Cardiorenal Syndrome
The Emerging Role of Protein-Bound Uremic Toxins
Suree Lekawanvijit, Andrew R. Kompa, Bing H. Wang, Darren J. Kelly, Henry Krum

Abstract: Cardiorenal syndrome is a condition in which a complex interrelationship between cardiac dysfunction and renal dysfunction exists. Despite advances in treatment of both cardiovascular and kidney disease, cardiorenal syndrome remains a major global health problem. Characteristic of the pathophysiology of cardiorenal syndrome is bidirectional cross-talk; mediators/substances activated by the disease state of 1 organ can play a role in worsening dysfunction of the other by exerting their biologically harmful effects, leading to the progression of the syndrome. Accumulation of uremic toxins is a hallmark of renal excretory dysfunction. Removal of some toxins by conventional dialysis is particularly problematic because of their high protein binding. In this review, we demonstrate that protein-bound uremic toxins may play an important role in progression of cardiovascular disease in the setting of chronic kidney disease. The highly protein-bound uremic toxin indoxyl sulfate has emerged as a potent toxin adversely affecting both the kidney and heart. Direct cardiac effects of this toxin have been recently demonstrated both in vitro and in vivo. Specifically, potent fibrogenic and prohypertrophic effects, as well as oxidative stress-inducing effects, appear to play a central role in both renal and cardiac pathology. Many of these adverse effects can be suppressed by use of a gut adsorbent, AST-120. Potential mechanisms underlying indoxyl sulfate–induced cardiorenal fibrosis are discussed. Future research and clinical implications conclude this review. (Circ Res. 2012;111:1470-1483.)

Key Words: cardiorenal syndrome ■ heart failure ■ interstitial fibrosis ■ myocardial infarction ■ uremic cardiomyopathy

A close relationship between cardiovascular disease (CVD) and renal insufficiency exists. Impairment of 1 organ can accelerate pathological processes in the other, which in turn accelerates the progression of failure of both. Because of its impact on the health care system, this interrelationship has been increasingly recognized in the past decade as a new entity called cardiorenal syndrome (CRS) or renocardiac syndrome.1,2

CVD is one of the most common causes of death.3 In 2009, the United States Centers for Disease Control and Prevention reported that 31% of all deaths are because of CVD.4 Many patients with CVD have development of heart failure (HF), which is a final common pathway of diverse diseases affecting the cardiovascular (CV) system. HF is a growing health problem worldwide, with prevalence of 1% to 2% in the general population in industrialized countries.5 The prevalence is much higher in the elderly population, a transitional shift that is increasing over time.6 An extremely poor prognosis exists in HF patients, with a 1-year mortality of 44.5%.7 Coexistent renal insufficiency is 1 of the strongest independent risk factors and predictors of mortality in these patients.8,9

In the setting of kidney disease, CVD is the most common cause of death, responsible for 40% to 50% of all deaths.10 A growing trend of increased chronic kidney disease (CKD) prevalence in the past 2 decades (from 11%–15.2% in the United States population 20 years of age or older), reported by the National Health and Nutrition Examination Survey has raised global concerns.11 Furthermore, patients with combined CV and kidney disease are at much higher risk for mortality than patients with either in isolation.12

Given that heart and kidney diseases pose a significant burden to society despite advances in treatment, research in this area needs to be conducted with a more specific focus on the heart–kidney interrelationship. Even though CRS has been intensively studied over the past decade, there still are significant questions and knowledge gaps that need to be explored. For example, are there any CKD-specific risk factors that contribute to CV morbidity and mortality in patients with CKD because CV mortality remains unacceptably high in this population? Importantly, more mechanistic-oriented investigations are needed to explain the observations made in clinical studies.

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Cardiovascular Disease in the Setting of CKD

Renal dysfunction, even mild, is associated with a 10- to 30-fold increase in risk for CV events and mortality compared with that of the normal population. Hypertension is common in CKD, largely contributing to the development and progression of LVH. Hypertension is noteworthy in that CV pathology in the setting of CKD is hypertension-independent. Specifically, cardiac hypertrophy and fibrosis, predominant features of uremic cardiomyopathy, are sustained in both CKD patients and animal models, even with well-controlled blood pressure. A reduction in cardiac capillary surface and volume density is also persistently observed in blood-pressure–controlled CKD animals but not in a model of renovascular hypertensive disease.

CKD-Related CV Risk Factors

Hypertension

Hypertension is common in CKD, largely contributing to the development and progression of LVH. Nevertheless, it is noteworthy that CV pathology in the setting of CKD is hypertension-independent. Specifically, cardiac hypertrophy and fibrosis, predominant features of uremic cardiomyopathy, are sustained in both CKD patients and animal models, even with well-controlled blood pressure. A reduction in cardiac capillary surface and volume density is also persistently observed in blood-pressure–controlled CKD animals but not in a model of renovascular hypertensive disease.

