INDOXYL SULFATE, A TRYPTOPHAN METABOLITE, INDUCES NEPHRO-VASCULAR TOXICITY

Toshimitsu Niwa
Nagoya University School of Medicine, Department of Advanced Medicine for Uremia, Nagoya, Japan
Correspondence to: Toshimitsu Niwa
E-mail: tniwa@med.nagoya-u.ac.jp

ABSTRACT
Indoxyl sulfate, a uremic toxin, is accumulated in the serum of chronic kidney disease (CKD) patients. A part of the dietary protein-derived tryptophan is metabolized by intestinal bacteria into indole, which is metabolized to indoxyl sulfate in the liver. Indoxyl sulfate stimulates progression of CKD by increasing expression of fibrogenic genes such as transforming growth factor (TGF)-β1. AST-120 delays the progression of CKD by adsorbing indoxyl sulfate in the intestines, and consequently reducing serum indoxyl sulfate. Indoxyl sulfate exhibits cellular toxicity in renal tubular cells, glomerular mesangial cells, vascular smooth muscle cells, vascular endothelial cells, and cardiac myocytes by inducing reactive oxygen species. Indoxyl sulfate induces cell senescence in renal tubular cells. Indoxyl sulfate stimulates aortic calcification and senescence in hypertensive rats. Thus, indoxyl sulfate is involved in the progression of CKD and cardiovascular disease (CVD).

Keywords: indoxyl sulfate, chronic kidney disease, cardiovascular disease, oxidative stress, oral adsorbent AST-120, senescence

Metabolism of Indoxyl Sulfate, a Uremic Toxin
We have demonstrated that indoxyl sulfate is a uremic toxin accelerating the progression of chronic kidney disease (CKD) (21, 30, 31, 37). Fig. 1 shows the metabolism of indoxyl sulfate and the effect of an oral sorbent (AST-120: Kremezin). Indoxyl sulfate is derived from dietary protein. A part of the protein-derived tryptophan is metabolized into indole by tryptophanase in intestinal bacteria such as Escherichia coli. Indole is then absorbed into the blood from the intestine, and is metabolized to indoxyl sulfate in the liver, while indoxyl sulfate is normally excreted into urine. In CKD, however, the reduced renal clearance of indoxyl sulfate leads to its elevation. In fact, the serum levels of indoxyl sulfate were found to be markedly increased in both CKD rats and patients (27, 28, 30). In serum, approximately 95% of indoxyl sulfate is bound to serum albumin. AST-120 reduces the serum and urine levels of indoxyl sulfate in uremic rats and patients by adsorbing indole in the intestines, consequently stimulating its excretion into feces (23, 27, 28, 29, 30, 32, 34, 35). The administration of indoxyl sulfate to 5/6-nephrectomized rats promoted the progression of CKD accompanied by enhanced gene expression of transforming growth factor (TGF)-β1, tissue inhibitor of metalloproteinase (TIMP)-1 and proα1(I)collagen (21, 22). These findings support the notion that indoxyl sulfate is a uremic toxin stimulating the progression of CKD by increasing renal expression of these fibrogenic genes.

We proposed the protein metabolite theory by which endogenous protein metabolites such as indoxyl sulfate play a significant role in the progression of CKD (33, 35, 36). The initial insult leads to a loss of functioning nephrons via a disease-specific pathophysiological process. A progressive decline in the glomerular filtration rate (GFR) leads to increased circulating levels of endogenous protein metabolites such as indoxyl sulfate, and to the adverse effects of their overload on the remnant nephrons, especially proximal tubular cells. Indoxyl sulfate, for example, stimulates progressive tubulointerstitial fibrosis, glomerular sclerosis, and consequent progression of CKD by increasing the gene expressions of TGF-β1, TIMP-1, and proα1(I)collagen, leading to a further loss of nephrons (21, 22), completing the vicious circle of progressive renal injury.

A low-protein diet delays the progression of CKD by suppressing renal TGF-β1 expression in uremic animals in which it also reduces the serum levels of indoxyl sulfate (30, 34). The administration of AST-120 decreases the serum and urine levels of indoxyl sulfate, and delays the progression of CKD by reducing the gene expression of TGF-β1, TIMP-1, and proα1(I)collagen (5, 6, 7, 8, 9, 10, 11, 12, 23).

