Protein-bound uremic toxins: a long overlooked culprit in cardiorenal syndrome

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1Department of Pathology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand; and 2Centre of Cardiovascular Research and Education in Therapeutics, Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia

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Lekawanvijit S, Kompa AR, Krum H. Protein-bound uremic toxins: a long overlooked culprit in cardiorenal syndrome. Am J Physiol Renal Physiol 2016; 311:F52–F62. First published May 4, 2016; doi:10.1152/ajprenal.00348.2015.—Protein-bound uremic toxins (PBUTs) accumulate once renal excretory function declines and are not cleared by dialysis. There is increasing evidence that PBUTs exert toxic effects on many vital organs, including the kidney, blood vessels, and heart. It has been suggested that PBUTs are likely to be a potential missing link in cardiorenal syndrome, based on the high incidence of cardiovascular events and mortality in the dialysis population, which are dramatically reduced in successful kidney transplant recipients. These data have led the call for more effective dialysis or additional adjunctive therapy to eradicate these toxins and their adverse biological effects. Indoxyl sulfate and p-cresyl sulfate are the two most problematic PBUTs, conferring renal and cardiovascular toxicity, and are derived from dietary amino acid metabolites by colonic microbial organisms. Therefore, targeting the colon where these toxins are initially produced appears to be a potential therapeutic alternative for patients with chronic kidney disease. This strategy, if approved, is likely to be applicable to predialysis patients, thereby potentially preventing progression of chronic kidney disease to end-stage renal disease as well as preventing the development of cardiorenal syndrome.

Conversely, renal dysfunction is a common complication of CVD. The degree of renal dysfunction is a powerful independent risk factor for all-cause as well as cardiovascular mortality in heart failure patients (29). In the setting of acute myocardial infarction, mild and transient renal impairment during hospitalization is associated with long-term (10-yr) mortality (68).

Both CVD and CKD share common pathophysiological mechanisms (e.g., persistent neurohormonal activation, hemodynamic derangement, and systemic inflammatory activation) and comorbidities (e.g., diabetes and hypertension). However, the occurrence of adverse clinical outcomes is still unacceptably high despite the advance in therapeutic treatment of these conditions and comorbidities. This may be for reasons that 1) the pathophysiology of cardiorenal syndrome is not well understood due to a relative lack of mechanistically oriented studies in the setting of combined cardiovascular and renal diseases, and 2) there are still missing culprits/links that play a role in cross talk between these two organ systems (Fig. 1).

Protein-bound uremic toxins (PBUTs) have recently been recognized as a potential missing link in cardiorenal syndrome. Strong evidence for the oxidative stress-inflammation-fibrosis processes in both cardiovascular and renal tissue is attributable to PBUTs. This has been well demonstrated with two of the most potent toxins: indoxyl sulfate (IS) and p-cresyl sulfate (pCS) (Table 1). Uremic toxins begin to accumulate in the circulation once renal excretory function declines, even with mild to moderate renal dysfunction (9, 42). Accumulated toxins are believed to be a cause of uremic syndrome in CKD patients, and their removal generally relies on dialysis when the patient reaches end-stage renal disease (ESRD) or stage 5 CKD (54). However, removal of the key PBUTs by dialysis is problematic due to their high protein (mostly albumin)-binding affinity (32).

Importantly, many PBUTs including IS and pCS are derived from colonic microbial metabolic products using dietary amino acids tryptophan and tyrosine/phenylalanine as a substrate, respectively. Thus the colon has become a potential novel target for treatment to reduce the toxicity caused by colon-derived PBUTs. This strategy, if applicable, could offer several benefits over conventional renal replacement therapy, in terms of easier accessibility to treatment, lower cost, reduced complexity, and fewer major adverse complications. In addition, targeting the colon may be an ideal preventive strategy since treatment can be applied before stage 5 CKD or renal failure is reached.

Discovery of PBUTs as a Missing Link in Cardiorenal Syndrome

Concern about the toxicity associated with PBUTs primarily originates from renal studies (Table 1). Both IS and pCS are implicated in CKD progression (94) and cardiovascular and...
all-cause mortality (12, 43). CKD progression induced by IS and pCS is likely to be associated with renal inflammatory activation (83), renal interstitial and glomerular fibrosis, and increased expression of profibrotic genes and proteins (82). IS also promotes renal proximal tubular cell senescence via the reactive oxygen species/NF-κB pathway (80). Renal fibrosis induced by IS and pCS has been demonstrated to be mediated via the Smad-dependent TGF-β pathway and is associated with epithelial-to-mesenchymal transition of renal tubular cells and intrarenal activation of the renin-angiotensin-aldosterone system (82). Interestingly, administration of losartan, an angiotensin II type 1 receptor blocker, reduced renal fibrosis and its associated pathways in nephrectomized mice (82).