Anemia

Anemia results from defective erythropoietin production of the dysfunctional kidney and is contributed to further by cardiac dysfunction associated with proinflammatory cytokine activation and generation of antierthyrogenic circulating factors. Beneficial cardiorenal effects of exogenous erythropoietin have been demonstrated by not only elevation of hemoglobin but also other nonhematopoietic actions, such as anti-inflammatory, antioxidative, and antiapoptotic effects. Exogenous erythropoietin has been reported to reduce cardiac fibrosis and hypertrophy in association with decreased B-type natriuretic peptide levels and to improve endothelial function. However, overcorrection is associated with an increased risk of stroke, hypertension, vascular thrombosis, CV events, progression to ESRD, and death.

Oxidative Stress

Increased oxidative stress plays an important role in multiple biological pathways activated in CKD. In this setting, there is an imbalance between nitric oxide (NO) and reactive oxygen species (ROS) because of decreased NO synthesis and increased ROS production. NO enhances vasodilation, inhibits platelet aggregation, and prevents neutrophil adhesion, thereby stabilizing vascular tone and preventing atherogenesis. Increased ROS production can be induced by inappropriate activation of the renin angiotensin aldosterone system (RAAS) via activation of nicotinamide adenine dinucleotide phosphate oxidase. This is linked to uremic cardiomyopathy via inducing cardiac inflammation and fibrosis, as well as intramyocardial capillary loss, endothelial damage, and atherosclerosis. Loss of permeability to macromolecules by oxidative stress–induced endothelial damage may be a mechanism driving CRS progression. It enhances intimal accumulation of oxidized low-density lipoprotein, the key initiating event in atherogenesis. Accelerated atherosclerosis is highly prevalent in CKD; the coronary and renal vessels are common targets of obstructive atherosclerosis that increase the risk of organ dysfunction in CRS. If glomerular capillaries are directly affected, then protein leakage through glomerular filtration can further damage the kidney by initiating tubular inflammation with subsequent tubular atrophy and interstitial fibrosis, ultimately leading to worsening of renal function.

Nonischemic cardiac death mainly associated with uremic cardiomyopathy and CKD-associated vascular pathology, such as coronary calcification and ossification.

Cardiorenal Syndrome

Increased oxidative stress plays an important role in multiple biological pathways activated in CKD. In this setting, there is an imbalance between nitric oxide (NO) and reactive oxygen species (ROS) because of decreased NO synthesis and increased ROS production. NO enhances vasodilation, inhibits platelet aggregation, and prevents neutrophil adhesion, thereby stabilizing vascular tone and preventing atherogenesis. Increased ROS production can be induced by inappropriate activation of the renin angiotensin aldosterone system (RAAS) via activation of nicotinamide adenine dinucleotide phosphate oxidase. This is linked to uremic cardiomyopathy via inducing cardiac inflammation and fibrosis, as well as intramyocardial capillary loss, endothelial damage, and atherosclerosis. Loss of permeability to macromolecules by oxidative stress–induced endothelial damage may be a mechanism driving CRS progression. It enhances intimal accumulation of oxidized low-density lipoprotein, the key initiating event in atherogenesis. Accelerated atherosclerosis is highly prevalent in CKD; the coronary and renal vessels are common targets of obstructive atherosclerosis that increase the risk of organ dysfunction in CRS. If glomerular capillaries are directly affected, then protein leakage through glomerular filtration can further damage the kidney by initiating tubular inflammation with subsequent tubular atrophy and interstitial fibrosis, ultimately leading to worsening of renal function. Increased endothelial permeability of myocardial capillaries to albumin has been demonstrated to be associated with diabetic cardiomyopathy but is not yet as well studied in uremic cardiomyopathy.
Dysregulation of Calcium and Phosphate Homeostasis

Abnormal calcium-phosphate metabolism is associated with the progression of CKD, as well as development of CV complications. The kidney, bone, and parathyroid gland act in concert in regulation of both calcium and phosphorus homeostasis; however, kidney disease appears to be a far more common cause of an imbalance of both minerals. Chronic hypocalcemia because of decreased vitamin D production in dysfunctional kidney induces subsequent secondary hyperparathyroidism. Parathyroid hormone (PTH) is cardiotoxic and is associated with increased risk of CV death. PTH promotes cardiac fibrosis by activating cardiac fibroblasts and interferes with cardiac contractility and heart rate by disturbing intracellular calcium. Hyperphosphatemia, an independent risk factor for CV events, usually occurs in advanced stage CKD. High phosphate levels are linked to vascular calcification in hemodialysis (HD) patients and increased cardiac fibrosis and intramyocardial arterial wall thickening in a CKD model using a high-phosphorus diet. The presence of coronary calcification is strongly predictive of ischemic heart disease despite absence of significant luminal obstruction.