Indoxyl Sulfate Induces Reactive Oxygen Species (ROS) in the Kidney
Indoxyl sulfate induces ROS production not only in renal tubular cells but also in glomerular mesangial cells (19, 24). Indoxyl sulfate-induced ROS in renal tubular cells activate nuclear factor (NF)-κB which upregulates the expression of plasminogen activator inhibitor (PAI)-1 (24). Indoxyl sulfate-induced ROS in mesangial cells activate mitogen-activated protein kinases (MAPK) and cell proliferation (19). Thus, indoxyl sulfate induces the generation of ROS in the kidneys. Administration of indoxyl sulfate reduces the superoxide scavenging activity in the kidneys of uremic rats. Therefore, indoxyl sulfate impairs the kidney’s anti-oxidative system (39).
AST-120 alleviates ROS in the kidneys of uremic rats by reducing serum levels of indoxyl sulfate, and reduces the urine level of acrolein, a lipid peroxidation end product (44). Furthermore, AST-120 increases NO synthesis in the kidneys of CKD rats by increasing the renal expressions of endothelial nitric oxide synthase and neuronal nitric oxide synthase through alleviation of indoxyl sulfate overload on the kidney (46).

**Nephrotoxicity of Indoxyl Sulfate**

Fig. 2 shows the mechanism underlying the nephrotoxicity of indoxyl sulfate. Approximately 95% of indoxyl sulfate accumulated in the blood of uremic patients is bound to serum albumin. Therefore, indoxyl sulfate is normally excreted into urine mainly via active secretion by the proximal tubular cells. Organic anion transporters (OAT1 and OAT3) play an important role in the transcellular transport of indoxyl sulfate in the tubular cells and in the induction of its nephrotoxicity (16, 17). Indoxyl sulfate in the blood is taken up by OAT1 and OAT3 at the basolateral membrane of tubular cells (OAT1: proximal, OAT3: proximal and distal), and is accumulated in the tubular cells at high concentration in CKD patients (43). The accumulation of indoxyl sulfate generates ROS, reduces superoxide scavenging activity, and consequently causes tubular cell injury by impairing the kidney’s anti-oxidative systems (39).

The damaged tubular cells produce TGF-β1 as well as chemokines such as monocye chemotactic protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), and osteopontin. These chemokines promote the infiltration of macrophages which produce TGF-β1. The secreted TGF-β1 stimulates the production of TIMP-1 and collagen. The damaged tubular cells are transformed into myofibroblasts through an epithelial-to-mesenchymal transition (14), these changes facilitate interstitial fibrosis. Thus, indoxyl sulfate accumulated in uremic serum accelerates tubular cell injury, and induces subsequent interstitial fibrosis, thus acting as a nephrotoxin.

**Indoxyl Sulfate Reduces Klotho and Induces Senescence in the Kidney**

Klotho is an anti-aging gene, and mutation of the mouse Klotho gene leads to premature aging syndrome. Klotho protein is expressed predominantly in the kidneys. The expression of Klotho is decreased in the kidneys of not only rat models such as hypertensive rats, 5/6-nephrectomized rats, and diabetic rats, but also in patients with CKD.

We studied to clarify if indoxyl sulfate could reduce Klotho expression, and contribute to cell senescence in the kidneys of hypertensive rats (4). Indoxyl sulfate-administered hypertensive rats showed decreased expression of Klotho, increased expression of senescence-associated β-galactosidase (SA-β-gal), p16, p21, p53 and retinoblastoma protein (Rb) in renal tubular cells, and increased tubulointerstitial fibrosis and mesangial expansion compared with hypertensive rats. Thus,
administration of indoxyl sulfate to hypertensive rats reduced renal expression of Klotho, and promoted cell senescence accompanied by renal fibrosis (4).

We demonstrated that AST-120-treated CKD rats showed increased renal expression of Klotho compared with CKD rats (3). Then, we examined whether the expression of Klotho in proximal tubular cells is regulated by indoxyl sulfate using the proximal tubular cell line (HK-2) (41), because main target of indoxyl sulfate is proximal tubular cells, and Klotho is expressed in the cells as well as inner medullary collecting duct cells and distal tubular cells. Indoxyl sulfate downregulates Klotho expression in proximal tubular cells by activating NF-κB through production of ROS (40).

