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REVIEW

From old uraemic toxins to new uraemic toxins: place of 'omics'

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ABSTRACT

Uraemic toxins seem to play an important role in the genesis of cardiovascular and renal damage in chronic kidney disease patients. This short article is divided into two thematic sections. The first part focuses on a selection of 'old' toxins for which recent data (published between 2016 and 2018) have provided a better understanding of the associated harmful mechanisms and which, in our opinion, nephrologists should be more aware of. The second part highlights new perspectives for identifying and quantifying these compounds using 'omics' techniques.

Keywords: biomarkers, metabolomics, proteomic, uraemic toxin

INTRODUCTION

Chronic kidney disease (CKD) is associated with a major health care burden [1]. According to the 2016 Global Burden of Disease study, CKD was the 30th leading cause of death worldwide in 1990 and the 22nd in 2016 [2]. Furthermore, CKD is a major risk factor for other morbidities and for all-cause mortality and cardiovascular mortality. The risks of hospitalization, adverse events and mortality increase as CKD progresses [3].

As kidney function becomes increasingly impaired, a variety of substances (commonly referred to as uraemic retention solutes) accumulate in the body. Solutes that exert adverse biological effects are referred to as uraemic toxins and have been classified into three broad groups as a function of their solubility, molecular weight and ability to bind to serum proteins: small, water-soluble compounds (such as phosphate); middle molecules (such as beta2- microglobulin) and protein-bound compounds [such as indoxyl sulphate (IS)]. A recent review by the European Uremic Toxin (EUTox) study group identified 56 new uraemic toxins in addition to the previous list of 90 and specified their normal and uraemic concentrations [4]. Uraemic toxins are thought to contribute to vascular disease; endothelial, platelet and immune dysfunction; and renal damage [5].

In recent decades, researchers have focused on uraemic toxins and their effects. The key hypothesis is that controlling toxin levels can reduce CKD complications and/or slow CKD progression. This short article is composed in two parts. The first part focuses on a selection of 'old' toxins for which recent data (publications between 2016 and 2018) provide a better understanding of their deleterious mechanisms and that—in our opinion—need to be underlined to the nephrologist community. The second part focuses on 'omics' methods that permit identifying new proteins or metabolites that could be part of uraemic toxins, leading to expansion of the list.

TRIMETHYLAMINE *N*-OXIDE

The metabolite trimethylamine *N*-oxide (TMAO) is generated by the gut microbiota in a two-step process. First, the gut microbiota produces trimethylamine through the metabolism of dietary choline and carnitine. Second, trimethylamine is absorbed in the gut and travels (via the portal circulation) to the liver, where it is oxidized to TMAO [6].

Preclinical data indicate that TMAO is a mediator of cardiovascular disease [7]. Dietary supplementation with choline or TMAO enhances the development of atherosclerotic lesions in mice, and there is a significant positive correlation between plasma TMAO levels and atherosclerotic plaque size [8]. TMAO promotes vascular inflammation through the mitogen-activated protein kinase and nuclear factor-κB signalling pathways [9]. Furthermore, this toxin modulates platelet function and prompts the generation of a prothrombotic phenotype *in vivo* [10].

A number of clinical studies have shown that circulating levels of TMAO are associated with cardiovascular risk [7]. For example, the Hemodialysis (HEMO) study of prevalent dialysis patients with little or no residual kidney function investigated the longitudinal association between TMAO and cardiovascular morbidity and mortality [11]. The TMAO concentrations in patients on dialysis were 10- to 20-fold higher than those observed in people with normal renal function. TMAO is associated

with cardiovascular morbidity and mortality, although its effects depend on ethnicity [11]. In a Canadian observational study of patients with Stages 3b and 4 CKD and a prospective 3-year follow-up period (the CanPREDDICT cohort), the median TMAO level for the whole cohort was high and independently associated with cardiovascular events after adjusting for all other potential risk factors [12].

INDOLE URAEMIC TOXINS: IDENTIFICATION OF A NEW TOXICITY PATHWAY

Various studies have demonstrated the harmful effects of indole toxins on renal and vascular cells [13]. Epidemiological studies have shown a link between indole uraemic toxins [such as IS and indole acetic acid (IAA)] and cardiovascular outcomes [5].

Preclinical data indicate that the kidney proximal tubule transporters of indole uraemic toxins include two 'drug' transporters from the organic anion transporter (OAT) family: OAT1 and OAT3 [14]. An untargeted metabolomics analysis of plasma and urine from wild-type mice and OAT1 knock-out mice showed that IS is an OAT1 substrate [15]. The functional activity of these transporters has a major impact on drug pharmacokinetics and nephrotoxicity. The partial blockade of OAT1- or OAT3-mediated transport of uraemic toxins by competing drugs has the potential to increase the levels of certain toxins and thus lead to cascade effects. For example, the commonly used non-steroidal anti-inflammatory drugs diclofenac and ketoprofen decrease the clearance of IS by 71% and 82%, respectively [16].

