Role of Gut-Derived Protein-Bound Uremic Toxins in Cardiorenal Syndrome and Potential Treatment Modalities

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Uremic toxins have been increasingly recognized as a crucial missing link in the cardiorenal syndrome. Advances in dialysis technologies have contributed to an enormous improvement in uremic toxin removal, but removal of protein-bound uremic toxins (PBUTs) by current conventional dialysis remains problematic because of their protein-binding capacity. Most PBUTs that have been implicated in cardiorenal toxicity have been demonstrated to be derived from a colonic microbiota metabolism pathway using dietary amino acids as a substrate. Currently, indoxyl sulfate and p-cresyl sulfate are the most extensively investigated gut-derived PBUTs. Strong evidence of adverse clinical outcomes, as well as biological toxicity on the kidney and cardiovascular system attributable to these toxins, has been increasingly reported. Regarding their site of origin, the colon has become a potential target for treatment of cardiorenal syndrome induced by gut-derived PBUTs. (Circ J 2015; 79: 2088–2097)

Key Words: Cardiorenal syndrome; Gut-derived PBUTs; Indoxyl sulfate; p-cresyl sulfate; Protein-bound uremic toxins (PBUTs)

The coexistence of cardiovascular disease (CVD) and kidney disease, the so-called “cardiorenal syndrome,” leads to synergistically poor clinical outcomes. Strong evidence of a link between renal dysfunction and cardiovascular (CV) morbidity and mortality has been reported by the European Uremic Toxin Work Group (EUTox), based on 85 publications covering 18 years and 552,258 subjects.1 CVD is the leading cause of death worldwide. A number of additional CV risk factors, including renal impairment, have been increasingly established. Renal impairment is highly prevalent and strongly predictive of major CV events and mortality in patients with CVD, especially myocardial infarction and heart failure. An independent association between CVD and a decline in kidney function or development of kidney disease has been demonstrated in a longitudinal, community-based study (n=13,826).2 In heart failure patients, renal dysfunction is a stronger predictor of mortality than cardiac dysfunction and New York Heart Association functional class.3 Importantly, the increased risk for major CV events, including myocardial infarction, stroke and hospitalization for heart failure and CV death, occurs very early during the process of renal impairment, even with only microalbuminuria or low normal estimated glomerular filtration rate (eGFR).4,5

Conversely, CV mortality is responsible for approximately half of all deaths in the dialysis population, which is 10–30-fold higher than in the general population.6 The prevalence of CVD in elderly patients aged ≥66 years with chronic kidney disease (CKD) is double that in their non-CKD counterparts (69.8% vs. 34.8%).7 A recent study in the US population aged >30 years reported that the prevalence of CKD is expected to increase from 13.2% presently to 16.7% by 2030.8 The residual lifetime incidence of CKD in this population is estimated to be 42–54%, mostly responsible for CKD stage 3a where the patients have an eGFR between 45 and <60 ml/min/1.73 m² and usually no symptoms.8 Left ventricular hypertrophy (LVH), an independent predictor of CV events and deaths in the CKD population, is found in 25–50% of stage 3–4 CKD patients, which drastically increases to 75% in stage 5 dialysis patients.9 One study of 822 incident dialysis patients reported prevalences of heart failure, angina pectoris, myocardial infarction and peripheral vascular disease of 35%, 21%, 18% and 16%, respectively.10 In fact, CV damage can start very early in asymptomatic CKD, long before it becomes symptomatic or reaches the dialysis stage where pharmacological treatment becomes limited.1 Therefore, early detection of renal impairment has the potential to delay CKD progression and to prevent its CV complications.

