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Critical review

Treatment

Indoxyl sulphate and p-cresyl sulphate: therapeutically modifiable nephrovascular toxins

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Abstract

Introduction

Over the past decade, indoxyl sulphate and p-cresyl sulphate have emerged as potentially important, therapeutically modifiable, toxins in patients with chronic kidney disease. Both are protein-bound uremic retention solutes that are generated from colonic bacterial fermentation of dietary protein and have been associated with cardiovascular disease, kidney disease progression and overall mortality in the chronic kidney disease population. This review provides an overview of the toxicokinetics and nephrovascular toxicity of indoxyl sulphate and p-cresyl sulphate and then focuses specifically on the strengths and weakness of proposed therapeutic opportunities for targeting these molecules, including inhibition of colonic bacterial biosynthesis (protein restriction and pre- and pro-biotic therapy), suppression of absorption (oral adsorbents), augmentation of clearance (enhanced dialysis) and modulation of cellular pathways (organic anion transporters and antioxidants).

Conclusion

The uremic retention solutes, indoxyl sulphate and p-cresyl sulphate, have been identified as potentially important nephrovascular toxins that are highly amenable to therapeutic manipulation. In particular, targeting biosynthesis through pre- and pro-biotic therapy holds great potential as a tolerable, targeted and cost-efficient therapy for reducing serum concentrations of indoxyl sulphate and p-cresyl sulphate in the clinical setting. Well-designed clinical trials in the chronic kidney disease population are warranted to investigate whether reductions in indoxyl sulphate and p-cresyl translate into improved cardiovascular outcomes.

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Introduction

Chronic kidney disease (CKD) is associated with a greatly increased risk of cardiovascular disease (CVD), which rises exponentially as kidney function declines[1]. Traditional cardiovascular risk factors, such as older age, hypertension, diabetes mellitus, obesity, dyslipidaemia and smoking, only account for part of this increased risk[2]. Moreover, therapies targeting traditional risk factors of cardiovascular risk, such as hyperlipidaemia, have yielded disappointing results in the CKD population[3,4]. Over the past decade, emerging knowledge regarding the aetiology of CVD in CKD has shifted the focus from traditional to novel risk factors, including uremic toxins, in an effort to explain this increased CVD burden.

This recognition has led to a surge in uremic toxin research with the annual number of publications in this novel area tripling between 2000 and 2012[5]. Many potential uremic toxins have been identified and variously classified according to their physicochemical properties[6] and/or their origin[7] (Table 1). From this research, two uremic toxins, indoxyl sulphate (IS) and p-cresyl sulphate (PCS), have surfaced as key nephro- and cardiovascular toxins that are potentially amenable to therapeutic manipulation by a variety of strategies. This review focuses on IS and PCS with respect to their toxicokinetics, nephrovascular

Table 1 Classification of common uremic toxins according to their origin and physiochemical properties

		Physiochemical properties		
		Small water soluble (<500 Da)	Middle molecules (≥500 Da)	Protein-bound molecules
Origin	**Endogenous**	Urea Creatinine Oxalate Phosphorous	Advanced glycation end-products Parathyroid hormone Beta2 microglobulin	Homocysteine
	Microbial			Indoxyl sulphate P-cresyl sulphate
	Exogenous	Oxalate Phosphorous	Advanced glycation end-products	

Adapted from European Uremic Toxin Working Group[6] and Evenepoel et al.[7] classification systems.

FOR CITATION PURPOSES: *Rossi M, Campbell KL, Johnson DW.* Indoxyl sulphate and p-cresyl sulphate: therapeutically modifiable nephrovascular toxins. OA Nephrology 2013 Jul 01;1(2):13.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to the concept on, design, and preparation of the manuscript, as well as read and approved the final manuscript.
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toxicity and potential strategies for therapeutic manipulation.

Discussion

The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects in these referenced studies gave informed consent to participate in these studies.

Toxicokinetics

IS (MW 213 Daltons) and PCS (MW 188 Daltons) emanate from colonic bacterial fermentation of dietary protein[8]. Colonic microbiota produce tryptophanase, which degrades tryptophan into indole, and 4-hydroxyphenylacetate decarboxylase (4-Hpd), which degrades tyrosine and phenylalanine into p-cresol (Figure 1)[9]. Indole and p-cresol are then absorbed into the blood from the colon where they are metabolised