Fibroblast growth factor (FGF)-23 is an emerging factor that appears to play a central role in the regulation of phosphorus and vitamin D homeostasis. Increased FGF-23 observed in CKD enhances phosphaturia by downregulating renal expression of sodium phosphate cotransporters and it reduces 1,25-dihydroxyvitamin D production by inhibiting renal 1-α hydroxylase. FGF-23 also acts on the parathyroid gland to reduce PTH secretion. However, decreased systemic levels of active vitamin D result in subtle hypocalcemia and lack of inhibitory feedback to the parathyroid gland, leading to chronic parathyroid gland stimulation. The counterbalancing PTH-reducing effect of FGF-23 may explain the late manifestation of secondary hyperparathyroidism. Mechanistically, FGF-23 acts on the kidney and parathyroid gland through FGF receptors, which usually require the coreceptor klotho (klotho-dependent). Consequently, hyperphosphatemia together with overt hyperparathyroidism ensues, producing a state at high risk for CVD. Interestingly, increased FGF-23 recently has been demonstrated to be independently associated with LVH in CKD patients and to induce LVH, both in vitro and in vivo, through the klotho-independent FGF receptors and the PLC-γ1–calcineurin–nuclear factor of activated T-cells signaling pathway.

Uremic Toxins

Excretory function is 1 of 3 main functions of the kidney (the other 2 being hormone production and regulation of fluid/electrolyte homeostasis). When renal function is impaired, waste...
products accumulate. Such products are called uremic toxins if potentially harmful to the body by exerting biological or biochemical activity.

Systemic accumulation of uremic toxins/solutes is a hallmark of CKD. Many of them can be eliminated by conventional dialysis treatment, whereas others are ineffectively removed because of their protein-binding capacity. Renal toxicity of such protein-bound solutes, particularly oxidative stress/inflammation/fibrosis effects in association with the progression of CKD, has been widely investigated; however, direct CV effects have not been well-studied.

**Role of Protein-Bound Uremic Toxins in the CRS**

Ninety retention uremic compounds have been listed by the European Uraemic Toxin Work Group, selected from 55 of 857 publications published between 1968 to 2002. Inclusion criterion for these 90 retention uremic compounds listed in this report is the ratio of their mean/median uremic concentration to the mean normal concentration being >1. Retention uremic solutes can be divided into 3 major groups.64

1. Small water-soluble compounds (molecular weight [MW] <500 Da) such as urea and phosphorus. Most toxins in this group are removable by conventional dialysis. Therefore, they generally are not problematic; however, some of them, such as urea and creatinine, have been used in the assessment of renal excretory function and monitoring removal efficiency of dialysis treatment.65

2. Middle molecules (MW >500 Da). β2-microglobulin is a prototype of this group. Clearance of middle molecules is limited by conventional HD but appears to be more effective with peritoneal dialysis.66 This is likely because of the larger pore sizes and the longer dialysis time of peritoneal dialysis compared with HD.66 Currently, improvements in HD technique by using higher permeability membranes (high-flux HD) or convection transport (hemofiltration/hemodiafiltration) provide considerably better clearance of these molecules, such as PTH, β2-microglobulin, and advanced glycosylation end products,67,68 A reduction in accumulation of middle molecules is independently associated with a reduced mortality risk.69

3. Protein-bound compounds, for example, 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid, indoxyl sulfate, p-cresol, and homocysteine. Twenty-five of the 90 listed compounds (27.8%) are protein-bound and 23 of them have an MW <500 Da. Overload of these toxins competitively inhibits albumin binding of drugs, such as digoxin, warfarin, furosemide, salicylic acid, and diazepam, thereby enhancing drug toxicity.70-72

Removal of these protein-bound compounds by conventional dialysis is limited because of the larger size of the compound–protein complex molecule than the pore size of dialysis membranes, and it is not improved by treatment with high-flux HD.65,73,74 Dialysis treatment with protein-leaking membranes has been proposed and it offers a greater clearance of retention protein–bound solutes. Nevertheless, large amounts (2-6 g/4 hours of session) of albumin loss are a major concern75 because hypoalbuminemia is associated with increased mortality in HD patients.76-78 Addition of an adsorbent system to standard dialysis seems promising in preliminary studies but still requires further development.79

**Evidence of Adverse CV Effects of Protein-Bound Uremic Toxins**

Among protein-bound uremic toxins (PBUT), indoxyl sulfate (IS), and p-cresyl sulfate (pCS) are the most extensively studied with regard to their negative impact on the CV system.

IS is associated with endothelial dysfunction,80,81 vascular smooth muscle cell (VSMC) proliferation,82 and increased risk of atherosclerosis in man.83 Strong evidence of p-cresol (the mother compound of pCS) toxicity to endothelial cells has been demonstrated in vitro.80,84,85 Recently, abnormal structural and functional changes in the gap junction, mediated via protein kinase-Cα, have been observed in cultured cardiomyocytes stimulated with p-cresol.86 Circulating levels of p-cresol are positively associated with CV events.87 However, p-cresol detected in humans actually represents its conjugated forms, mainly pCS (>95%) and p-cresyl glucuronide (<4%), because the conjugated bonds are hydrolyzed during acid and heat deproteinization in the sample preparation step of measurement.88 True unconjugated p-cresol is below the limit of detection using acetone deproteinization to avoid deconjugation. Thus, significant CV effects related to increased p-cresol levels either in CKD patients or in experimental CKD models are most likely because of pCS and p-cresyl glucuronide. By contrast, in vitro study of p-cresol appears to be of less clinical significance.