PBUTs are also implicated in the pathogenesis of atherosclerosis as well as nonatherosclerotic vascular diseases commonly found in the setting of CKD, such as vascular stiffness, calcification, and ossification (Fig. 2 and Table 1). In atherogenesis, IS and pCS promote endothelial dysfunction (15, 53), oxidative stress (17), vascular leakage by increasing endothelial permeability (70), impaired blood flow, and leukocyte adhesion (70). IS can stimulate vascular smooth muscle cell proliferation, one of the hallmarks of atherogenesis (96). For nonatherosclerotic vascular diseases, IS has been implicated for contributing to aortic calcification and osteoblastic transformation of aortic smooth muscle cells (5, 59).

Direct cardiac effects of PBUTs have recently been reported (Table 1). IS has been demonstrated to have profibrotic and prohypertrophic effects in cardiac cells as well as a proinflammatory effect in monocytes by increasing gene expression of key inflammatory cytokines involved in the progression of heart failure (37). The cardiac profibrotic effect of IS has also been observed in renal failure and animal models of myocardial infarction with concomitant renal impairment that is likely to be mediated through the oxidative stress/NF-κB/TGF-β pathway similar to that mediating IS-induced renal fibrosis (20, 38, 40). Furthermore, p-cresol, the parent compound of pCS, has been shown to induce gap junction abnormalities in cultured cardiomyocytes, reducing myocyte conduction frequency and connexin 43 staining (69).

Therapeutic Prospects

Although the issue of inadequate removal of PBUTs originates from the ESRD population receiving dialysis, PBUTs actually start accumulating at earlier stages of renal dysfunction. Hence, their biological toxicity can develop long before the commencement of renal replacement therapy, which may cause irreversible damage to the target organs of PBUTs.

Ideally, any additional therapeutic strategies to conventional dialysis that could improve clinical outcomes closer to successful kidney transplantation has the potential to be included as part of the standard treatment for cardiorenal syndrome, especially in patients at high risk for progression to ESRD. PBUTs, which play an important role in the pathophysiology of CVD and renal disease, are believed to be an important missing link of cardiorenal syndrome, and targeting them could offer better outcomes (Table 2). The two most damaging PBUTs with regard to their renal and cardiovascular toxicity are IS and pCS, which originate from gut microbial metabolism (7, 92). Therefore, addressing their production in the colon could prevent the progression of cardiorenal syndrome (Fig. 2). In addition, information regarding signaling pathways involved in PBUT-associated cardiorenal toxicity may provide selective targets for treatment (Fig. 2); however, this still requires further well-designed experimental studies and randomized controlled trials.

Preventing toxin production. Targeting colonic production of gut-derived PBUTs appears to be the most feasible approach to prevent their biological toxicity. A previous study demonstrated using liquid chromatography/mass spectrometry an absence or reduced levels of ≥30 uremic solutes in dialysis patients with a colectomy compared with those with an intact colon (7). Several of these suspected gut-derived PBUTs were inadequately removed by dialysis, as indicated by a substantially lower reduction ratio of pre- and postdialysis concentrations compared with the reduction ratio of urea (7).

Prevention of gut-derived PBUT production can be divided into three main strategies: dietary protein restriction, maintenance of gut homeostasis, and oral sorbents (Fig. 2 and Table 2).

