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Uraemic toxins and cardiovascular disease across the chronic kidney disease spectrum: An observational study

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Abstract *Background and aims:* There is a growing body of evidence supporting the nephrovascular toxicity of indoxyl sulphate (IS) and p-cresyl sulphate (PCS). Nonetheless, a comprehensive description of how these toxins accumulate over the course of chronic kidney disease (CKD) is lacking.

Methods and results: This cross-sectional observational study included a convenience sample of 327 participants with kidney function categorised as normal, non-dialysis CKD and end-stage kidney disease (ESKD). Participants underwent measurements of serum total and free IS and PCS and assessment of cardiovascular history and structure (carotid intima-media thickness [cIMT, a measure of arterial stiffness]), and endothelial function (brachial artery reactivity [flow-mediated dilation (BAR-FMD); glyceryl trinitrate (BAR-GTN)]).

Across the CKD spectrum there was a significant increase in both total and free IS and PCS and their free fractions, with the highest levels observed in the ESKD population. Within each CKD stage, concentrations of PCS, total and free, were significantly greater than IS (all $p < 0.01$). Both IS and PCS, free and total, were correlated with BAR-GTN (ranging from $r = -0.33$ to -0.44) and cIMT ($r = 0.19$ to 0.21), even after adjusting for traditional risk factors (all $p < 0.01$). Further, all toxins were independently associated with the presence of cardiovascular disease (all $p < 0.02$).

Conclusion: More advanced stages of CKD are associated with progressive increases in total and free serum IS and PCS, as well as increases in their free fractions. Total and free serum IS and PCS were independently associated with structural and functional markers of cardiovascular disease. Studies of therapeutic interventions targeting these uraemic toxins are warranted.

Introduction

Chronic kidney disease (CKD) remains a major public health burden with one in ten adults in developed countries suffering some level of kidney dysfunction [1]. Despite significant advances in CKD treatment, cardiovascular-associated mortality and morbidity continue to rise. The relentless nature of CKD-associated cardiovascular disease (CVD) and the limited response to traditional cardiovascular risk management in this population have driven research to explore novel risk factors, such as uraemic toxins.

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Two uraemic toxins in particular, indoxyl sulphate (IS) and p-cresyl sulphate (PCS), have attracted attention over the past decade not only for their pro-inflammatory and pro-oxidative properties but their potential therapeutic amenability [2]. Both IS and PCS have demonstrated the ability to induce endothelial dysfunction through inhibition of endothelial proliferation and wound repair at uraemic concentrations [3], along with dose-dependent stimulation of oxidative stress and increased expression of inflammatory cytokines [4,5] *in vitro*. In addition, the administration of either IS or PCS in animal models have illustrated significant increases in renal fibrosis and nephrosclerosis, compared to controls [6]. These findings have been supported by observational studies which have shown independent associations between at least one or both of these toxins and all-cause mortality [7,8] and cardiovascular events [9,10]. Furthermore, controlled clinical intervention studies have demonstrated slowed CKD progression [11] and improvement in endothelial dysfunction [12] with reduction in IS levels following administration of oral adsorbent therapy.

Both IS and PCS are by-products of dietary protein fermentation by bacteria in the large intestine (collectively known as the gut microbiota), which undergo sulphation and hydroxylation in the liver and intestinal wall, respectively, before entering the circulatory system [13]. The toxins' hydrophobic nature ensures both are predominantly bound to albumin in the systemic circulation and therefore mainly depend on active renal excretion. Like many uraemic toxins, both are detectable in the non-CKD population. However, the decreased renal excretion of the toxins, along with the CKD-specific gut dysbiosis [14], resulting in increased bacterial production of IS and PCS, gives rise to the heightened concentrations observed in the CKD population.

IS and PCS are often referred to collectively given their notable similarities including synthesis, transportation (via albumin binding and organic anion transporters) and mechanism of action (notably nephrovascular damage). Despite this, important differences between these two toxins have recently been identified, including mechanisms of renal clearance and rates of intestinal absorption [15] as well as differing concentrations across ethnic groups [16]. These differences may be key to explaining the lack of consensus between findings in observational studies reporting associations with one toxin, but not the other. Wu et al. [8] demonstrated that PCS, but not IS, was an independent predictor of all-cause mortality in an Asian pre-dialysis population, whereas Melamed et al. [17] found this association with IS but not PCS in an American haemodialysis cohort. Intervention studies have also drawn conflicting conclusions, indicating potential for population-specific benefit of treatment [18].

Identifying the differences between these toxins may help to explain some of the conflicting findings between studies as well as providing a better understanding for potential mechanisms of action and opportunities for therapeutic manipulation. This paper aims to compare the total and free serum concentrations of IS and PCS, and the free fractions across the CKD spectrum, as well as exploring their associations with CVD markers.

Methods

Study population

Baseline data were analysed from a convenience sample of participants enrolled in one of three trials at a single tertiary centre's renal outpatient department. All patients from Study 1 who had completed 2 years of follow up were included in the study. All baseline patients from Studies 2 and 3 were included, except for when serum samples were not available for analyses (Study 2 $n = 5$, Study 3 $n = 86$). There was no systematic exclusion of patients. The three trials included participants from different CKD stages (Study 1: normal kidney function (control), Study 2: moderate kidney dysfunction and Study 3: end stage kidney disease (ESKD) including peritoneal- and haemo-dialysis). Participants from Study 1 ($n = 42$) were prospective living kidney donors. Studies 2 and 3 were participants enrolled in randomised controlled trials of cardiovascular risk modification ($n = 171$ & $n = 114$, respectively). Inclusion into Study 2 required an estimated glomerular filtration rate (eGFR) between 25 and 75 ml/min/1.73 m^2 and at least one of the following modifiable risk factors: blood pressure not at target (>130/80, or >120/75 for those with diabetes or proteinuria >1 g/24 h), overweight (classified as a body mass index (BMI) of 25 or above), poor diabetic control (HbA1c > 7%), or hyperlipidaemia (defined as low density lipoprotein (LDL) < 2.5, or <2.0 in those with diabetes or existing coronary heart disease). Study 3 included participants with an eGFR below 30 ml/min/1.73 m^2 or receiving maintenance dialysis [19]. Exclusion criteria for Study 2 were a previous kidney transplant or anticipated dialysis within 6 months. The current study was approved by the institution's Human Research Ethics Committee (HREC/12/QPAH/216).

Laboratory assessment

Venous blood was collected from all patients following an overnight fast. Serum creatinine, cholesterol, low-density lipoproteins (LDL), triglycerides, high-density lipoproteins (HDL), albumin, phosphate were measured using Beckman DxC800 general chemistry analysers (Beckman coulter Brea, CA, USA) and haemoglobin using a Sysmex XE- 5000 haematology analyser (Sysmex Cooperation, Kobe, Japan). The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula. CKD stage was defined by the KDIGO criteria for CKD diagnosis [20].

Uraemic toxins

Serum total and free concentrations of both uraemic toxins, IS and PCS, were analysed by ultra-performance liquid chromatography (UPLC) using a fluorescence detection method (Waters Corporation, Milford, MA, USA). This recently validated method allowed for low limits of detection (as low as 0.1 μmol/L) as detailed by Pretorius et al. [21] The free fraction of each toxin was defined as a