Importantly, CVD in the setting of CKD has its own unique characteristics. LVH with extensive cardiac interstitial fibrosis or uremic cardiomyopathy, as well as non-obstructive vascular disease such as arterial stiffness and calcification, is highly prevalent in the CKD population, compared with the general population, and all this CKD-associated CVD is independent of high blood pressure.11,12 Nearly half of the CKD patients with symptomatic ischemic heart disease have only trivial or no coronary artery occlusion on coronary angiographic evaluation,13 despite a high prevalence of accelerated atherosclerosis being well known in the CKD population. Interestingly, traditional CV risk factors such as diabetes, hypertension and dyslipidemia are not sufficient to explain CKD-associated...
PBUTs in Cardiorenal Syndrome

2089

Circulation Journal Vol.79, October 2015

into IS and pCS. In the healthy kidney, both circulating IS and pCS are excreted via renal tubular cells into the urine. Whenever renal function declines, their circulating levels rise. In fact, increased circulating IS levels have been demonstrated in early stages 2 and 3 of CKD.20

Most of the circulating IS (~90%) and pCS (>90%) is in a protein-bound form,21 and the free functional (toxic) forms are maintained at very low or even undetectable levels in healthy subjects.18 However, the free-form levels can substantially increase with the severity of renal dysfunction in CKD patients. The free IS level can increase from undetectable in healthy subjects to a mean uremic concentration of 3.22 mg/L, and the uremic free pCS level can increase up to 20-fold higher than the normal level (0.08 vs. 1.75 mg/L).30 Of note, apart from the severity of renal dysfunction, the free-form fraction of PBUTs may be affected by serum albumin levels. Low serum albumin concentrations result in an increase in the free fraction of PBUTs.22,23 A higher free fraction of PBUTs may be involved in the association between hypoalbuminemia and CV events and mortality in CKD patients.24

It is also noteworthy that evidence of poor clinical outcomes such as CV events and mortality related to p-cresol actually represent its conjugates: mostly pCS (>$95%) and p-cresyl glucoronide (<4%). Actually, p-cresol is not present in the circulation but is an artifact caused by strong acid hydrolysis of its conjugates during measurement.24 Thus, clinical and in vivo (not in vitro) experimental studies of p-cresol might be interpreted as effects of pCS.

Adverse Clinical Outcomes and Biological Effects of Gut-Derived PBUTs

As previously mentioned, the detrimental effects of IS and pCS have been the most extensively studied among the PBUTs (Table) (for review see Liabeuf et al26 and Lekawanvijit et al27). IS appears to be best systematically described by a number of clinical and experimental studies with regard to renal and CV effects (Figure 1). The following sections of this review will therefore mainly focus on these 2 toxins.

Adverse Clinical Outcomes

Briefly, an increase in circulating IS and pCS levels is significantly associated with a decline in eGFR.28–30 Both the IS and pCS levels are predictive of CKD progression,30,33 and all-cause and CV mortality.29,31 A recent study reported that the IS level can predict major CV events from stage 3 CKD where kidney dysfunction is mild-to-moderate.28 The predictive performance of the IS level on overall and CV mortality is also associated with the presence of aortic calcification and vascular stiffness determined by carotid-femoral pulse wave velocity.29