First, protein restriction diets limit the amount of substrate for PBUT production. A very low-protein diet (0.3 g·kg body wt·day⁻¹) has been demonstrated to significantly reduce serum IS levels in predialysis CKD patients after only 1 wk (47). Of note, supplementary ketoanalogs and essential amino
### Table 1. Summary of studies on protein-bound uremic toxins in cardiovascular and renal disease

<table>
<thead>
<tr>
<th>Protein</th>
<th>Cardiac Effects</th>
<th>Cardiac Effects</th>
<th>Cardiovascular Outcomes</th>
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<tbody>
<tr>
<td><strong>Indoxyl sulfate</strong></td>
<td>Cardiac Effects: Profibrotic and prohypertrophic effects in NCF and NCM, respectively, with OATs 1 and 3 as potential intracellular transporters of IS (44).</td>
<td>Cardiac fibrosis in association with diastolic dysfunction via TGF-β/NF-κB pathway in a ½ nephrectomy model (38).</td>
<td>Increased risk for cardiovascular and all-cause mortality (12).</td>
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<td></td>
<td>Renal Effects: Increased expression of renal proinflammatory genes encoding major cytokines and intracellular signal mediators as well as target genes connected to TGF-β1 (83).</td>
<td>Renal Effects: Impair renal function (56, 57, 64).</td>
<td>Progression of CKD (12, 94).</td>
</tr>
<tr>
<td><strong>p-Cresyl sulfate</strong></td>
<td>Cardiac Effects: Profibrotic and prohypertrophic effects (39).</td>
<td>Cardiac fibrosis and hypertrophy in association with increased cardiac oxidative stress in ½ nephrectomy plus adriamycin and ½ nephrectomy models (20).</td>
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<tr>
<td></td>
<td>Vascular Effects: Enhanced oxidative stress, and activate NF-κB and PAI-1 promoter in human renal proximal tubular cells (58).</td>
<td>Vascular Effects: Enhance endothelial-leukocyte adhesion, leukocyte extravasation, and interrupted blood flow without a vasoactive effect, fibrin deposition, or capillary plugging in rats with IS-peritoneal superfusion (70).</td>
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<td></td>
<td>Renal Effects: Enhance oxidative stress, and activate NF-κB and PAI-1 promoter in human renal proximal tubular cells (58).</td>
<td>Renal Effects: Promote oxidative stress by reducing urine and renal nitric oxide in nephrectomy rats (87) and reduce renal superoxide scavenging activity in both normal and uremic rats (67).</td>
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<tr>
<td></td>
<td>Enhance renal oxidative stress in mesangial cells (22).</td>
<td>Glomerulosclerosis (56, 57, 64), tubular atrophy (56), and renal interstitial fibrosis (56) with increased expression of profibrotic genes [TGF-β1, TIMP-1 and pro-α(I) collagen] (56, 57).</td>
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<tr>
<td></td>
<td>Enhance oxidative stress, and activate NF-κB and PAI-1 promoter in human renal proximal tubular cells (58).</td>
<td>Induce renalinfibrosis with renalin-angiotensin activation and increased renal TGF-β1 protein expression in IS-administered mice (82).</td>
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<tr>
<td></td>
<td>Promote cell senescence by downregulation of renal Klotho gene and protein expression in human proximal tubular cells, which is inhibited by antioxidant and NF-κB inhibitors (79).</td>
<td>Renal fibrosis (81) in association with decreased Klotho gene (a renoprotective antiaging gene) expression (79, 81) due to DNA hypermethylation in IS-administered mice (81).</td>
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<tr>
<td></td>
<td>Increased expression of renin, angiotensinogen, and angiotensin 1 receptor genes as well as activation of Smad-associated TGF-β1 pathway in IS-treated renal tubular cells (82).</td>
<td>Induce epithelial-to-mesenchymal-like transition (82).</td>
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acids are recommended together with protein restriction to maintain the nitrogen balance.

Second, maintenance of normal gut homeostasis has been shown to decrease circulating levels of PBUTs in both CKD animal models and patients. In the setting of CKD, changes in colonic microbial fermentation from a saccharolytic to proteolytic pattern (11) as well as prolonged colonic transit time (95) enhance the production of PBUTs from dietary amino acids. There are three major ways to modify gut homeostasis: probiotic, prebiotic, and synbiotic treatments.