The cellular receptor for indole solutes has been identified as the aryl hydrocarbon receptor (AHR). In endothelial cells and vascular smooth muscle cells, activation of the AHR increases the expression and activity of tissue factors and leads to a procoagulant state [17]. Hence AHR activation by IAA and IS could contribute to vascular dysfunction [17]. Recently Dou *et al.* [18] developed the AHR activating potential (AHR-AP) concept as a quantitative guide to the overall load of AHR agonists in serum. Serum AHR-AP levels were strongly elevated in mice and patients with renal insufficiency. Antagonizing the AHR might be a useful therapeutic approach [19]. However, since AHR is present in a large number of human cells, and unselective inhibition could lead to beneficial and negative effects, this needs to be taken into account in drug development.

In the same line, it was demonstrated that IS activates the AHR pathway in primary human aortic vascular smooth muscle cells and further that AHR interacts directly with and stabilizes functional tissue factor. This finding delineates a mechanism of the prothrombotic property of IS and, in doing so, defines AHR as an antithrombotic target and AHR antagonists as a novel class of antithrombotics [20]. Recently Kolachalama *et al.* [21] studied 473 participants with advanced CKD from the Dialysis Access Consortium Clopidogrel Prevention of Early AV Fistula Thrombosis trial. Participants with subsequent arteriovenous thrombosis had significantly higher levels of IS and kynurenine, another uraemic solute, and greater activity of AHR than those without thrombosis.

Furthermore, the AHR pathway seems to be involved in IS's effect on P-glycoprotein (PGP; an efflux pump that exports lipophilic substrates, including many drugs) [22]. IS appears to increase PGP expression. In transplanted patients treated with cyclosporine (a PGP substrate), the patients with higher serum IS levels needed higher doses of cyclosporine to achieve the target concentration [22]. The modulation of drug transporters by uraemic toxins is an important issue and might explain the differences in efficacy and safety between patients with and without CKD.

N-METHYL-2-PYRIDONE-5-CARBOXAMIDE: A COMEBACK BY AN 'OLD' URAEMIC TOXIN

N-methyl-2-pyridone-5-carboxamide (2PY) is a low molecular weight, water-soluble and non-protein-bound uraemic toxin. It appears to inhibit poly (adenosine diphosphate ribose) polymerase-1, which is involved in the cell's response to DNA injury [23]. We recently used a sensitive, specific liquid chromatography–tandem mass spectrometry (LC-MS/MS) method to assay 2PY concentrations in healthy volunteers ($n = 65$) and in patients with CKD (60 non-dialysed and 80 on haemodialysis). Our data confirmed that 2PY levels rise progressively with the CKD stage; the highest concentration was observed in patients on haemodialysis and greatly exceeded the value determined for healthy subjects [24]. This finding is important in view of the renewed interest in nicotinamide for the treatment of hyperphosphataemia in patients with advanced CKD [25]. Indeed, the CKD Optimal Management with BInders and NicotinamidE (COMBINE) Study tests the hypothesis that the use of nicotinamide combined with lanthanum carbonate on a background of reduced dietary phosphate intake safely reduces serum phosphate and fibroblast growth factor 23 levels over 12 months in 200 patients with Stages 3–4 CKD [25]. In a recent clinical trial we compared nicotinamide with sevelamer in patients on haemodialysis. The two drugs were equally effective in lowering serum phosphate levels, although the patients' tolerance of nicotinamide was markedly worse than that of sevelamer. Extremely high 2PY levels were observed in patients in the nicotinamide arm [26]. It remains to be seen whether low-dose nicotinamide treatment will show clinically meaningful efficacy together with a lower increase of 2PY.

OMICS TECHNIQUES TO IDENTIFY URAEMIC TOXINS

Omics analysis offers multiple possibilities in the medicine area, from the understanding of physiological processes, to identification of pathogenesis conditions, to the screening or prognostics of different diseases. The use of new analytical technologies, such as capillary electrophoresis (CE) coupled with mass spectrometry (MS), permit the identification of large numbers of polypeptides in biological samples within <1 h in a single-run analysis.

Proteomic techniques can be used to identify additional uraemic toxins. In one study, researchers used CE-MS and CE-MS/MS to show that >30 polypeptides were present in the plasma of dialysis patients but absent in non-CKD controls [27].

Two polypeptides were formally identified: a fragment of fibrinogen alpha and a fragment of the complement factor peptide C3f (produced by proteolytic cleavage). It is noteworthy that the C3f fragment might enhance vascular permeability. Indeed, C3f fragments have been found in the sera of patients with myocardial infarction but not in healthy controls [28].

The impact of clinical proteomics will depend on the choice of samples, their technical qualities, sample transport, storage and analysis and clinical validation phases. Before the routine use of proteomics can be advocated in CKD, it will be imperative to validate the clinical utility of proteomic profiling in a blind manner against a large set of samples and establish comparability criteria and standards for quality control.