Adverse Biological Effects

Renal Effects IS can promote renal dysfunction, oxidative stress, cell senescence, inflammatory activation, and renal structural damage, including inflammation, extracellular matrix deposition, glomerulosclerosis and interstitial fibrosis.27,32 IS-induced renal toxicity, usually progressing towards renal fibrosis, is most likely mediated by the reactive oxygen species (ROS)/nuclear factor-κB (NF-κB)/transforming growth factor-β1 (TGF-β1) pathway. Likewise, pCS is also implicated in the development of renal inflammation and fibrosis. An epithelial-to-mesenchymal transition mechanism has been demonstrated to be a potential cellular mechanism in IS- and pCS-induced renal fibrosis in both glomeruli and the
| Compounds                  | Cardiac effects                                                                                                      | Vascular effects                                                                                       | Renal effects                                                                                             |
|----------------------------|---------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
| **Indoxyl sulfate**        | - Increase collagen synthesis in NCF<sup>46</sup>                                                                     | - Decrease endothelial proliferation and wound repair in vitro<sup>45</sup>                           | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - Increase protein synthesis in NCM<sup>46</sup>                                                                      | - Oxidative stress-induced endothelial dysfunction and damage<sup>45</sup>                            | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - Abnormal changes in the gap junction in cultured cardiomyocytes<sup>59</sup>                                      | - Increase expression of vascular inflammatory and thrombogenic mediators<sup>79</sup>               | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - Induce oxidative stress in vivo<sup>46</sup>                                                                       | - MAPK-mediated VSMC proliferation<sup>40</sup>                                                    | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                                       | - Promote calcium deposition and MAPK-mediated VSMC proliferation<sup>40</sup>                     | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - Enhance oxidative stress determined by an increase in ROS production and NADPH oxidase activity, and a reduction in glutathione levels in cultured HUVEC<sup>53</sup> | - Increase expression of vascular inflammatory and thrombogenic mediators<sup>79</sup>               | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - Promote ROS production and a senescence in cultured HUVEC<sup>44</sup>                                           | - MAPK-mediated VSMC proliferation<sup>40</sup>                                                    | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - Promote VSMC proliferation in vitro<sup>19</sup>                                                                    | - Promote ROS generation and osteoelastic transformation of aortic smooth muscle cell in vitro by increasing expression of osteoblast-specific proteins such as core binding factor 1, osteopontin and alkaline phosphatase<sup>56</sup> | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - Promote aortic calcification and cell senescence in vivo, in association with an increased expression of senescence-related proteins such as p16(INK4a), p21(WAF1/CIP1), p53 and retinoblastoma protein<sup>44</sup> | - Enhance leukocyte adhesion and extravasation and interrupted blood flow<sup>55</sup>               | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - Enhance leukocyte adhesion and extravasation and interrupted blood flow<sup>55</sup>                            | - Induce oxidative stress determined by an increase in ROS production and NADPH oxidase activity, and a reduction in glutathione levels in cultured HUVEC<sup>53</sup> | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - Increase leukocyte rolling in vivo<sup>55</sup>                                                                     | - Oxidative stress-induced endothelial dysfunction and damage<sup>45</sup>                            | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - Impaire blood flow and cause vascular leakage, in the presence of p-cresyl glucuronide<sup>60</sup>               | - Increase endothelial permeability to albumin in vitro, in the presence of p-cresyl glucuronide<sup>65</sup> | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - Potentially induce renal tubular adenoma<sup>41</sup>                                                                | - Increase inflammatory gene expression in cultured renal proximal tubular cells<sup>59</sup>        | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
| **p-cresyl sulfate**       | - Increase collagen synthesis in NCF<sup>46</sup>                                                                      | - Increase inflammatory gene expression in cultured renal proximal tubular cells<sup>59</sup>        | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - Increase protein synthesis in NCM<sup>46</sup>                                                                      | - Glomerulosclerosis and renal interstitial fibrosis with activation of pro-fibotic gene and protein expression in vivo<sup>33</sup> | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - Abnormal changes in the gap junction in cultured cardiomyocytes<sup>59</sup>                                      | - Renal fibrosis in association with CpG hypermethylation of the Klotho gene (a renoprotective antiaging gene) and decreased Klotho expression in renal tubular cells both in vitro and in vivo<sup>34</sup> | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - Induce oxidative stress in vivo<sup>46</sup>                                                                        | - Potentially induce renal tubular adenoma<sup>41</sup>                                               | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
| **p-cresol (present in the body as its conjugated forms, mainly p-cresyl sulfate)** | - Induce CD133+ cell apoptosis in vitro<sup>51</sup>                                                                 | - Function impairment<sup>74</sup>                                                                     | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - All-cause mortality<sup>66</sup>                                                                                  | - Glomerular sclerosis and interstitial fibrosis<sup>14</sup>                                        | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - CV events<sup>97</sup>                                                                                            | - Enhance renal oxidative stress in vitro<sup>82</sup>                                               | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
| **Phenylacetic acid**      | - Increase protein synthesis in NCM<sup>46</sup>                                                                      | - Induce inflammatory cytokine gene expression in vitro<sup>72</sup>                                 | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - NA                                                                  | - Glomerular sclerosis and interstitial fibrosis<sup>14</sup>                                        | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
| **Indole-3-acetic acid**   | - NA                                                                  | - Enhance renal oxidative stress in vitro<sup>82</sup>                                               | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - Induce CD133+ cell apoptosis in vitro<sup>51</sup>                                                                  | - Induce inflammatory cytokine gene expression in vitro<sup>72</sup>                                 | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
| **Homocysteine**           | - Induce inflammatory cytokine gene expression in vitro<sup>72</sup>                                                 | - Glomerular sclerosis and interstitial fibrosis<sup>14</sup>                                        | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - All-cause mortality<sup>73</sup>                                                                                  | - Enhance renal oxidative stress in vitro<sup>82</sup>                                               | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - CV events<sup>75–77</sup>                                                                                          | - Potential inflammatory cytokine gene expression in vitro<sup>72</sup>                              | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - All-cause mortality<sup>73</sup>                                                                                  | - Functional impairment<sup>74</sup>                                                                     | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - NA                                                                  | - Glomerular sclerosis and interstitial fibrosis<sup>14</sup>                                        | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - NA                                                                  | - Enhance renal oxidative stress in vitro<sup>82</sup>                                               | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
| **Hippuric acid**          | - NA                                                                  | - Functional impairment<sup>74</sup>                                                                     | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - NA                                                                  | - Glomerular sclerosis<sup>74</sup>                                                                     | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
| **Phenol**                 | - NA                                                                  | - NA                                                                                                  | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |
|                            | - Suppress contractility of cardiac muscle in vitro<sup>92</sup>                                                      | - NA                                                                                                  | - Defective endothelial proliferation and wound repair in vitro<sup>45</sup>                            |