Administering microorganism or “probiotics” such as lactic acid bacilli (28) and *Bifidobacterium longum* (84) in dialysis patients can reduce circulating IS levels. A randomized controlled trial reported an improvement of renal function in CKD patients (n = 46) receiving 6 mo of probiotic treatment; however, this study did not investigate serum levels of PBUTs. On the other hand, “prebiotics” which use nondigestible dietary fiber to modify the gut microbial milieu have been demonstrated to lower serum IS levels and improve renal injury in an animal CKD model after administration of galacto-oligosaccharides for 2 wk (21). A clinical study using oligofructose-enriched inulin for 4 wk in dialysis patients showed a significant reduction of serum pCS levels but not IS levels (52). Synbiotics, a combination of probiotic and prebiotic treatment, significantly decreases circulating levels of p-cresol in both predialysis and dialysis CKD patients (25, 60). In addition, decreasing colonic transit time of amino acid substrates by simply using laxa-

Table 1.—Continued

<table>
<thead>
<tr>
<th>In Vitro Studies</th>
<th>In Vivo Studies</th>
<th>Clinical Studies</th>
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<tr>
<td><strong>Vascular Effects</strong></td>
<td><strong>Vascular Effects</strong></td>
<td><strong>Renal Outcomes</strong></td>
</tr>
<tr>
<td>Promote endothelial dysfunction by inducing Rho kinase-mediated microparticle shedding in HUVEC (53).</td>
<td>Impair blood flow with vascular leakage, in the presence of p-cresyl glucuronide in peritoneal toxin-supfusion model (70).</td>
<td>Progression of CKD (43, 94).</td>
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<tr>
<td>Increase endothelial permeability to albumin in the presence of p-cresyl glucuronide (63).</td>
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<tr>
<td>Increase leukocyte oxidative burst activity in healthy human blood exposed to pCS (50, 76).</td>
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<tr>
<td><strong>Renal Effects</strong></td>
<td><strong>Renal Effects</strong></td>
<td><strong>Cardiovascular Outcomes</strong></td>
</tr>
<tr>
<td>Increase expression of renal proinflammatory genes encoding major cytokines and intracellular signal mediators as well as target genes connected to TGF-β1 (83).</td>
<td>Induce renal fibrosis with increased renal renin, angiotensinogen, and angiotensin 1 receptor mRNA expression and TGF-β1 protein expression in pCS-administered mice (82).</td>
<td>Increased risk for cardiovascular events (51) and all-cause mortality (10).</td>
</tr>
<tr>
<td>Increased expression of renin, angiotensinogen, and angiotensin 1 receptor genes, and activation of Smad-associated TGF-β1 pathway in pCS-treated renal tubular cells (82).</td>
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<tr>
<td>Induce epithelial-to-mesenchymal-like transition (82).</td>
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**p-Cresol**

<table>
<thead>
<tr>
<th>Cardiac Effects</th>
<th>Vascular Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prohypertrophic effects in cultured cardiac myocytes (39).</td>
<td>Defective endothelial proliferation and wound repair without effects on cell viability and apoptosis in cultured HUVEC (15).</td>
</tr>
<tr>
<td>Reduce spontaneous contraction with enhanced irregular beating of cardiac myocytes in association with abnormal structural and functional changes in the gap junction (69).</td>
<td>Defective leukocyte adhesion and inhibition of endothelial adhesion molecule expression in cytokine-stimulated HUVEC (16).</td>
</tr>
<tr>
<td><strong>Renal Effects</strong></td>
<td><strong>Renal Effects</strong></td>
</tr>
<tr>
<td>n/a</td>
<td>Induce renal tubular adenoma (74).</td>
</tr>
</tbody>
</table>

AhR, aryl hydrocarbon receptor; CKD, chronic kidney disease; HUVEC, human umbilical vein endothelial cells; IS, indoxyl sulfate; NCF, neonatal rat cardiac fibroblast; NCM, neonatal rat cardiac myocyte; n/a, no data available; NO, nitric oxide; OAT, organic anion transporter; PAI-1, plasminogen activator inhibitor-1; pCS, p-cresyl sulfate; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; TIMP-1, tissue inhibitor of metalloproteinase-1; TGF-β1, transforming growth factor-β1; VSMC, vascular smooth muscle cell.
tives in conjunction with colonic microbiome modification strategies will help decrease colonic production of PBUTs.