Increased levels of free pCS per se have been reported to be predictive of all-cause and CV mortality,89 pCS is directly linked to vascular damage by inducing Rho kinase–mediated microparticle release from endothelial cells.89 A recent publication90 has reported that both pCS and p-cresyl glucuronide together cause endothelial dysfunction by increased permeability to albumin, but not each of them individually.

Other than IS and pCS, there have been reports of other PBUT associated with CVD. Increased homocysteine levels in ESRD patients are correlated with CV events and mortality.92 Hyperhomocysteinemia is mainly associated with hallmarks of atherosclerosis. Homocysteine causes oxidative stress–induced endothelial dysfunction and damage,93 increased expression of vascular inflammatory and thrombogenic mediators,94 and mitogen-activated protein kinase (MAPK)–mediated VSMC proliferation.95 High prevalence of vascular calcification and calcified atherosclerotic plaque in the CKD population may be explained by homocysteine promoting calcium deposition and osteogenic differentiation demonstrated in vitro in VSMCs cocultured with THP-1 cells (human leukemia monocytic cell line).96 Adverse vascular effects of homocysteine are exaggerated with the deficiency of folate and vitamins B6 and B12.94 However, beneficial CV effects of homocysteine-lowering therapy by folate and vitamin B supplementation are still controversial. A metaanalysis has recently reported a 15% CV risk reduction in CKD patients receiving folate treatment (n=3386),97 but results from other large clinical trials (n=619 and 2056) with folate and vitamin B therapy have been neutral,98,99 with 1 study even reporting adverse CV outcomes.100
Nevertheless, there are ongoing issues still to be verified, such as optimal doses and forms of supplementation, baseline renal function and homocysteine levels, the fortification of grain, and genetic polymorphisms of crucial enzymes in the homocysteine metabolism pathway.\textsuperscript{101} Notably, most investigations of the CV effects of PBUTs have primarily focused on vascular pathology, whereas study of direct cardiac effects are extremely rare. For example, there have been no known studies of direct effects of well-described PBUTs, such as homocysteine on the heart. In contrast, phenol was demonstrated many years ago to suppress contractility of cardiac muscle isolated from piglets.\textsuperscript{102} More recently, our group examined profibrotic and prohypertrophic effects of IS, cresol, and cresol conjugates in cultured neonatal rat cardiac fibroblasts and myocytes using 'H-proline and 'H-leucine incorporation, respectively.\textsuperscript{103} Angiotensin II served as positive control. IS had strong dose-dependent profibrotic and prohypertrophic effects, whereas other PBUT, including pCS, p-cresol, m-cresol, m-cresyl sulfate, and phenylacetic acid, had little or no effect (Figures 1 and 2).

**Indirect Evidence of Adverse CV Effects Possibly Associated With Nondialyzable Uremic Toxins Based on Treatment Modalities**

**Hemodialysis**

Despite considerable advances in HD technology, CVD still remains the major cause of morbidity and mortality in CKD patients on maintenance HD.\textsuperscript{104} Progression of LVH has been demonstrated in patients who have received long-term conventional HD for ≥4 years.\textsuperscript{105} Even with hypertension and anemia correction, increased left ventricle mass is still observed in one-third of patients, whereas one-fifth show no improvement within an average follow-up period of 54 months after 5.5 years of HD treatment.\textsuperscript{106} Furthermore, LVH is still observed in three-quarters of patients who have been on long-hour HD (24 hours per week) for >10 years.\textsuperscript{107} Increased severity of cardiac interstitial fibrosis over time in patients on chronic HD also has been reported.\textsuperscript{24}

**Kidney Transplantation Versus Long-Term Dialysis Treatment**

Successful kidney transplantation results in significant improvements in left ventricle dilation, left ventricle mass, cardiac function, and HF symptoms, thereby preventing HF progression and decreasing CV mortality.\textsuperscript{108,109} Three-quarters of patients who have experienced symptomatic HF become asymptomatic after transplantation.\textsuperscript{110} Interestingly, the pretransplantation duration of HD is negatively associated with the degree of left ventricle function recovery posttransplantation.\textsuperscript{110} HD patients clearly receive benefits from the treatment but are also at high risk for CV events because of fluid and electrolyte shift during a dialysis session. Chronic exposure to electrolyte and hemodynamic

**Figure 1. Profibrotic effect of protein-bound uremic toxins in cultured neonatal rat cardiac fibroblasts.** Ang II indicates angiotensin II. \textsuperscript{**}P<0.01, \textsuperscript{***}P<0.001 vs control.
fluctuations activates neurohormonal processes and also makes blood pressure control in dialyzed patients more complicated.\textsuperscript{111} Hemodynamic stress caused by dialysis and even dialysis itself can promote the production of proinflammatory cytokines, such as interleukin (IL)-6, fibrinogen, and C-reactive protein in both peritoneal dialysis and HD patients.\textsuperscript{112} Elevated IL-6 levels are associated with high risk for CV events in CKD patients\textsuperscript{113} and enhance cardiac fibrosis and hypertrophy in rats infused with IL-6.\textsuperscript{114} In addition, an arteriovenous shunt in HD patients can increase cardiac workload that enhances LVH progression.