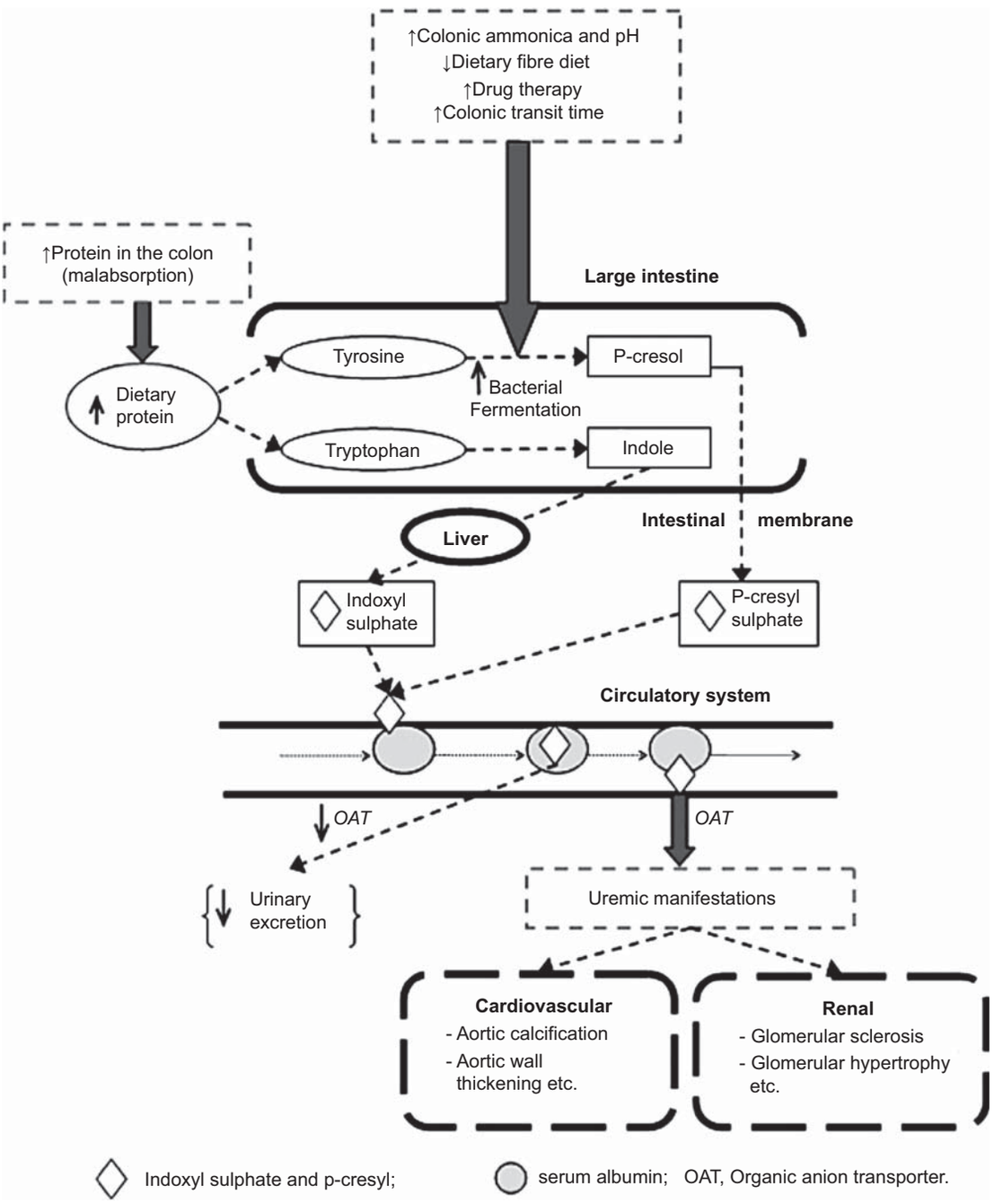

Figure 1: Toxicokinetics of indoxyl sulphate and p-cresyl sulphate in chronic kidney disease

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by the liver and intestinal membrane to IS and PCS, respectively. Both are predominantly bound to albumin in the serum and share the same albumin-binding site, Sudlow II, to which they are non-covalently bonded[10]. In the healthy population, more than 99% of the toxins are protein-bound in serum. However, as their concentrations rise, such as in CKD, binding saturation occurs resulting in an increase in the unbound fraction of IS and PCS (free IS and free PCS), reaching levels of approximately 10% in haemodialysis patients[11].

Organic anion transporters (OAT) play an important role in the uptake of IS and PCS by a diverse range of tissues, including renal proximal tubules, vascular endothelial cells, vascular smooth muscle cells and osetoblasts[12]. This aligns with the sites vulnerable to clinical manifestations of uremia[13]. OAT1 and 3 are the main isoforms from the OAT family responsible for the transport of IS and PCS[14,15]. OAT are also involved in renal elimination via active tubular secretion from the proximal tubules.

Patients with CKD exhibit an exponential rise in serum levels of IS and PCS with declining kidney function due to augment levels of toxin-producing intestinal micro-organisms[16], impaired protein assimilation in the small intestine[17] and reduced renal clearance (Table 2)[7].