Last, administration of oral sorbents can inhibit gastrointestinal absorption of PBUT precursors by adsorbing and enhancing their excretion into the feces. AST-120 is a synthesized carbon adsorbent, selectively adsorbing low-molecular weight compounds in the lower gastrointestinal tract where PBUT precursors are metabolized by colonic microbiota. AST-120 has been demonstrated in preclinical and nonrandomized clinical studies to reduce serum levels of PBUTs, mostly focusing on IS, improve clinical outcomes, and inhibit their biological toxicity on the kidney, blood vessels, and heart (Table 2). Two large-scale randomized controlled trials of AST-120 have been conducted in moderate to severe CKD patients. The first study (n = 460) did not observe a substantial delay in CKD progression within 1 yr of follow-up, although the decline in estimated creatinine clearance was greater in placebo than AST-120-treated groups (6). The recent randomized placebo-controlled EPPIC trial (n = 2,035) also reported that AST-120 did not show renal benefits using the composite end point of dialysis initiation, kidney transplantation, and serum creatinine doubling (77). Major limitations of both trials were infrequent primary end point events due mainly to a longer actual than estimated median time to primary end point events. The actual
Table 2. Summary of studies on targeting production of protein-bound uremic toxins in cardiovascular and renal disease

<table>
<thead>
<tr>
<th>Animal Studies</th>
<th>Clinical Studies</th>
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<tr>
<td><strong>Protein Restriction Diet</strong></td>
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<tr>
<td>Improved renal function and delayed progression of glomerulosclerosis as well as reduced serum and urine IS levels in a model of uremia (64).</td>
<td>Reduced serum IS levels with a low-protein diet (consisting of a mixture of ketoanalog and amino acid supplements) in predialysis CKD patients (47).</td>
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<tr>
<td><strong>Maintain Gut Homeostasis</strong></td>
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<tr>
<td><strong>Prebiotics</strong></td>
<td><strong>Prebiotics</strong></td>
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<tr>
<td>Reduce serum IS levels, cecal indole, endoplasmic reticulum stress, and apoptosis, and improve renal injury in association with gut microbiota modification after 2 wk administration of galacto-oligosaccharides in 5⁄6 nephrectomized rats (21).</td>
<td>Reduce circulating IS levels by using lactic acid bacilli (28) and <em>Bifidobacterium longum</em> (84) in hemodialysis patients.</td>
</tr>
<tr>
<td><strong>Laxatives</strong></td>
<td><strong>Laxatives</strong></td>
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<tr>
<td>Accelerate intestinal transit with alterations of gut microbiota in association with a decrease in IS levels, improved renal function, attenuated renal fibrosis, and a reduction in the expression of renal fibrotic and inflammatory cytokine genes by using lubiprostone in mice with renal failure (55).</td>
<td>Improve renal function and quality of life in stages 3 and 4 CKD patients (double-blind randomized-controlled trial, probiotic treatment for 6 mo, n = 46) (72).</td>
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<tr>
<td><strong>Oral sorbents</strong></td>
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<tr>
<td><strong>CV End Points</strong></td>
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<tr>
<td>Prevent atherosclerotic plaque extension, inflammation, and necrosis in apolipoprotein E-deficient mice with CKD (97).</td>
<td>Reduce serum levels of pCS, but not IS, with administration of prebiotic oligofructose-enriched inulin for 4 wk in hemodialysis patients (nonrandomized phase I/II study, n = 22) (52).</td>
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<tr>
<td>Reduce plasma levels of cholesterol and very low density lipoprotein in rats with spontaneous focal glomerulosclerosis (75).</td>
<td>Synbiotics</td>
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<tr>
<td>Reduce cardiac fibrosis, cardiac TGF-β protein expression, cardiac NF-κB phosphorylation, and cardiac oxidative stress in association with reduced serum IS levels in 5⁄6 nephrectomized rats (20, 38).</td>
<td>Reduce plasma p-cresol levels on days 15 and 30 after administration of a commercial synbiotic, Probioin neutro in stages 3 and 4 CKD patients (double-blind, randomized placebo-controlled trial, n = 30) (25).</td>
</tr>
<tr>
<td><strong>Renal End Points</strong></td>
<td><strong>Renal End Points</strong></td>
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<tr>
<td>Decrease serum IS levels in association with improved renal function and glomerulosclerosis (56, 64), decrease oxidative stress by restoring urine and renal nitric oxide (87), reduce interstitial fibrosis and tubular injury with decreased expression of profibrotic genes (TGF-β1, TIMP-1, and pro-α1(I) collagen) (56), attenuate renal fibrosis with concomitant inhibition of TGF-β1 gene expression and renal cortical NF-κB-DNA binding activity in a model of uremia (85).</td>
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</tr>
<tr>
<td><strong>Synbiotics</strong></td>
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<tr>
<td>Reduce serum p-cresol and improved bowel habits by using <em>Lactobacillus casei</em> and <em>Bifidobacterium breve</em> as probiotics and galacto-oligosaccharides as prebiotics for 2 wk in 7 hemodialysis patients (60).</td>
<td>Reduce vascular stiffness (pulse wave velocity), and carotid intima-media thickness (the surrogate markers for atherosclerosis) after 12- and 24-mo duration of AST-120 treatment, respectively, in predialysis patients (62).</td>
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<tr>
<td>Improve the aortic calcification index with AST-120 treatment duration of at least 6 mo starting from the predialysis stage (24).</td>
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<tr>
<td>Prevent atherosclerotic plaque extension, inflammation, and necrosis in apolipoprotein E-deficient mice with CKD (97).</td>
<td>Improve endothelial dysfunction by increasing flow-mediated endothelium-dependent vasodilatation in association with decreased plasma IS levels and GSSG/GSH ratio with 24 wk AST-120 administration in CKD patients, preferentially effective in those with low baseline high-sensitivity C-reactive protein levels and absence of diabetes (98).</td>
</tr>
<tr>
<td>Reduce plasma p-cresol levels on days 15 and 30 after administration of a commercial synbiotic, Probioin neutro in stages 3 and 4 CKD patients (double-blind, randomized placebo-controlled trial, n = 30) (25).</td>
<td>Reduce vascular stiffness (pulse wave velocity), and carotid intima-media thickness (the surrogate markers for atherosclerosis) after 12- and 24-mo duration of AST-120 treatment, respectively, in predialysis patients (62).</td>
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<td>Improve renal function after 1-yr duration of AST-120 treatment in predialysis CKD patients (45).</td>
</tr>
<tr>
<td>Reduce risk for initiation of dialysis or diagnosis with renal failure after 2 yr of AST-120 administration in predialysis patients (89).</td>
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Continued
Cardiorenal End Points

