Editorial Comments

The gut–kidney axis: indoxyl sulfate, \( p \)-cresyl sulfate and CKD progression

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The special position assigned to urea appears to date from the time at which urea could not be formed synthetically and was believed to arise only in living matter. Now that urea is formed artificially, it has been robbed of much of its mystery. (A. R. Cushny, The secretion of urine, 1917)

The fundamental insights into uraemic toxicity have evolved little since publication of the classical monograph by Cushny nearly a century ago [1]. Organic metabolites are still thought to substantially contribute to uremia (albeit urea might not be the culprit), yet evidence unequivocally demonstrating toxicity of any single uraemic constituent is lacking [2].

To advance research in uraemic toxicity, the European Toxin work group (EUTox) developed a classification of uraemic blood constituents according to characteristics that affect their removal during dialysis [3]. Besides small water-soluble molecules (e.g. urea and creatinine) and peptides/proteins (e.g. \( \beta _2 \)-microglobulin), they identified a group of solutes that circulate in equilibrium between free solute versus bound to carrier proteins. Tight protein binding severely limits solute clearances by dialysis [4]. Intriguingly, a substantial number of these so-called protein-bound uraemic retention solutes originate from protein fermentation in the large intestine, including \( p \)-cresyl sulfate and indoxyl sulfate [5]. Recently, several groups demonstrated direct associations between \( p \)-cresol, mainly reflecting \( p \)-cresyl sulfate, and overall mortality and cardiovascular disease in end-stage renal disease [6,7] and in chronic kidney disease (CKD) [8,9]. Likewise, direct associations between indoxyl sulfate and overall mortality and cardiovascular disease were reported [10].

While indoxyl sulfate and \( p \)-cresyl sulfate are frequently thought of as independent uraemic retention solutes, they share common ground. First, as mentioned before, \( p \)-cresyl sulfate and indoxyl sulfate both originate from bacterial protein fermentation in the large intestine. Colonic microbiota degrade tryptophan to indole. Further hydroxylation results in 3-hydroxy-indole, the majority of which is sulfonated to indoxyl sulfate. In parallel, fermentation of tyrosine results in \( p \)-cresol and ultimately \( p \)-cresyl sulfate [11].

Recently, we reported on sulfate conjugation of \( p \)-cresol in CKD [12,13]. Second, most \( p \)-cresyl sulfate and indoxyl sulfate circulate noncovalently bound to albumin and competes for the same albumin-binding sites (Sudlow site II) [14] (Figure 1). Indoxyl sulfate and \( p \)-cresyl sulfate are interchangeable marker molecules to study behaviour of protein-bound solutes during dialysis [15].

In this issue of Nephrology Dialysis Transplantation, Wu et al. demonstrate that serum indoxyl sulfate is associated with progression of CKD, confirming previous findings. Niwa et al. first advanced the hypothesis that accumulation of indoxyl sulfate accelerates glomerular sclerosis and progression of kidney disease [16,17]. Animal and small-scale human studies on CKD patients suggested retardation of CKD progression by adsorption of indole in the large intestine [18,19]. Intriguingly, Wu et al. equally demonstrate that \( p \)-cresyl sulfate is associated with CKD progression. Does this indicate that indoxyl sulfate and \( p \)-cresyl sulfate be considered equally valid markers for CKD progression?

This illustrates one of the key problems we are faced with when investigating uremia. One of the hallmarks of uraemic retention solutes is that they all move more or less in the same direction. When glomerular filtration rate falls, concentrations of the uraemic retention solutes we measure, and most likely a host of solutes that we are not aware of, all rise. Indeed, in the current study, Wu et al. observed a moderate correlation between indoxyl sulfate and estimated glomerular filtration rate (eGFR) (\( r = -0.72, P < 0.001 \)), between \( p \)-cresyl sulfate and eGFR (\( r = -0.64, P < 0.001 \)) and between indoxyl sulfate and \( p \)-cresyl sulfate (\( r = 0.66, P < 0.001 \)). From a statistical point of view, if nominally related measures actually quantify the same phenomenon, then they are redundant, i.e. collinear. This might lead us to conclude that indoxyl sulfate and \( p \)-cresyl sulfate are plain markers of kidney function.

The strongpoint of the study by Wu et al. is that they went to great length to correct for residual confounding, including by correction for related protein-bound uraemic retention solutes. They thus demonstrate that, while indoxyl sulfate is independently associated with CKD progression, this association is lost after correction for \( p \)-
Indoxyl sulfate and 2-cresyl sulfate [hazard ratio (HR) 1.025, P = 0.2]. On the contrary, the independent association between 2-cresyl sulfate and CKD progression persists after correction for indoxyl sulfate (HR 1.07, P = 0.0009). Moreover, while in multivariate Cox regression 2-cresyl sulfate remains directly associated with all-cause mortality (HR 1.16, P < 0.001), they observe near significance of an ‘inverse’ relation between indoxyl sulfate and all-cause mortality when corrected for 2-cresyl sulfate (HR 0.90, P = 0.059). In addition, they formally excluded collinearity according to prespecified criteria. Combined, these data suggest that—at least from a statistical viewpoint—total serum concentrations of 2-cresyl sulfate might be a better predictor of CKD progression than indoxyl sulfate. This effect is independent of eGFR, a traditional marker of kidney function, or more accurate, of glomerular function.