Dialysis efficiency that is routinely determined by the clearance of small water-soluble solutes as a marker actually does not reflect clearance of all accumulated toxins. Compared with long-term dialysis patients, kidney transplantation recipients have a better CV prognosis.\textsuperscript{115} Cardiac fibrosis regresses over time after successful transplantation, whereas it increases with time on HD.\textsuperscript{24} In addition, CV mortality is 2- to 10-times lower in kidney transplantation than in age-matched dialysis patients.\textsuperscript{21} There may be bias with regard to selection criteria for kidney transplantation. However, such an observation suggests a possible role for nondialyzable uremic toxins, herein the protein-bound compounds, in the pathogenesis and progression of CV complications.

Among PBUTs, IS appears to be the most likely potential candidate with which to test this hypothesis for 2 reasons. First, it has been most extensively investigated with particular regard to its fibrogenic and oxidative stress-inducing effects on the kidney. Increased circulating IS levels have been linked to the progression of CKD, as well as the functional impairment of other organs, such as the thyroid and blood vessels.\textsuperscript{81-83,116,117} Interestingly, a strong profibrotic effect of IS has been well-established in the kidneys but not well-studied in the heart. Second, IS has the strongest cardiac profibrotic and prohypertrophic effects compared with other potential toxins, including pCS, as previously mentioned.\textsuperscript{103}

Is Indoxyl Sulfate a Culprit in Uremic Cardiomyopathy?

IS is derived from dietary tryptophan, an essential amino acid. Tryptophan is converted into indole by tryptophanase from intestinal flora, such as \textit{Escherichia coli} (\textit{E. coli}), at the distal intestine. Indole is absorbed across the gastrointestinal tract before being metabolized to IS by hepatic sulfation.\textsuperscript{116} Use of an oral gut adsorbent (AST-120) prevents IS synthesis by inhibiting gastrointestinal effects of indole (Figure 3).

In healthy kidneys, circulating IS is excreted by renal tubules via organic anion transporters (OATs) 1 and 3,\textsuperscript{118} at a rate of \approx 170 \mu mol per day.\textsuperscript{119} IS accumulates whenever renal tubular excretory function is impaired, initially in the kidney as well as in the circulation (Figure 3).
Detrimental Effects of IS

Kidney

Renal toxicity of IS has been extensively studied. The kidney, specifically the renal tubular cells, is most likely to be the first target of injury caused by retention of IS.\textsuperscript{120-131} IS accelerates the progression of CKD mainly because of its profibrotic and oxidative stress-inducing effects.

Renal fibrosis is a common contributor as well as sequelae of progressive kidney disease and leads to disturbances of renal function irrespective of the nature of the initial injury.\textsuperscript{132} A profibrotic effect of IS administration has been widely reported on the kidney. Oral administration of IS in 5/6-STNx rats induced renal tubular injury, renal interstitial fibrosis, and glomerular sclerosis, leading to functional impairment, determined by increased serum creatinine and blood urea nitrogen.\textsuperscript{116,133,134} Such findings are associated with increased expression of profibrotic genes, such as transforming growth factor (TGF)-β1, tissue inhibitor of metalloproteinases-1, and pro-α1(I) collagen, in the kidney.\textsuperscript{113} Glomerular sclerosis in association with renal impairment also has been demonstrated in 5/6-STNx rats receiving indole.\textsuperscript{135} The presence of IS, not indole, in the urine of indole-loaded animals confirms the protein metabolite hypothesis of IS production\textsuperscript{135} in which indole absorbed across the gastrointestinal tract into the body is metabolized by liver conjugation before urinary excretion. Treatment with AST-120 reduces renal macrophage infiltration and fibrosis in 3/4-STNx rats.\textsuperscript{136} This suggests a role for IS in activation of the inflammation–fibrosis pathway in the kidney. A recent study has supported this pathway by showing that IS can activate genes regulating inflammatory and profibrotic processes, such as IL-6 and TGF-β1, in renal proximal tubular cells.\textsuperscript{137}

IS may be implicated in the renal imbalance of NO and ROS in CKD. The net effect is in favor of ROS predominance that is linked to renal vasoconstriction, abnormal sodium and water handling, abnormal glomerular filtration and tubular function, and systemic hypertension.\textsuperscript{138} Increased IS levels are related to a significant decrease in urine levels of NO and the expression of glomerular and tubulointerstitial NO synthase in 4/5-STNx rats.\textsuperscript{139} IS has been demonstrated to induce intracellular production of ROS in cultured rat mesangial cells.\textsuperscript{140} IS-induced free radical production is associated with increased expression of plasminogen activator inhibitor and nuclear factor-κB (NF-κB) in human proximal tubular cells in a dose-dependent manner.\textsuperscript{141} In this study, activation of plasminogen activator inhibitor-1 promoter could be inhibited by an antioxidant.\textsuperscript{141} IS administration also impaired renal superoxide scavenging activity that is associated with renal dysfunction in uremic (3/4- and 5/6-STNx) rats.\textsuperscript{126}

There also is evidence that the profibrotic and oxidative stress-inducing effects of IS may be interrelated. One study demonstrated that an antioxidant can suppress IS-induced NF-κB activation in proximal tubular cells, and inhibition of NF-κB in turn suppressed TGF-β1 protein expression in uremic rats.\textsuperscript{130} The authors in this study concluded that the ROS/NF-κB/TGF-β1 pathway is likely involved in adverse renal effects induced by IS.

