Dysregulated Oxygen Metabolism of the Kidney by Uremic Toxins: Review

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Because kidneys consume a large amount of oxygen and are relatively inefficient in oxygen uptake, they are susceptible to hypoxia, especially in patients with advanced chronic kidney disease accompanied by loss of peritubular capillaries. Accumulating evidence suggests that chronic tubulointerstitial hypoxia acts as a final common pathway leading to end-stage renal disease. Some biologically active uremic retention molecules, considered as uremic toxins, accumulate as the renal function declines, and at this moment, more than 90 bioactive uremic toxins have been identified. Uremic toxins per se have been proven to accelerate the progression of renal failure. However, the causal relationship between uremic toxin and tubulointerstitial hypoxia remains unclear. Our studies provided direct evidence that uremic toxin dysregulates oxygen metabolism in the kidney. Indoxyl sulfate (IS), a representative protein-bound uremic toxin, increased oxygen consumption in proximal renal tubules, decreased renal oxygenation, and consequently aggravated hypoxia in the remnant rat kidneys. The increase in tubular oxygen consumption by IS was dependent on sodium–potassium adenosine triphosphatase and oxidative stress. Our work also indicated a possible connection between IS and the desensitization of the oxygen-sensing mechanism in erythropoietin-producing cells, which may partly explain inadequate erythropoietin production in hypoxic kidneys of end-stage renal disease patients. Studies of uremic toxins will open a new avenue in development of novel therapeutic approaches of kidney disease.

IN NORMAL SUBJECTS, glomerular filtration and reabsorption process contribute to maintenance of proper electrolyte, fluid, and solute homeostasis, but this energy-consuming process costs approximately 10% of the body's energy consumption.¹ Kidneys are also relatively insufficient in oxygen uptake owing to the presence of arterial-venous oxygen shunt.² Such high basal energy consumption and inefficiency of oxygen uptake are the reasons for the kidney susceptibility to hypoxia, especially in advanced tubulointerstitial damage with progressive glomerulosclerosis and tubulointerstitial fibrosis, which are associated with the loss of peritubular capillaries.³,⁴ A decrease in oxygen tensions of the kidney was demonstrated in a number of experimental models of chronic kidney disease (CKD). These findings in turn have led to the broad recognition that chronic hypoxia of the kidney is the final common pathway of kidney failure.

Uremia is now defined as the accumulation of organic waste products that are normally cleared by the kidney, instead of the previous definition, that is, “those signs and symptoms of derangements in extracellular volume, inorganic ion concentrations, or lack of known renal synthetic products in the advanced CKD.” Some
biologically active uremic retention molecules are called uremic toxins. A widely accepted classification, endorsed by the European Uremic Toxin (EUTox) Work Group, is based on the molecular weight and plasma protein-binding characteristics of uremic toxins. In this scheme, European Uremic Toxin Work Group identified 90 organic compounds reportedly associated with uremia, including (1) 45 water soluble, low molecular weight (MW ≲ 500 Da), and nonprotein bound; (2) 23 water soluble, low molecular weight (MW ≲ 500 Da), and protein bound; (3) 22 “middle” molecular weight (MW ≳ 500 Da), with 2 of them being protein bound (Table 1). Recently, newly developed mass spectrometry techniques have been successfully applied to uremic toxin research, with the discovery of novel uremic toxins that range from low-molecular-weight solutes to small-molecule proteins. Current evidence suggests that uremic toxins promote the progression of CKD in both clinical and experimental studies. The mechanisms of chronic hypoxia in the kidney are multifactorial and a role of uremic toxins in oxygen metabolism in the kidney remains inconclusive. In this review, we will focus on the potential mechanisms of abnormal oxygen metabolism in the kidney induced by uremic toxins.

### Chronic Hypoxia in the Uremic Kidney

Energy utilization by the kidney, as indexed by kidney oxygen consumption, is primarily devoted to the process of electrolyte and solute reabsorption. The significant electrolyte transport in the proximal tubule relies on adenosine triphosphate, which mainly originates from oxygen-consuming oxidative phosphorylation. Tubular metabolism is a major determinant of intrarenal oxygen consumption and oxygenation, and medullary reabsorptive work is at least in part responsible for renal medullary hypoxia observed under basal conditions. In addition to significant loss of peritubular capillaries leading to tissue hypoxia at the late phase of kidney diseases, functional mechanisms inducing tubulointerstitial hypoxia at an early stage of kidney disease have been identified. Glomerular sclerosis and vasoconstriction of efferent arterioles as a result of imbalances in vasoactive substances decrease postglomerular peritubular capillary blood flow. Angiotensin II not only constricts efferent arterioles but, through its induction of oxidative stress, also hampers the efficient utilization of oxygen in tubular cells. Furthermore, relative hypoxia in the kidney also results from increased metabolic demand in tubular cells. These factors can affect the kidney before the appearance of significant pathologic changes in the vasculature and predispose the kidney to tubulointerstitial injury. Finally, CKD-related anemia may also aggravate the tubulointerstitial hypoxia in diseased kidney.