Nephrovascular toxicity

Both IS and PCS have demonstrated extensive pro-inflammatory and oxidative stress properties *in vitro* and have shown to be independent predictors of all-cause mortality, CVD and kidney disease progression in the CKD population.

Convincing findings from *in vitro* studies have illustrated dose-dependent stimulation of oxidative stress and increased expression of inflammatory cytokines with ranging concentrations of IS[18,19] and PCS[20].

Animal studies have shown further support for the nephro- and cardiovascular toxicity of IS and PCS. Rat studies have demonstrated IS stimulates the proliferation of vascular smooth muscle[21] as well as promotes aortic calcification and wall thickening[22]. In addition, oral administration of IS has resulted in increased serum creatinine with decreased clearance[23], increased glomerular sclerosis[24], increased glomerular hypertrophy and expression of transforming growth factor[22]. Although few animal studies have investigated the effects of PCS, a recent study reported similar effects to IS, with increased fibronectin and α-smooth muscle actin expression in the kidney, resulting in significant nephrosclerosis[25].

Observational studies have consistently shown independent associations between at least one of the toxins (IS, free IS, PCS or free PCS) and all-cause mortality. In a cohort of 175 haemodialysis patients, Bammens et al. reported high free PCS serum levels (above median) was an independent predictor of all-cause mortality (HR = 2.28, 95% confidence interval [CI] = 1.12–4.64)[26], which was similarly reported in a cohort of 139 CKD patients (CKD Stage II–IV [dialysis])[27]. Interestingly, total PCS was not associated with mortality in these studies, which further supports the important role and the metabolic activity of the free form. IS has also demonstrated an independent association with all-cause mortality in a cohort of 521 haemodialysis patients (HR = 1.30, 95% CI = 1.01, 1.69)[28]. Furthermore, free IS was associated with all-cause mortality in 112 haemodialysis patients, following adjustment for age, diabetes status and albumin, whereas no association between mortality and IS was found[29].

An association between free PCS and cardiovascular events was demonstrated in 499 CKD patients, independent of both Framingham risk factors (HR = 1.40, 95% CI = 1.07–1.84) and kidney function (HR = 1.49, 95% CI = 1.09–2.04)[30]. Similarly, an independent association with cardiovascular events and IS was reported in a cohort of 70 CKD patients[31]. In addition, both total IS and PCS independently predicted renal progression (defined as decrements in estimated glomerular function [Egfr] >50% or progression to end-stage renal disease) in 268 CKD patients[32].

These findings, though not conclusive, indicate the role of IS and PCS as nephrovascular toxins.

Therapeutic opportunities

With the growing body of evidence for the nephrovascular toxicity of IS and PCS, a number of therapeutic

Table 2 Factors contributing to the rise in serum indoxyl sulphate and p-cresyl sulphate in chronic kidney disease

Kidney-disease-specific features	Mechanism
Decreased urinary excretion	Injured proximal tubule cells limiting the active excretion of toxins
Increased dietary protein entering the colon	Decreased protein assimilation in the small intestine
Increased 'toxin-producing' gut microbiota	Increased colonic ammonia and pH Decreased dietary fibre (dietary potassium restriction) Increased drug therapy Increased colonic transit time Increased protein in the colon

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strategies have been developed to decrease their generation and increase their excretion (Table 3). These treatment options are discussed in more detail below.

Protein restriction

It has been known for decades that a decrease in dietary protein intake results in decreased production of uremic toxins[33]. In particular, a recent study has shown a 37% reduction in serum concentrations of IS following a very low protein diet (0.3 g/kg/day) compared to a low-protein diet (0.6 g/kg/day) in the CKD population[34]. Nonetheless, the efficacy of low-protein diets in early-to-moderate-stage CKD is controversial, and in the dialysis population, best-practice guidelines recommend moderate-to-high protein intakes (1.0–1.2 g/kg/day)[35].

Low-protein and very low protein diets have been associated with modest improvement in outcomes in the CKD population, including reducing mortality[36]. Nonetheless, concerns with the overall value of achieving such low intakes have been raised. First, malnutrition associated with low-protein diets was observed in the largest CKD study assessing protein intake to date, Modification of Diet in Renal Disease (MDRD) study, even under close monitoring[37]. Without close monitoring, the risk of malnutrition on low-protein diets in this population is even greater. Second, protein-restricted diets are often plagued by poor dietary compliance, with few studies achieving greater than 50% of targeted restriction[38]. These figures suggest the risks of limited monitoring and poor compliance may challenge the overall benefit of recommending a low-protein diet in this population.

