus on safety

See related article: Burmakin M, Fasching A, Kobayashi H, Urrutia AA, Damdimopoulos A, Palm F, and Haase VH. 2021.Pharmacological HIF-PHD inhibition reduces renovascular resistance and increases glomerular filtration by stimulating nitric oxide generation. *Acta Physiol* (Oxf). e13668.

and on noninferiority in the treatment of anaemia compared with recombinant human EPO. Burmakin et al set out to map the acute effects on kidney physiological parameters by PHD inhibitors. Such effects are relevant but difficult to predict due to the highly pleiotropic effects of HIF as also confirmed in the Acta study by Burmakin, where 322 genes showed differential regulation by HIF-PHD inhibition.

Beneficial renal and cardiovascular effects of several HIF-derived signalling molecules like VEGF, adrenomdullin and EPO could be hypothesized. The other side of the coin is that several molecules are potent vasodilators and cell cycle regulators, and HIF is not confined to the kidneys but would be affected in multiple tissues. In the most common type of renal cancer, clear cell renal cell cancer, the vast majority of tumours exhibit somatic loss of function mutations in von Hippel Lindau factor and elevated HIF protein and its downstream mediators including angiogenic molecules.[7] Thus, elevated HIF could sustain malignant transformation. On the other hand, the pharmacological approach permits highly controlled periods of intervention although selectivity towards PHD enzyme subtypes is not clear at present. The international group of authors in the Acta study joined forces to exploit their combined expertise within determination of tissue oxygen tension, tubular transport metabolic efficiency and GFR and plasma flow. The study used a thorough and systematic pharmacological approach since two different within-class drugs were tested and with different doses given in vivo to rats. Authors show in anaesthetized rats that both drugs lowered mean arterial pressure, increased renal plasma flow, oxygen delivery and GFR. This led to increased filtered sodium, increased tubular Na^+ transport and increased oxygen consumption with lower metabolic efficiency.[1] PHD blockers elevated tissue HIF protein abundance and EPO concentration increased in plasma along with haemoglobin. Higher doses of roxadustat elevated cortical and medullary tissue oxygen tension. Nitric oxide synthases increased in organs and isolated endothelial cells and plasma nitrite and nitrate increased.[1] The renal functional changes were largely mitigated by L-NAME. Based on this set of data, authors concluded that nitric oxide (NO) must play an important role in acute responses to PHD blockers in kidney. Despite blood pressure decrease, GFR increased. Renal autoregulation is efficient and the fact that plasma flow and GFR increase indicates direct and selective effects of NO on glomerular arterioles. Can changes in NO account for such effects alone or are the changes in NO secondary to other contributory signalling molecules? This is not clarified by the present experiments but is likely. It was shown previously that experimental hypoxic hypoxia and hypoxia by carbonmonoxide treatment for 6 hours induce differential NOS responses at mRNA level with upregulation of eNOS (NOS-3) and NOS-1 which the present data corroborate.[7] Will it be safe in the long run to induce glomerular hyperfiltration? The change in GFR is likely a matter of drug dosing and needs confirmation in humans. The pharmacological approach allows rapid lowering of dose, intermittent use or withdrawal. The present observations open a whole avenue of other potential applications for short time HIF-PHD inhibitors to patients at high risk of renal injury or perhaps even cardiovascular injury in addition to the proven indication of renal anemia. Also, it seems as an attractive option to use HIF-PHD inhibitors to prime transplant kidneys and mitigate ischemic injury. It is hoped that HIF-PHD inhibitors will become a more effective (and cheaper!) alternative to the use of recombinant EPO in CKD patients. The first data from large randomized clinical trials suggest that PHD-inhibitors are equally efficient as recombinant EPO in the treatment of renal anaemia, for example.[8] In this context the present findings are reassuring. The -dustats have potential for organ protection and will likely gain use in many and different pathophysiological settings. Misuse in performance sports must also be anticipated.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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