CKD, chronic kidney disease; CV, cardiovascular; HUVEC, human umbilical vein endothelial cells; LV, left ventricular; MAPK, mitogen-activated protein kinase; NA, no data available; NADPH, nicotinamide adenine dinucleotide 3-phosphate; NCF, neonatal rat cardiac fibroblast; NCM, neonatal rat cardiac myocyte; ROS, reactive oxygen species; VSMC, vascular smooth muscle cell. (Reproduced with kind permission from Springer Science & Business Media: Lekawanvijit S, et al; p.202, Table 19.1.94)
PBUTs in Cardiorenal Syndrome

• Vascular Effects  Adverse vascular effects of PBUTs can be simply divided into atherosclerotic and non-atherosclerotic vascular disease.

  - Atherosclerosis  Both IS and pCS promote endothelial dysfunction, the major step in the development of atherosclerosis, which is associated with increased oxidative stress in endothelial cells, increased expression of endothelial adhesion molecules and shedding of endothelial microparticles.\textsuperscript{36,37} pCS can also activate free radical production in human leukocytes.\textsuperscript{38} Vascular smooth muscle proliferation, another hallmark of atherosclerosis, has been demonstrated to be induced by IS.\textsuperscript{39}

    A recent study showed that IS enhances expression of interleukin-6, a key pro-inflammatory cytokine playing a role in the development of atherosclerotic lesions, in both vascular endothelial and smooth muscle cells mediated via the organic anion transporter 3 (OAT3), responsible for the intracellular uptake of IS/AhR/NF-κB pathway.\textsuperscript{40} This is consistent with a previous study demonstrating that IS can activate AhR and increase expression of monocyte chemoattractant protein-1 in human umbilical vein endothelial cells.\textsuperscript{41}

    In addition, an inverse correlation between the serum IS level and high-density lipoprotein cholesterol has been reported in hemodialysis patients.\textsuperscript{42} High plasma IS levels are also independently predictive of increased carotid intima-media thickness,\textsuperscript{43} a surrogate marker of carotid atherosclerosis and future CVD.

  - Vascular calcification  Vascular stiffness, calcification and ossification are common in the CKD population. Vascular stiffness occurring in various stages of CKD has been demonstrated to be correlated with IS levels,\textsuperscript{29} and vascular calcification correlated with both IS and pCS levels.\textsuperscript{30,31} IS-induced vascular calcification is associated with activation of cell senescence.\textsuperscript{44} IS is also involved in osteogenic differentiation of vascular smooth muscle cells.\textsuperscript{45}

Cardiac Effects  Direct detrimental cardiac effects of PBUTs have been demonstrated for the first time in an in vitro study.\textsuperscript{46} The study showed pro-fibrotic, pro-hypertrophic and pro-inflammatory effects of IS in cultured cardiac fibroblasts, cardiomyocytes and monocyte THP-1 cells, mediated via activation of p38 mitogen-activated protein kinase (MAPK), p44/42 MAPK and NF-κB. Data from 2 other in vivo studies in an experimental CKD model induced by 5/6 subtotal nephrectomy demonstrated an increase in serum IS levels in association with diastolic left ventricular dysfunction, cardiac
Potential Treatment Modalities Targeting Gut-Derived PBUTs

Treatment targeting gut-derived PBUTs can be mainly divided into 2 strategies: (1) prevention of toxin production and (2) enhancing toxin removal by renal replacement therapy (RRT) (Figure 2).