Figure 3. Indoxyl sulfate metabolism. The diagram shows the protein metabolite hypothesis of indoxyl sulfate (IS) production that is derived from dietary tryptophan. Inhibition of indole uptake by a gut adsorbent AST-120 reduces IS synthesis. GI tract indicates gastrointestinal tract; OAT, organic anion transporter. Modified from Enomoto et al.\textsuperscript{120}
Blood Vessel
IS is implicated in the development of accelerated atherosclerosis in the setting of CKD by inducing endothelial dysfunction and VSMC proliferation, hallmarks of atherogenesis. Increased oxidative stress determined by an increase in ROS production and nicotinamide adenine dinucleotide phosphate oxidase activity and a reduction in glutathione levels have been demonstrated in human umbilical vein endothelial cells stimulated with IS. IS-induced VSMC proliferation is likely to be mediated via activation of p44/42 MAPK and platelet-derived growth factor. OAT 3 that is expressed in VSMCs may be the site of intracellular IS uptake.

IS may be involved in vascular calcification/ossification, a common CKD-associated vascular abnormality. Increased expression of osteoblast-specific proteins that are linked to osteoblastic transformation has been demonstrated in human aortic smooth muscle cells. Increased oxidative stress determined by an increase in ROS production and nicotinamide adenine dinucleotide phosphate oxidase activity and a reduction in glutathione levels have been demonstrated in human umbilical vein endothelial cells stimulated with IS. IS-induced VSMC proliferation is likely to be mediated via activation of p44/42 MAPK and platelet-derived growth factor. OAT 3 that is expressed in VSMCs may be the site of intracellular IS uptake.

Heart
Our group has demonstrated direct cardiac effects of IS. Stimulation with IS in neonatal rat cardiac myocytes and fibroblasts increased protein and collagen synthesis, respectively. This suggests that IS may be directly implicated in adverse cardiac remodeling processes. A proinflammatory effect of IS also was demonstrated in cultured THP-1 cells, determined by a significant increase in the mRNA expression of the 3 key inflammatory cytokines, IL-1β, IL-6, and tumor necrosis factor-α. The role of these cytokines in the progression of HF is well-known. Further mechanistic study indicated that IS might exert its adverse cardiac as well as proinflammatory effects via activation of the p38 MAPK, p44/42 MAPK, and NF-κB pathways. MAPK signaling cascades are regulators of pathological cardiac remodeling. OATs 1 and 3 are likely to be responsible for intracellular IS uptake in cardiac cells because inhibition of these transporters with probenecid and cilastatin suppressed IS-stimulated cardiac myocyte hypertrophy and fibroblast collagen synthesis.

An in vivo study to assess the cardiac effects of IS in CRS was conducted in a severe CKD model induced by 5/6-STNx. At 12 weeks, the 5/6-STNx rats had a significant increase in serum IS levels in association with renal dysfunction determined by an increase in serum creatinine and urine total protein levels and a reduction in glomerular filtration rate and creatinine clearance. The STNx animals had development of diastolic dysfunction determined by Doppler and tissue Doppler echocardiography. Morphologically, 5/6-STNx induced cardiac fibrosis and hypertrophy, associated with a significant increase in cardiac protein expression of TGF-β, phospho-NF-κB, phospho-p44/42, and phospho-p38, as well as gene expression of profibrotic (TGF-β, connective tissue growth factor) and hypertrophic (atrial natriuretic peptide, β-myosin heavy chain, and α-skeletal muscle actin) markers. Treatment with AST-120 significantly reduced serum IS levels and improved renal function. AST-120-treated STNx animals showed a significant reduction in cardiac fibrosis (by 68%; Figure 4A), which was accompanied by reduced TGF-β and phosphorylated NF-κB protein expression (back to sham levels; P<0.05) despite no significant improvement in cardiac function and hypertrophy at that time point. Interestingly, reduction of IS with AST-120 was correlated with the extent of cardiac fibrosis (Figure 4B) independently of blood pressure and renal function parameters.

Consistent results of IS-lowering therapy preventing cardiac fibrosis in a 5/6-STNx model have been reported. In that study, adverse cardiac effects of IS were demonstrated to be strongly associated with increased cardiac expression of oxidative stress markers, 8-hydroxydeoxyguanosine and acrolein.

Collectively, it is suggested that: IS is likely to be at least partly responsible for the development of cardiac fibrosis in the CKD setting; the reduction of cardiac fibrosis by AST-120 treatment is partially directly because of its IS-lowering effect and not secondary to improved renal function; and IS-induced cardiac fibrosis may be mediated via the proposed ROS/NF-κB/TGF-β1 pathway of IS-induced renal fibrosis. However, further investigation is needed to confirm this mechanistic pathway. Specifically, increased ROS production caused by IS has been demonstrated in mesangial cells and in endothelial cells but has not yet been demonstrated in cardiac cells.

