Uremic Toxins, Oxidative Stress, and Renal Fibrosis: An Interwined Complex

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The prevalence of end-stage renal diseases is currently on the rise globally, and finding the way to curb this tide is urgently needed. Tubulointerstitial fibrosis is a common pathway for essentially all the nephropathy categories known to date, and the manifestations of renal fibrosis include excessive deposition of extracellular matrix with distortion of renal microstructures and functional deterioration. Uremic toxins have been gradually found to play an important role in the development of progressive renal fibrosis, with protein-bound indoxyl sulfate, *p*-cresol, and *p*-cresyl sulfate receiving the most attention. However, the contribution of oxidative stress among the pathogenesis of uremic toxins and renal fibrosis has not been evaluated much until recently. In this review, we will discuss about the nature and sources of oxidative stress in the kidney and how uremic toxins use oxidative stress to orchestrate the processes of renal fibrosis.

RENAL FIBROSIS, OR tubulointerstitial (TI) fibrosis, is characterized by progressive interstitial hypercellularity, extracellular matrix deposition, loss of peritubular capillaries, and tubular atrophy.[1] Multiple cellular and humoral mediators of renal fibrosis have been implicated,[2,3] and reactive oxygen species (ROS) are also key players.[4] Recently, a renaissance view on protein-bound uremic toxin, with a focus on its influence on oxygen biology, is purported to be responsible for progressive renal damage.[5] In the current article, we will discuss the relationship and clinical implications of these uremic wastes in the pathogenesis of renal fibrosis.

Oxidative Stress in the Kidney: Physiology and Pathology

ROS are oxygen derivatives, with unpaired electrons and possessing tendency to attack susceptible moieties. The main sources of ROS include nicotinamide adenine dinucleotide phophate hydrogen (NADPH) oxidase, xanthine oxidase (XO), cyclo-oxygenase (COX), lipoxygenase, and cytochrome P450 (CYP450) enzymes with mitochondria as major producers. ROS play a dual role in cellular physiology because in low amount, they could regulate cellular growth, adhesion, differentiation, apoptosis, as well as senescence, whereas too much ROS leads to cellular dysfunction.

It is well recognized that oxygen metabolism is deranged in diseased kidneys, and increased oxidative stress might take part in the progression of many renal diseases, including diabetic nephropathy, chemotherapeutics-related kidney damages, and so forth.[6,7] A brief discussion about relevant ROS producers is provided in the following.

NADPH Oxidase

NADPH oxidase family is the most abundant ROS producer in the kidney. Nox1, Nox2, and Nox4 are the predominant subunits expressed, and Nox4 significantly outweighs the others.[8] Renal Nox expression is found in cortical interstitial fibroblasts, endothelial cells, vascular smooth muscle cells, mesangial cells, tubular cells, and podocytes.[8] Among all tubular segments, macula densa, thick ascending limb, distal tubules, and collecting ducts are responsible for Nox expression, with region specificity and cell specificity.[9] Nox expressions are involved in renal glucose metabolism, renal hemodynamics, and electrolyte absorption.[4] Researchers have demonstrated that Nox inhibitors could impair tubular epithelial gluconeogenesis in diabetic rabbits.[10] Similarly, inhibition of NADPH oxidase is found to block the activity of sodium-glucose cotransporters in proximal tubules (PT) and reduce the high salt-induced sodium–hydrogen exchanger intensity, leading to less macula densa depolarization.[11,12]

In pathologic kidney statuses, members of NADPH oxidase could be culprits in some, whereas beneficial in others. Nox2 expression is significantly associated with lower superoxide dismutase activity and greater cardiac/renal fibrosis in C57BL/KsJ diabetic mice.[13] Others also demonstrated that diabetic mice have higher Nox2 levels

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in renal cortex, and treatment with angiotensin-converting enzyme inhibitors could reduce oxidative stress, hypertension, and renal fibrosis.[14] On the other hand, manipulating Nox4 expression in mice PT cells could induce renal fibronectin and transforming growth factor-beta (TGF-β) upregulation.[15] Being a main source of renal ROS, Nox4 inhibition/knock-out (KO) effectively confers renoprotection in Streptozocin-induced diabetic mice although studies with contrary results also exist.[16,17] Nonetheless, it is still widely believed that NADPH oxidase is important for promotion/progression of diabetic nephropathy, synergistic with renin-angiotensin systems.[18,19]

However, the role of Nox in other kidney injury models could be unexpected. In unilateral ureteral obstruction models, Nlandu Khodo et al[20] discovered that Nox4 deficiency increases renal oxidative stress and aggravates renal fibrosis, through decreasing hypoxia-inducible factor-1α and nuclear factor-like 2 levels. Experimental hyperuricemia-induced kidney damage is also partially mediated through increasing oxidative stress and ameliorated by Nox4 inhibition.[21] On the contrary, Nox4 KO mice with 5/6 nephrectomy seem to behave similarly with their wild-type counterpart.[17] The exact role of NADPH oxidase family in different kinds of renal injuries is still under active investigation.