Strategy 1: Prevention of Toxin Production

Low-Protein Diet  To date, study of the effects of a protein-restricted diet on renal and CV outcomes in association with a reduction in gut-derived solute production in the setting of CKD is extremely rare. However, serum IS levels have been demonstrated to be reduced by a very low protein diet, with a mixture of ketoanalog and amino acid supplements, in predialysis CKD patients.

Modification of Colonic Microbiome Homeostasis  Alterations in the gut microbiome are common in the setting of CKD. Impaired intestinal absorption in CKD increases the amount of protein substrates delivered to the colon, leading to an increase in uremic solute production and a shift of colonic microbiome from a saccharolytic to proteolytic fermentation pattern. In addition, the production of gut-derived solutes is further accelerated by the prolonged colonic transit time that is commonly observed in long-term hemodialysis patients. Strategies to modif...
Prebiotic and synbiotic treatments, as well as the use of laxatives.

**Probiotics**  “Probiotic” treatment uses live microorganisms for health benefit. Use of lactic acid bacilli and *Bifidobacterium longum* in hemodialysis patients can lower circulating IS levels. A double-blind randomized-controlled trial of probiotic treatment for 6 months in 46 patients with CKD stages 3 and 4 demonstrated a significant improvement in renal function as determined by blood urea nitrogen and creatinine levels and quality of life.

**Prebiotics**  “Prebiotic” treatment uses a non-digestible food ingredient or dietary fiber to selectively promote the activity of colonic bacteria. In 5/6 nephrectomized rats, 2 weeks administration of galacto-oligosaccharides can modify the gut microbiota by increasing 3 bacterial families and decreasing 5 bacterial families, including Clostridiaceae. This finding is associated with a reduction in serum IS levels, cecal indole, endoplasmic reticulum stress, apoptosis, and an improvement of renal injury. A non-randomized phase I/II study of hemodialysis patients demonstrated that administration of prebiotic oligofructose-enriched inulin for 4 weeks significantly reduced serum levels of pCS, but not IS.

**Synbiotics**  “Synbiotic” treatment uses a combination of probiotics and prebiotics. A few studies examining effects of synbiotic treatment in CKD patients have been conducted with levels of p-cresol, currently accepted as a representative of pCS, being monitored. The first study in hemodialysis patients using *Lactobacillus casei* and *Bifidobacterium breve* as probiotics and galacto-oligosaccharides as prebiotics demonstrated that treatment for 2 weeks significantly reduced serum p-cresol and improved bowel habits. Another double-blind, randomized placebo-controlled trial (n=30) using a commercial synbiotic, Probiunal neutro, in stages 3 and 4 CKD patients also demonstrated a significant reduction in plasma p-cresol on days 15 and 30 in the treated group but no change in the placebo group.

**Laxatives**  Impaired intestinal absorption is common in CKD patients, resulting in an increase in protein substrates passing to the colon for solute production. A recent study using lubiprostone, a chloride-channel activator used for chronic constipation, in renal failure mice demonstrated that lubiprostone-treated renal failure mice showed accelerated intestinal transit, 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.

Collectively, treatments that maintain colonic microbiome homeostasis have shown preliminary favorable results on reducing problematic colon-derived PBUTs, preserving renal function, attenuating renal damage and improving quality of life. However, the beneficial effect of such treatments on CV or cardiorenal outcomes has not been reported.

**Oral Sorbents**

Activated charcoal has been widely used to adsorb organic toxins from the GI tract. AST-120 is a microspherical carbon adsorbent with high porosity and adsorptive capacity characterized by being selective to low MW molecules (<10kDa), not interfering with GI enzymes or nutritional status, and most effectively binding in the lower GI tract where organic compounds, including indole and p-cresol, are produced.