Reduction in IS levels potentially can be achieved in 3 ways. First, preservation of residual renal function is a fundamental goal to preserve tubular excretory function. The second strategy appears to rely on promoting IS removal with renal replacement therapy. Unfortunately, the effectiveness in removal of PBUTs by current conventional dialysis including new strategies is not yet satisfactory. Reducing IS production, the third strategy, may be the best choice for IS control at present.

Based on the protein metabolite hypothesis of IS metabolism (Figure 3), IS production can be reduced by a low-protein diet, specifically tryptophan that is the precursor of IS, by modifying intestinal microflora to reduce indole production, and by blocking IS precursors from intestinal absorption. Restriction of dietary tryptophan has limitations because tryptophan is an essential amino acid unable to be synthesized by the body, and it is ubiquitously found in basic foods, such as milk, eggs, nuts, soy, fish, and chicken. Furthermore, disadvantages of dietary protein restriction (especially risk of malnutrition) may outweigh the desired advantages. Treatment with bifidobacteria to replace E. coli (tryptophanase-producing bacteria) can significantly reduce serum IS levels in HD patients. However, bacterial capsules are easily inactivated by gastric acid before reaching the intestinal target.

Prevention of IS precursors from being absorbed across the intestinal tract has been extensively studied in the renal literature by use of oral adsorbents. AST-120 (Kremezin), an oral charcoal, has been demonstrated to effectively reduce circulating and renal IS levels. AST-120 specifically adsorbs low-MW molecules at the distal intestine where tryptophan is metabolized. An improvement in renal structure and function with AST-120 treatment has been observed in uremic animal models. In CKD patients, AST-120 has been shown to improve estimated creatinine clearance, to delay the progression to ESRD, and to improve survival. Two large-scale trials are currently ongoing testing the role of AST-120 in CKD populations.

Cardio renal-protective effects of AST-120 recently have been demonstrated in a small, uncontrolled trial of chronic
HF patients with moderate CKD. These patients showed an improvement in renal function, cardiothoracic ratio, atrial natriuretic peptide levels, length of hospital stay, and a reduced number of admissions after long-term (2 years) AST-120 treatment, compared with before treatment.159 Our data from an in vivo MI study support the cardiorenal beneficial effects of AST-120.160 At 16 weeks, MI animals had development of profound systolic dysfunction and renal impairment in association with an increase in serum IS levels, renal interstitial fibrosis, and cardiac fibrosis in noninfarct myocardium. Reduction of IS levels by AST-120 treatment partially prevented renal fibrosis and normalized cardiac TGF-β1 and tumor necrosis factor-α mRNA expression despite absence of effect on cardiac fibrosis (Figure 5).

Other Related Mechanisms/Mediators of IS-Induced Cardiorenal Fibrosis

Neurohormonal activation is a well-known contributor to the progression of CVD, kidney disease, and CRS. Several well-known mediators of neurohormonal processes, such as angiotensin II, aldosterone, and endothelin-1, are linked to cardiac and renal fibrosis.161–165 Marinobufagenin, an endogenous cardiorenal steroid induced by angiotensin II,166 was recently identified in uremic patients.167 Increased circulating levels of marinobufagenin in a STNx model have been demonstrated to induce cardiac fibrosis and hypertrophy, and has been shown to impair diastolic function,168 in association with increased systemic oxidative stress,169 similar to IS effects. Whether IS-induced cardiac fibrosis is associated with activation of such neurohormonal processes is not clear and requires further investigation.

Renal fibrosis induced by IS and pCS recently has been demonstrated to be mediated via the epithelial-to-mesenchymal transition mechanism (changes in the epithelial-to-myofibroblastic phenotype of renal tubular epithelial cells), which is associated with intrarenal RAAS activation determined by an increased expression of renin, angiotensinogen, and angiotensin II type 1 receptor.170 Such IS-induced renal fibrosis is prevented by the angiotensin II receptor blocker losartan in association with decreased renal expression of TGF-β1 expression. The authors170 suggested that intrarenal RAAS activation is most likely because of CKD-associated oxidative stress, which has been shown to be enhanced by IS.130 Interestingly, a superior renoprotective effect of combined RAAS blocker and AST-120 therapy compared with AST-120 treatment alone has been reported in CKD patients.154 Thus, it is of importance to investigate whether neurohormonal pathways activated in CRS are involved in IS-induced cardiac fibrosis because their pharmacological blockade is readily available. This may be simply achieved by use of RAAS blockade in IS-stimulated cultured cardiac fibroblasts or in an experimental CKD model treated with or without IS-lowering agents. Prevention of IS effects via early treatment with neurohormonal blockade or AST-120, because IS starts accumulating in early-stage CKD, may be important in progression of heart and kidney disease.