Xanthine Oxidase

XO possesses an essential role in cellular metabolism and uric acid production. Several lines of evidence suggest that xanthine oxidoreductase contributes to oxidative stress generation in kidneys.[22] Xanthine oxidoreductase converts xanthine or hypoxanthine to uric acid or xanthine, and the resultant xanthine oxidase produces free radicals via electron transfer to O_2. Studies indicate that during ischemia–reperfusion injury, XO activity significantly increases and causes higher oxidative stress.[23] Febuxostat, an XO inhibitor, prominently reduces TL fibrosis in different models.[23] Consistently, epidemiologic researches suggest that serum uric acid level is associated with renal function deterioration, whereas treatment with allopurinol/febuxostat potentially ameliorates these negative influences.[24]

Cyclo-oxygenase

COX1/COX2 are important mediators of inflammation, and their products are potent vascular tones modifiers. COX produces oxidative stress through NADPH oxidase dependent or independent pathways. Increased renal COX expression during aging has been linked with aging-associated ROS increase, and renal COX2 is significantly upregulated during ischemia–reperfusion injury.[25] COX might serve both as sources of renal ROS and mediators of downstream proinflammatory cascades in multiple nephropathy models. COX2 KO mice have diminished ROS production and less tendency to develop renal hypertension.[26]

CYP450 Enzymes

The family of CYP450 enzymes reside mainly in mitochondria, whose ROS production is most abundant in the outer medulla, where the ROS contribute to medullary blood flow fine-tuning/electrolyte excretion. Indeed, CYP4502 $\times$ 10^1 is found to be activated by hydroxyl ion ($OH^{\star}$) and acts as an iron donor, contributing to hydrogen peroxide–related PT injury.[27] CYP450 monooxygenases are also involved in the pathogenesis of renovascular hypertension through mitochondrial dysfunction in spontaneous hypertensive rats.[28] The source of renal oxidative stress in 5/6 nephrectomized mice was also found to be mitochondrial superoxide production.[29] However, scanty researches exist regarding the role of CYP450 in other models.

Uremic Toxin and Its Impact on Extrarenal Tissues: ROS as Collateral Damage or Mediators?

Uremic toxins are namely waste products that accumulate in the blood during renal failure. They could be classified according to molecular sizes and water solubility, including small and water soluble molecules (urea, phosphorus), middle molecules (β-microglobulins), and protein-bound compounds (indoxyl sulfate [IS], *p*-cresyl sulfate [pCS], homocysteine),[30] among which *p*-cresol (parent compounds of pCS) and IS are the most extensively evaluated.[31,32]

p-Cresol reduces spontaneous beats of cardiomyocytes through elevating intracellular Ca^{2+} levels and subsequent protein kinase C alpha activation.[33] pCS, a metabolite of *p*-cresol, directly induces endothelial microparticles shedding via Rho kinase.[34] Clinically, IS is also associated with endothelial dysfunction, atherosclerosis, and peripheral artery diseases.[32] IS further causes elevated oxidative stress in human umbilical vein endothelial cells through NADPH oxidase stimulation and glutathione reduction.[35] In addition, IS activates p44/42 mitogen-activated protein kinase (MAPK) pathways and elevates platelet-derived growth factor/platelet-derived growth factor receptor concentrations, causing vascular smooth muscle proliferation and aggravating atherosclerosis.[36] Moreover, IS stimulates interleukin 1, interleukin 6, and tumor necrosis factor α production from cardiomyocytes. The subsequent nuclear factor kappaB (NF-κB) activation and the increases in collagen synthesis contribute to unfavorable cardiac remodeling.[37] Similarly, IS could upregulate intercellular adhesion molecule 1 and monocyte chemotactic protein 1 (MCP-1) expression through NADPH oxidase induction and NF-κB activation,[38] while ameliorated by N-acetylcysteine.[39] Finally, administration of IS increases oxidative stress markers in vascular smooth muscles,[40] leading to p53 upregulation and cell senescence. Thus, the induction of oxidative stress seems to play an essential role in the uremic toxin–induced endothelial/cardiac injuries.