We investigated how indoxyl sulfate promotes CKD using cultured HK-2 cells and CKD rats (40). Indoxyl sulfate inhibited serum-induced cell proliferation, and promoted the activation of SA-β-gal, a marker of cellular senescence, and the expression of α-smooth muscle actin (α-SMA), a marker of fibrosis, through inducing p53 expression and phosphorylation. Further, we have demonstrated that NF-κB plays an important role in indoxyl sulfate-induced cellular senescence, fibrotic gene expression and inhibition of proliferation in proximal tubular cells (42). More notably, indoxyl sulfate accelerates proximal tubular cell senescence with progression of CKD through ROS-NF-κB-p53 pathway (42), AST-120, which reduces serum indoxyl sulfate level, suppressed the expression of phosphorylated NF-κB p65, p53, p21, SA-β-gal, TGF-β1 and α-SMA in the kidneys of CKD rats.

Fig. 2. Nephrotoxicity of indoxyl sulfate

Fig. 3. Uremic toxicity of indoxyl sulfate
Vascular Toxicity of Indoxyl Sulfate

Indoxyl sulfate has been shown to inhibit endothelial proliferation and wound repair (15), and to stimulate the proliferation of rat vascular smooth muscle cells (49) and human aortic smooth muscle cells (26). The serum level of indoxyl sulfate has been associated with pentosidine and HDL-cholesterol, the risk factors of atherosclerosis in hemodialysis patients (45).

We demonstrated that indoxyl sulfate promotes aortic calcification and aortic wall thickening in hypertensive rats (1). Osteoblast-specific proteins such as osteopontin, core binding factor 1 (Cbfa1), alkaline phosphatase (ALP), and osteocalcin are expressed in the cells embedded in the aortic calcification area. More notably, indoxyl sulfate promotes cell senescence with increased expression of SA-β-gal, p53, p21, p16, and Rb in the cells embedded in the calcification area in hypertensive rats (2). Thus, indoxyl sulfate is a nephro-vascular toxin that may be responsible for progression of not only CKD but also cardiovascular disease (CVD) (38).

Indoxyl sulfate stimulates the generation of ROS such as superoxide by up-regulating NADPH oxidase Nox4, and induces the expressions of osteoblast-specific proteins such as Cbfa1, ALP, and osteopontin in human aortic smooth muscle cells (25). ROS derived from NADPH oxidase Nox4 are important in inducing the transdifferentiation of human aortic smooth muscle cells into cells with a more osteoblastic phenotype. These effects of indoxyl sulfate have been observed even at a concentration of indoxyl sulfate found in hemodialysis patients (30).

Indoxyl sulfate inhibits NO production and cell viability by inducing ROS such as superoxide through the induction of NADPH oxidase Nox4 in human vascular endothelial cells (47). Indoxyl sulfate has been shown to induce the expression of Nox4 mRNA and the production of superoxide and peroxynitrite in human vascular endothelial cells. Further, indoxyl sulfate upregulates expression of ICAM-1 and MCP-1 by oxidative stress-induced NF-κB activation (48).

Indoxyl sulfate may play a significant role in vascular disease and its higher rate of mortality observed in CKD patients (13). Indoxyl sulfate levels exhibited an inverse relationship to renal function and a direct relationship to aortic calcification and pulse-wave velocity. The highest indoxyl sulfate tertile has proved to be a powerful predictor of overall and cardiovascular mortality in CKD patients.

Indoxyl sulfate shows pro-fibrotic, pro-hypertrophic, and pro-inflammatory effects on cardiac fibroblasts and myocytes, indicating that indoxyl sulfate might play an important role in adverse cardiac remodeling mediated via activation of the p38 MAPK, p42/44 MAPK, and NF-κB pathways (20).

AST-120 lessens the extent of atherosclerosis induced by kidney injury and alters lesion characteristics in apolipoprotein E-deficient mice, resulting in plaques with a more stable phenotype with less necrosis and reduced inflammation (50).

Oxidative stress plays a key role in the development of cardiac hypertrophy and fibrosis in CKD. AST-120 suppresses oxidative stress and reduces cardiac damage in CKD (18).

Conclusions

Fig. 3 shows the cell toxicity of indoxyl sulfate. It induces the cellular production of ROS such as superoxide by activating NADPH oxidase, especially Nox4, by its uptake through OAT1 and OAT3, consequently impairing the cellular anti-oxidative system. It induces ROS in renal tubular cells and glomerular mesangial cells, and stimulates the progression of CKD. It also induces ROS in vascular smooth muscle cells, vascular endothelial cells and cardiomyocytes, and aggravates CVD.

REFERENCES