Reduce IS-induced renal and cardiac fibrosis, as well as the expression of both renal and cardiac profibrosis markers including normalizing cardiac microRNA-21, -29b, and angiotensin-converting enzyme and angiotensin receptor 1a gene expression in a myocardial infarction model with coexisting renal impairment (42, 71).

INHIBITION OF INTRACELLULAR SIGNAL MEDIATORS. PBUTs—induced renal and cardiovascular toxicity is most likely mediated through the oxidative stress-inflammation-fibrosis pathway. Antioxidant and NF-κB inhibitors have been shown to dose dependently inhibit the IS-induced activation of plasminogen activator inhibitor-1, a factor known to promote tubulointerstitial fibrosis, in cultured renal proximal tubular cells (58). The aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, may be another possible target of treatment for IS-induced renal and vascular toxicity. AhR activation by IS in endothelial cells promotes oxidative stress (48) and cellular senescence (34), as well as expression of monocyte chemoattractant protein-1, a chemokine involved in atherosclerosis which is abolished by AhR inhibitors in a dose-dependent manner (91). IS has recently been demonstrated to be involved in renal glomerular podocyte injury by increasing activity of the AhR (30). This suggests that podocyte injury may be a cellular mechanism of IS-induced glomerulosclerosis, which may be prevented by AhR inhibitors.

One of the greatest difficulties regarding cell signaling inhibition is to selectively target the specific site of activation in the affected organs. Data on targeting signal transduction pathways activated by PBUTs are mainly based on experimen-
Value and Roadblocks in Cardiorenal Research Focusing on PBUTs

Most reports investigating the biological effects of PBUTs on renal and/or cardiovascular systems have been derived from preclinical research that has demonstrated a potential causative role of these nondialyzable toxins in the development and progression of cardiorenal syndrome. Such cumulative evidence has been initiated a research direction to further find therapeutic strategies to eradicate accumulated PBUTs and reduce their toxicity in CKD patients. Ideally, successful kidney transplantation is the most effective way but donor kidneys are in extremely short supply. In parallel with ongoing studies to improve dialysis performance, targeting colonic production of PBUTs has been increasingly investigated. However, there have been many clinical concerns and major research hurdles in this area that need a sophisticated approach.