Investigation using AST-120 to reduce the production of gut-derived PBUTs has been conducted in a number of clinical and experimental studies. Evidence of its renoprotective and CV protective effects, especially in preclinical studies, has been continuously reported.

**Renal Endpoints in Preclinical Studies**

In uremic rats, AST-120 lowered serum, renal and urinary IS levels, and improved renal function, inflammation, tubulointerstitial fibrosis and glomerular sclerosis. Such findings are associated with decreased expression of renal pro-fibrotic (TGF-β1, tissue inhibitor of metalloproteinase-1 and pro-α1(I) collagen), pro-inflammatory (intercellular adhesion molecule-1, osteopontin, monocyte chemotactic protein-1) and apoptosis-related (clusterin and osteopontin) genes. Attenuation of renal cortical NF-κB expression activated in the uremic state is associated with all antiinflammatory, antibacterial and antioxidative effects of AST-120.

**CV Endpoints in Preclinical Studies**

Prevention of atherosclerotic plaque extension, inflammation and necrosis by AST-120 was observed in apolipoprotein E-deficient mice with CKD. AST-120 has been demonstrated to lower plasma cholesterol and very low density lipoprotein in rats with spontaneous focal glomerulosclerosis.

For cardiac endpoints, 5/6 nephrectomy rats treated with AST-120 showed a significant reduction in serum IS levels, cardiac fibrosis, cardiac TGF-β protein expression, cardiac NF-κB phosphorylation and cardiac oxidative stress. These findings support the proposed ROS/NF-κB/TGF-β1 mechanistic signaling pathway of IS-induced cardiac fibrosis.

**Clinical Outcomes in Clinical Studies**

In predialysis CKD patients, an improvement in renal function was observed after 1 year of AST-120 treatment, and a risk reduction for initiation of dialysis or reaching ESRD was achieved after 2 years of treatment. Improved renal function and delayed CKD progression by AST-120 are associated with decreased levels of serum and urinary IS, and plasma TGF-β1. In diabetic patients with CKD, AST-120 preserved renal function, and improved survival and cost reduction were also observed in advanced stages. Interestingly, AST-120 significantly improved the 5-year survival in hemodialysis patients who received AST-120 treatment from the predialysis stage with an average treatment duration of 15 months.

Beneficial CV effects have been reported in CKD patients receiving AST-120. A significant improvement in the surrogate markers for atherosclerosis and vascular stiffness determined by carotid intima-media thickness and pulse wave velocity has been demonstrated in predialysis patients who are on AST-120 for 2 years, but not at 1 year. However, treatment duration of at least 6 months initiated from the predialysis stage can improve the aortic calcification index, restore IS-induced endothelial dysfunction in association with a reduction in oxidative stress, and delay progression of LVH.

Nutritional status is also improved with AST-120 treatment, by increasing albumin and transferrin levels as well as decreasing the free fraction of tryptophan, which is a substrate for serotonin (an appetite suppressor) production. AST-120 provides synergistic benefits on preserving renal function in CKD patients who are on a low-protein diet. The regimen of a low-protein diet can be adjusted from strict to mild when AST-120 is combined to achieve a comparable treatment effect.

A phase III multicenter, randomized, controlled trial demonstrated significantly better preservation of renal function in moderate to severe predialysis CKD patients after a 1-year administration of AST-120. This study did not observe benefits on delaying progression of CKD or survival, probably because of inadequate treatment duration and follow-up time. Recently, 2 large-scale multicenter randomized trials of AST-120 (Evaluating Prevention of Progression In...
Chronic Kidney Disease, EPPIC) in 2,035 moderate to severe CKD patients showed no benefit of adding AST-120 to standard therapy on a composite endpoint of dialysis initiation, kidney transplantation and serum creatinine doubling. However, subgroup analysis observed a fast decline in renal function in patients who had high proteinuria and hematuria.115 The authors discuss the factors that may contribute to the differences in the results between this trial and previous studies, including (1) underestimation of the time to primary endpoints, (2) regional differences in the initiation of dialysis, which appears to be more delayed in Russia and Ukraine than in North America and Europe, (3) covariate imbalances because there is no correlation between CKD progression and indicators (at enrolment) of disease severity and (4) compliance issue because of a high pill burden (30 capsules/day).115 From the cardireno point of view, adding CV endpoints could provide data on cardiorenal protective effects of AST-120 and confirm/strengthen the causative role of gut-derived PBUTs in the pathogenesis of cardiorenal syndrome.