### Abnormal Oxygen Metabolism in the Uremic Kidney

Uremic toxins per se have been proven to accelerate the progression of renal failure. Wu et al. showed accumulation of p-cresyl sulfate and indoxyl sulfate (IS), two prototypic protein-bound uremic toxin molecules, associated with renal disease progression during 2 years of follow-up. Niwa and colleagues showed that the oral administration of IS and its precursor “indole” to uremic rats increased glomerulosclerosis in association with aggravation of renal dysfunctions. An oral sorbent AST-120 (Kremezin; Kureha, Tokyo, Japan) reduced the serum and urine levels of IS in CKD rats and patients, which in turn delayed the progression of CKD by reducing the profibrotic gene expression in the rat remnant kidney. Although chronic hypoxia in the

<table>
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<th>Classification</th>
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<tr>
<td>Small water-soluble</td>
<td>MW ≲ 500 Da, easily removed by any</td>
<td>Urea, creatinine</td>
<td>Not necessarily toxic</td>
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<tr>
<td>molecules</td>
<td>dialysis strategy</td>
<td></td>
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</tr>
<tr>
<td>Middle molecules</td>
<td>MW ≳ 500 Da, removed only through</td>
<td>β2-M, NY</td>
<td>Potential biological impacts</td>
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<td></td>
<td>high-flux membranes</td>
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</tr>
<tr>
<td>Protein-bound molecules</td>
<td>Any MW, difficult to remove with any</td>
<td>Phenols, indoles</td>
<td>Potential biological impacts</td>
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<td></td>
<td>dialysis strategy</td>
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EUTox, European Uremic Toxin; N, number; MW, molecular weights; β2-M, β2-microglobulin; NY, neuropeptide Y.
tubulointerstitium has been intensively studied and validated as a final common pathway to end-stage kidney injury,\textsuperscript{3,4,17,18} the causal relationship between uremic toxin and tubulointerstitial hypoxia remains controversial. Our collaborative works with Fredrik Palm from Sweden connected these gaps and demonstrated that IS increased oxygen consumption in proximal renal tubules, decreased renal oxygenation, and consequently induced hypoxia in the remnant rat kidneys.\textsuperscript{19} Studies using apocynin, the nicotinamide adenine dinucleotide phosphate hydrogen oxidase inhibitor, as well as the in vivo gene-silencing approach to knock down a subunit of nicotinamide adenine dinucleotide phosphate hydrogen oxidase showed that the increase in tubular oxygen consumption by IS was dependent on oxidative stress. We validated these findings in experimental animals. We administered AST-120 to reduce serum IS levels and observed improvement of the tissue oxygenation and decreased oxidative stress in the remnant kidney.\textsuperscript{19}

A Role of Uremia in Renal Anemia

Recently, Bernhardt et al.\textsuperscript{20} conducted a phase I clinical trial of a prolyl-4-hydroxylase domain enzymes’ inhibitor to enhance hypoxia-inducible transcription factor (HIF) availability in hemodialysis patients. They found that inhibition of prolyl-4-hydroxylase domain enzymes significantly enhanced erythropoietin (EPO) production in hemodialysis patients, and suggested that inadequate responses of EPO-producing cells, presumably as a result of desensitization of the oxygen-sensing mechanism, rather than destruction of the cells per se, may cause the inappropriately low production of EPO in patients with advanced CKD.\textsuperscript{20} Our recent works demonstrated that IS plays a pathologic role in inappropriate EPO production by disturbing the oxygen-sensing system, both in vitro and in vivo, by suppressing nuclear translocation of HIF–α protein and transcriptional activity of HIF thereof.\textsuperscript{21} These findings suggested that accumulation of uremic toxins directly enhances tubulointerstitial hypoxia by increasing oxygen consumption and dampens hypoxia response in EPO-producing cells. In addition to dysregulated oxygen metabolism in the kidney, mitochondrial oxygen consumption, oxygen delivery to the muscle, and aerobic capacity were also impaired in uremic skeletal muscles.\textsuperscript{22} We believe that uremic toxins might disturb cellular oxygen metabolism in multidisciplinary systems.

Uremia and Inhibition of Tubular Cell Proliferation

Niwa and colleagues demonstrated that IS inhibits tubular cell proliferation by activation of p53.\textsuperscript{23} In addition to achieving the oxygen-dependent control of EPO production, HIF is at the center of the cellular response to hypoxia, inducing many adaptive genes, including vascular endothelial growth factor, glycolytic enzymes, and antioxidative enzymes. However, cells are endowed with another mechanism against hypoxia: endoplasmic reticulum (ER) stress response. ER stress shuts down translation of proteins and induces hibernation of hypoxic cells. We found that CCAAT/enhancer binding protein homologous protein (CHOP), a representative downstream effector of the ER stress response, was upregulated by IS and inhibits proliferation of tubular cells.\textsuperscript{24} This is an adaptive response to save energy in a short term, but will cause a trouble of repairmen in the damaged kidney in a long term.

Conclusion

Uremic toxins increase oxygen consumption and aggravate local hypoxia in renal tubular cells through enhancement of oxidative stress. Uremia may also play a pathologic role in inappropriate EPO production by disturbing the oxygen-sensing system. Uremia per se may accelerate progression of renal dysfunction through aggravation of chronic hypoxia as a final common pathway to end-stage renal disease.

Practical Application

Metabolites of bacterial metabolism in the gut may play an important role as potential uremic toxins, and uremic toxins per se accelerate the progression of renal failure. The renal dietitian’s knowledge and dietary services can make a difference in CKD patients.

References

2. Gardner BS, Smith DW, O’Connor PM, Evans RG. A mathematical model of diffusional shunting of oxygen from