The report by Wu et al. thus suggests that serum concentrations of indoxyl sulfate and 2-cresyl sulfate are not interchangeable prognostic factors. Importantly, an aspect that was not touched upon in the study by Wu et al. is the role of protein binding. In haemodialysis patients, free 2-cresyl sulfate concentrations were an even stronger predictor of overall mortality and of cardiovascular disease than total serum concentrations [6,7]. To complicate matters, indoxyl sulfate and 2-cresyl sulfate compete for the same albumin-binding sites. It is an unresolved question whether free serum concentrations of either indoxyl sulfate or 2-cresyl sulfate would be even better predictors of CKD progression.

How should we understand their findings in terms of ureaemic toxicity? Although tempting, the findings by Wu et al. do not permit us to judge the degree of toxicity of 2-cresyl sulfate as compared to toxicity of indoxyl sulfate. In addition, the cross-sectional study design precludes inference on causation. The apparent association of 2-cresyl sulfate with CKD progression could be accounted for by at least three different hypotheses.

First, clearance of protein-bound solutes depends heavily on specific transporters located in the tubuli. Serum concentrations of indoxyl sulfate and 2-cresyl sulfate may thus be markers of tubular function. In this case, serum concentrations of either 2-cresyl sulfate or indoxyl sulfate are markers of CKD progression independent of GFR not because they are toxic but because they reflect tubular damage in addition to glomerular function.

Second, serum concentrations of indoxyl sulfate and 2-cresyl sulfate, to a certain degree, depend on nutrient intake. To what extent nutrient intake directly accelerates progression of CKD, while at the same time influencing serum concentrations of these protein-bound ureaemic retention solutes, remains largely unexplored.

Finally, 2-cresyl sulfate and indoxyl sulfate might be more than ureaemic retention solutes, i.e. ureaemic toxins affecting progression of CKD. To answer the question whether and to what extent these solutes are true ureaemic toxins, additional studies are needed. In vitro research provides insight into the pathways involved, thus elucidating the role of individual ureaemic toxins. So far, most studies focused on indoxyl sulfate. Indoxyl sulfate induces free radicals in renal tubular cells and glomerular mesangial cells [20], activating the nuclear factor-kappa B pathway [21]. In contrast, little is known about the direct effects of 2-cresyl sulfate on the kidney. The report by Wu et al. thus shows us new avenues for in vivo research.

The ultimate proof of toxicity would be to demonstrate in vivo that reduction of serum concentrations results in improved clinical end points.

As indoxyl sulfate and 2-cresyl sulfate originate from bacteria in the intestines, bacterial metabolism may therefore be an important therapeutic target in CKD [5]. Factors promoting generation and absorption include an increased ratio of dietary protein to carbohydrate due to insufficient intake of fibre and/or reduced intestinal protein assimilation, as well as prolonged colonic transit time. Up to now, only two strategies exist to reduce intestinal absorption: interventions that modulate intestinal bacterial growth and metabolism (e.g. probiotics, prebiotics and dietary modification) and adsorbent therapies that bind precursors of the various ureaemic retention solutes in the intestines to reduce their absorption.
Several small studies indicated retardation of CKD progression by AST-120, an orally ingested activated charcoal adsorbent [22,23]. Currently, a large phase III trial is being run to investigate the efficacy of AST-120 to retard progression of CKD [24]. Yet, if such a therapy would prove effective, this will leave us with open questions. As can be expected from the characteristics of AST-120, which is an activated charcoal, various uraemic retention solutes are adsorbed. A recent paper demonstrates that oral intake of AST-120 reduces serum concentrations of several uraemic retention solutes apart from indoxyl sulfate [25]. It will prove a daunting task to tease out the effects of individual uraemic retention solutes, unless specific therapies become available.

Interventions that modulate bacterial metabolism alter serum concentrations of specific uraemic retention solutes, while serum concentrations of other solutes are unaffected. Ingestion of the prebiotic oligofructose-enriched inulin significantly reduced p-cresyl sulfate serum concentrations, in contrast to indoxyl sulfate serum concentrations [26]. So far, clinical end point studies investigating the role of prebiotics and/or probiotics in patients with CKD are lacking.

The quest for the uraemic retention solute(s) that determine uraemic toxicity is ongoing. Insight into the individual solutes that contribute to uraemic toxicity might help us to identify new therapeutic targets in CKD.

Conflict of interest statement. None declared.

(See related article by Wu et al. p-Cresyl sulphate and indoxyl sulphate predict progression of chronic kidney disease. Nephrol Dial Transplant 2011; 26: 938–947.)

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