Study investigating treatment effects of AST-120 on both renal and CV outcomes is extremely rare. A small non-randomized trial in heart failure patients with moderate CKD demonstrated an improvement in renal function and heart failure symptoms after a 2-year administration of AST-120.116 Two studies in a myocardial infarction model with coexisting renal impairment showed that AST-120 treatment can reduce IS-induced renal and cardiac fibrosis, as well as the expression of both cardiac and renal pro-fibrosis markers.117,118 The latter study also demonstrated that treatment with AST-120 can normalize overexpression of cardiac microRNA-21, angiotensin-converting enzyme and angiotensin receptor 1a genes, and restore decreased expression of cardiac microRNA-29b.118

Strategy 2: Enhancing Toxin Removal by RRT

Dialysis Removal of uremic retention solutes to relieve uremic syndrome has mainly relied on dialysis. The removal efficacy of dialysis generally depends on the characteristics of the membrane (e.g., permeability, pore size, pore size distribution, surface area and material), mechanism of solute transport (diffusion or convection or both), frequency of dialysis and time spent in each dialysis session.119,120 Despite a wide variety of options, removal of PBUTs, especially those with high protein-binding capacity such as IS and pCS, by current conventional dialysis is far less effective compared with non-PBUTs. Removal of most PBUTs is either not significantly or only marginally improved with frequent dialysis, use of a high-flux membrane or a convective strategy.121–123 A protein-leaking membrane allows better clearance of PBUTs; however, large amounts (2–6 g/4 h) of albumin loss can increase the toxic free-fraction of PBUTs and worsen the state of protein malnutrition.

Addition of a sorbent system to conventional dialysis has been developed in order to improve the removal of large MW solutes and PBUTs. Promising results from a preliminary study show an improvement in the clearance of only middle molecules and cytokines with a MW range from 12,000 to 21,000 D.124 Another study using coated carbon hemoperfusion failed to demonstrate better removal of PBUTs.125 However, using a dual-layer hollow fiber membrane with embedded adsorptive carbon particles has been recently demonstrated to effectively clear the daily production of IS and pCS.126

IS and pCS are particularly difficult to clear from the circulation, largely because of their high protein-binding affinity and large distribution volume. Therefore, strategies to decrease the protein-binding capacity of the plasma may enhance PBUTs removal by dialysis. The potential approach for this propose, including increasing ionic strength using sodium chloride, increasing temperature from room to body temperature and dilution, has been demonstrated to decrease the protein-bound fraction and increase the free fraction of IS, thereby likely contributing to better removal during dialysis.126

Kidney Transplantation Successful kidney transplantation has shown benefits on survival,128 preventing progression of CKD, recovery of cardiac function129 and regression of cardiac fibrosis.130 Study demonstrating such outcomes in relation to reduced levels of problematic non-dialyzable PBUTs is extremely rare in post-kidney transplantation patients. Nevertheless, it can be assumed that all kidney functions, including excretion of PBUTs, are restored. However, the major limitation of kidney transplantation is that donor kidneys are in short supply.

Conclusions

Advances in technologies and methodologies continue to discover more retention solutes in uremic patients. Using the EUTox approach indicating that uremic retention solutes should have a uremic to normal concentration ratio >1, the newly identified uremic solutes can be further classified by physicochemical characteristics and their biological relevance should be investigated. If applicable, the next step in the search for specific potential preventive/therapeutic strategies would contribute to tangible beneficial clinical effects in CKD patients who are at high risk for developing cardiorenal syndrome. At present, non-dialysis treatment appears to be the potential option for reducing the accumulation of gut-derived PBUTs. Decreasing colon-derived PBUTs by targeting the colon offers many advantages to the patient because it is much simpler, safer and cheaper than dialysis treatment. This strategy can also be initiated in the early predialysis stages of CKD, thereby being superior to RRT in terms of prevention of both CKD progression and its complications.

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Conflict of Interest

None.

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