Metabolic profiling refers to the high-throughput MS analysis of plasma metabolites. Interest in metabolomics-based discovery of CKD biomarkers has been growing because of the broad impact that kidney function has on levels of circulating metabolites and because metabolites themselves may have a functional impact on the pathogenesis of CKD. Metabolomic techniques have been used to characterize novel uraemic metabolites and identify those associated with cardiovascular risk [29, 30]. In addition, these techniques have been used to identify potential biomarkers of kidney disease progression. In type 1 diabetic patients, van der Kloet *et al.* [31] looked for urinary biomarkers that differentiate the progressive form of albuminuria from the non-progressive form in humans. The most significant discriminating metabolites were as follows: kynurenic acid (indole compound and metabolite of tryptophan), tryptophan (precursor of IS) and substituted carnitine (precursor of TMAO) and hippuric acid. Thus we could argue that the same molecules were identified by both the metabolomic and 'non-omics' approaches. In diabetic rats, Zhao *et al.* [32] identified a number of abnormal metabolites in the diabetic kidney, including groups of amino acids, carbohydrates, polyols, glucuronides and other unidentified metabolites. Of them, an increase in intrarenal organic toxins, including uraemic toxins, glucuronides and glucotocixity-associated metabolites, are highly correlated with diabetic kidney injury. Treatment with fosinopril (an angiotensin-converting enzyme inhibitor) significantly attenuated diabetic kidney injury and simultaneously blocked the intrarenal accumulation of these organic toxins, especially hippurate and glucuronides [32]. In a recent study performed in subjects with type 2 diabetes, the majority of whom had normal renal function at baseline, half progressed to ESRD while half did not during a decade of follow-up. In baseline plasma, progressors could be distinguished from non-progressors by high concentrations of metabolites referred to as uraemic solutes and low concentrations of certain amino acids and their derivatives. Once again, among the metabolites identified, a large part were already studied by the EUTox group. Phenyl compounds (p-cresol sulphate and phenylacetylglutamine), solute derivatives of amino acids that are synthesized in the gut, phenol sulphate, indole acetate and 3-IS were elevated in the plasma of progressors compared with non-progressors [33]. In addition, associations between metabolites and CKD progression were revealed by metabolite profiling of high-quality samples as part of a nested case–control study of a well-phenotyped, racially diverse cohort [34].

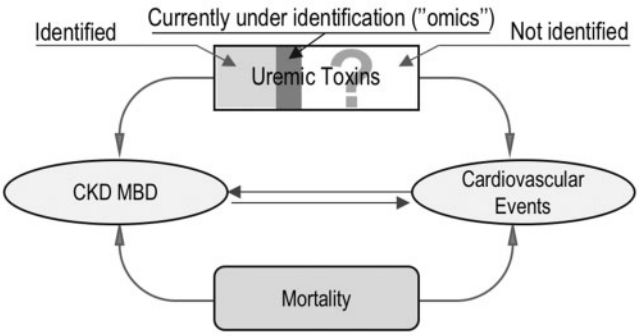

FIGURE 1: A schematic view of the role of identified and not identified uraemic toxins.

A recent study used metabolic profiling to identify and validate uraemic metabolites associated with cognitive impairment in two cohorts (a discovery cohort and an independent replication cohort) of patients on maintenance dialysis. Four metabolites (related to phenylalanine, benzoate and glutamate metabolism) were identified as potential markers of cognitive impairment [35].

Given that kidney function affects many aspects of metabolic health, an abnormal metabolomic profile may mediate the pathogenesis and prognosis of CKD. Although unbiased metabolomic profiling appears to be an excellent way of evaluating a broad range of metabolites, its precision is limited because the level of quantification is relative rather than absolute. While it is clear that omics techniques can usually address clinical questions about uraemic toxins and help to identify new unknown toxins, the main long-term challenge is to explore ways in which omics can be most effectively integrated into current methods for assaying uraemic toxins and thus maximize the synergy for disease detection. The discovery of a marker only designates the beginning of the problem. In order to test its toxicity, the exact and measurable concentration of the molecule is needed. To determine its uraemic concentration, the molecule *per se*, purchased or made, is required. Finally, *in vitro* and/or *in vivo* studies must be conducted to determine the effective toxicity.

CONCLUSION

At present, we suspect that only a small proportion of the pathophysiologically relevant mediators with significant organ effects in the uraemic milieu have been identified. Clearly knowledge of these mediators is essential for understanding the damage-inducing mechanisms and then preventing and/or treating this damage. Proteomics and metabolomics appear to be effective for profiling uraemic retention solutes. However, it seems important for the restricted community of researchers in uraemic toxins to choose the most pertinent toxin to study. As we have shown in the first part of the present review, new data related to uraemic toxins considered as old have recently extended our understanding of the deleterious effects of these solutes. Moreover, proteomics and metabolomics continue to extend the list of uraemic toxins by adding new proteins and metabolites (Figure 1).

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