Figure 4. AST-120 reduces cardiac fibrosis in the 5/6 subtotal nephrectomy (STNx) model. Upper row, left ventricular cross-sections (magnification, ×0.8) show focal interstitial and perivascular fibrosis stained in red with Picrosirius red. STNx+vehicle animals demonstrated significantly increased compared with sham animals. A, A significant reduction in cardiac fibrosis is observed in STNx animals treated with AST-120. B, The extent of cardiac fibrosis is positively correlated with changes in serum indoxyl sulfate (IS) level. *P<0.05, ***P<0.001 vs sham; #P<0.01 vs STNx+vehicle. Modified from Lekawanvijit et al.147

HF patients with moderate CKD. These patients showed an improvement in renal function, cardiothoracic ratio, atrial natriuretic peptide levels, length of hospital stay, and a reduced number of admissions after long-term (2 years) AST-120 treatment, compared with before treatment.159 Our data from an in vivo MI study support the cardiorenal beneficial effects of AST-120.160 At 16 weeks, MI animals had development of profound systolic dysfunction and renal impairment in association with an increase in serum IS levels, renal interstitial fibrosis, and cardiac fibrosis in noninfarct myocardium. Reduction of IS levels by AST-120 treatment partially prevented renal fibrosis and normalized cardiac TGF-β1 and tumor necrosis factor-α mRNA expression despite absence of effect on cardiac fibrosis (Figure 5).

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IS recently was found to be the first identified potent endogenous agonist for the human aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor. AHR activation induced by IS increases expression of IL-6 gene in MCF-7 (human breast cancer cell line) and drug metabolism genes in cultured liver cells. AHR also is involved in the cardiovascular system. Specifically, AHR activation by a solely AHR-mediated exogenous toxin 2,3,7,8-tetrachlorodibenzo-p-dioxin has been demonstrated to rapidly enhance genes that regulate cardiac proliferation, contractility, and metabolism. Whether cardiac and renal AHR could be activated by IS and thus may be involved in the pathogenesis of IS-induced cardiorenal fibrosis is unknown.

**Conclusion**

CV pathology in the setting of CKD has its own unique characteristics, of which the pathogenesis is not yet fully understood. This review has focused on the role of PBUTs emerging as novel risk factors for the development of CKD-associated CVD. There has been recent growing evidence of adverse cardiac, vascular, and renal effects induced by such toxins, with the main focus being on a few potential toxins such as IS and pCS (Table). At present, IS appears to be the strongest evidence-based PBUT involved in the pathophysiology of CRS (types 2 and 4 according to Ronco et al.). IS cardiorenal toxicity is mainly attributable to its profibrotic effects. Cardiac and renal fibrosis are considered important surrogates representing the pathological remodeling processes underlying the progression of CKD and HF.

Despite strong evidence of adverse CV effects caused by PBUTs, little mechanistic explanation has been uncovered, particularly regarding their direct effects on the heart. The most promising potential mechanism of IS-induced cardiac fibrosis currently points to activation of the ROS/NF-κB/TGF-β1 axis, a similar pathway proposed in IS-induced renal fibrosis. However, this needs to be further elucidated, especially the evidence of increased cardiac ROS production and other cellular signaling mediators in this pathway. Cardiac fibrosis and renal fibrosis caused by IS may share a common pathogenesis pathway; however, underlying mechanism may not necessarily be the same because of differences in resident cell types and the intrinsic milieu that exists in both organs. For example, the epithelial-to-mesenchymal transition mechanism is commonly involved in pathological fibrogenesis caused by the various causes of kidney disease, but this mechanism is not well documented in the heart. Interestingly, the question whether IS-induced cardiac fibrosis may be linked to other common mediators described in the pathophysiology of the CRS, particularly neurohormonal processes, remains currently unanswered.

As previously mentioned, increased IS levels can occur in early-stage CKD. The harmful biological activity of IS, therefore, needs urgent attention before consideration of renal replacement therapy. At present, AST-120 appears to be the treatment of choice for adverse cardiorenal effects caused by IS. Unfortunately, there has been little investigation of both CV and renal effects within the same study. Most clinical studies of AST-120 in the CKD population have mainly focused on renal outcomes and all-cause mortality.

In addition to AST-120, OAT inhibition may be another potential treatment for IS-induced cardiac fibrosis. However, more solid evidence of cardiac OAT expression and OAT-mediated cardiac toxicity caused by IS is required.

In summary, the concept of the heart and the kidney as individual organs has progressively shifted to consideration of an integrated cardiorenal system because of the complex interrelationship of both organs. Cardiorenal toxicity of nondialyzable PBUTs has been poorly studied until recently, despite strong evidence being still limited to a few toxins. More intense investigation should yield a list of PBUTs adversely affecting the cardiorenal system and important mechanistic insights as well as novel therapeutic opportunities in parallel with ongoing development of dialysis technology.
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In the *Circulation Research* article by Lekawanvijit et al (Cardiorenal syndrome: the emerging role of protein-bounduremictoxins. *Circ Res*. 2012;111:1470–1483. DOI:10.1161/CIRCRESAHA.112.278457), on page 1472, right column, line 14, the text should be: “…phosphaturic and PTH-suppressing effects without…”

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