In vitro study on PBUTs in cardiovascular research should use concentrations of toxin within their clinical uremic range. Regarding their protein binding capacity, ideally culture conditions should replicate the conditions observed in CKD patients by using human serum albumin at the average uremic concentration of 35 g/l in any cell-based system not containing protein as recommended by the European Uremic Toxin Work Group (13). To test effects of phenyl derivatives in vitro, p-cresol should be avoided because p-cresol is not present in the human circulation but it is an artifact caused by the acid hydrolysis of its conjugate during the measurement (14). Although in vitro analysis is of importance to explore the mechanistic pathway of PBUT toxicity, it has no relevance for the investigation of interventions preventing colonic production of these toxins.

The in vivo investigation of PBUT administration to animals with healthy kidneys presents difficulties in obtaining sufficient levels of toxin accumulation in the circulation. A surgery-induced 5/6 nephrectomy CKD model with or without PBUT administration is the most commonly used; this model development requires a skillfully performed surgery utilizing a renal artery ligation technique or a renal parenchymal ablation technique to achieve an actual 5/6 nephrectomy. Moreover, a cardiorenal model, such as combined nephrectomy and coronary artery ligation model, adds more substantial difficulty and provides a challenge to interpret the study results. At present, there is no model reproducing the pathophysiology of cardiorenal syndrome observed in humans. In addition, studies of PBUTs with simultaneous assessment of renal and cardiovascular end points is very rare.

In clinical studies and trials in patients with CKD, heterogeneity of the complex CKD population despite strict inclusion criteria and a proper sample size appear to be problematic especially in large-scale trials. CKD patients commonly have concomitant comorbidities, such as diabetes, hypertension, and cardiovascular disease, which can vary in number, subtype, and degree of severity. This may indirectly but substantially contribute to an inadequate incidence rate of study end points within a defined study period (6) or to an incorrect underestimate of the time to events (77). This was reflected in the EPPIC trials with an increase in an actual average time to event of 170–190 wk from an estimated time to event of 124 wk (77). Adjusting the inclusion criteria to recruit participants at high risk for progression of CKD or for adverse CV outcomes, when it comes to evaluating CV end points, should reduce the heterogeneity of the study population.

In addition, clinical studies with renal outcomes commonly use serum creatinine concentration to monitor renal function. Serum creatinine concentration has been considered suboptimal or not sensitive enough to detect minimal renal parenchymal damage due to the functional reserve capacity of the kidney, although creatinine levels are not disturbed by AST-120, and has been proved to be valid for renal function monitoring in AST-120-treated patients (46). There have been studies reporting potential novel biomarkers which directly represent renal parenchymal damage such as kidney injury molecule-1 (90) and neutrophil gelatinase-associated lipocalin (35) or reflect renal filtration function with less bias than serum creatinine, such as cystatin C (19). Use of such novel biomarkers in addition to conventional serum creatinine may be useful in refining renal outcome assessment.

Importantly from the cardiorenal viewpoint, adding cardiovascular outcomes/end points could provide additional clinically useful information to clinical trials on CKD population. For instance, hospitalization rate for CVD has been demonstrated to be approximately twice as common in CKD patients with concomitant CVD compared with those without, which is independent of the stage of the disease (3). Noting that all three large randomized trials with AST-120 in CKD patients (6, 77) did not virtually exclude all types of CVD, but only severe heart failure, arrhythmias, and recent cardiovascular events, suggests a percentage of the participants had underlying CVD at baseline; however, subgroup analysis (groups with the presence vs. the absence of CVD at baseline) was not demonstrated in the study results.

Last, measurement of PBUT levels is usually performed in clinical studies on PBUTs’ effects without interventions/treatment, which rarely occurs in clinical trials. However, PBUT levels in clinical trials provide supportive evidence of the causative role of PBUTs in humans and can be used to monitor compliance of the participants.

Conclusions

With emerging evidence of the toxicity associated with PBUTs, monitoring them as a marker for evaluating dialysis adequacy in addition to conventional biomarkers such as urea and creatinine should be considered. More mechanistic insights into cardiorenal toxicity induced by individual PBUTs are needed, including the toxins newly discovered by high-throughput mass spectrometry, for which the physicochemical characteristics and function are not yet identified. Finally, potential therapeutic strategies for cardiorenal syndrome still require supportive in vivo studies and translation by appropriately designed randomized controlled trials.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.L. prepared figures; S.L. drafted manuscript; S.L., A.R.K., and H.K. edited and revised manuscript; S.L. and A.R.K. approved final version of manuscript.

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Perspective