Table 3 Summary of therapeutic options for targeting indoxyl sulphate and p-cresyl sulphate

Therapeutic target	Strengths	Weaknesses
Biosynthesis		
Protein restriction	Positive studies the in chronic kidney disease (CKD) population Cost efficient	Risk of malnutrition Compliance issues
Pre- and pro-biotics	Positive studies in the CKD population Minimal side effects Cost efficient	Requires select probiotic strains and/or prebitoic varieties and dosing Limited studies in CKD
Absorption		
Oral adsorbents	Positive studies in the CKD population Cost efficient	Potential lack of absorbent specificity Contraindicated side effects
Clearance		
Dialysis	Positive studies in the dialysis population	Only applicable in end-stage kidney disease Cost Quality of life
Intra- and inter-cellular pathways		
Tran-cellular transporters	Mechanistic plausibility demonstrated *in vitro*	Lack of *in vivo* translation
Inhibition of intracellular pathways (e.g. Oxidative stress)	Mechanistic plausibility demonstrated *in vitro*	Lack of *in vivo* translation

Pre- and pro-biotics

The use of prebiotics, 'indigestible' carbohydrates that stimulate gut bacteria, and probiotics, supplements of living beneficial bacteria, is becoming increasingly popular in mainstream medicine. Furthermore, in nephrology the increased intestinal generation of IS and PCS, coupled with the disturbed microbiota observed in the CKD population[16], suggests a theoretical benefit for pre- and pro-biotic therapy. Support for the mechanism behind this therapy was demonstrated in a probiotic intervention study in a cohort of haemodialysis patients, which reported both serum IS and faecal flora as outcome measures. This approach illustrated a 30% decrease in serum IS ($p < 0.01$), in conjunction with a decline in faecal *enterobacteria (E. coli)* ($p < 0.05$), which has one of the highest observed enzymatic activities for IS production[39]. Furthermore, a prebiotic intervention in 12 healthy volunteers demonstrated a significant reduction in faecal p-cresol (65%) and indole (25%) along with increases in faecal *Bifidobacteria* and *Lactobacillus* and a decrease in *Bacteroidaceae*[40]. The *Bacteroidaceae* family includes the *Bacteroides fragilis* species, which has a high enzymatic activity for p-cresol production[41]. The combination of the decrease in PCS-producing bacteria

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(*Bacteroidaceae*) and the increase in PCS-and-IS-suppressing bacteria (*Bifidobacteria and Lactobacillus*) supports the proposed mechanistic rationale for the significant reduction in faecal p-cresol and indole.

Our group recently published a systematic review that included 19 intervention studies measuring the effectiveness of pre-, pro- and/or syn-biotics on IS and/or PCS levels in adults[42]. Despite the significant limitations of the reviewed studies, including case-series study design and limited studies in kidney disease, pre-, pro- and/or syn-biotics appeared to be beneficial for suppressing IS and PCS production. Following publication of this review, the results of a further randomised, placebo-controlled, cross-over trial in the healthy population was published, illustrating a significant decrease in urinary p-cresol (a marker of p-cresol generation) and an increase in faecal *bifidobacteria* with prebiotic therapy[43].

Despite the limited number of studies in kidney disease populations, the clear mechanistic plausibility of this therapy makes this a promising treatment opportunity warranting further investigation.

Dialysis

Dialytic removal of uremic toxins has been shown to improve both life expectancy and quality of life in patients with end-stage kidney disease[44]. Nonetheless, even in the early stages of dialysis, the limited clearance of protein-bound solutes was identified[45]. Indeed, it wasn't until two large-scale randomised-controlled trials, HEMO[46] and ADEMEX[47], failed to show a survival advantage associated with increased removal of small water-soluble uremic toxins and even middle molecules above current standards that the focus turned to protein-bound uremic toxins.

The high protein-binding affinity of IS and PCS markedly limits their dialytic removal, averaging <15% of the dialytic urea clearance[48]. To date, a number of studies have evaluated the clearance of protein-bound uremic toxins using different types of dialysis. The introduction of high-flux dialysis membranes showed no improvement in clearance of these two toxins[49]. This finding was not unexpected, given that the increased permeability of high-flux membranes is isolated primarily to large, non-protein-bound solutes. The addition of convective transport to conventional dialysis has also been investigated, albeit with conflicting findings. Two cross-over studies reported opposing effects of haemodiafiltration on dialysis clearance, although both studies failed to show a superior benefit on post-treatment plasma concentration of the toxins[50,51]. In contrast, Meert et al. reported a 20% reduction in serum PCS following 9 weeks of haemodialfitration[52]. This disparity, however, may be owing to the lower dialysate flow rate used for the conventional haemodialysis (500 vs. 620 mL/min), thereby exaggerating the effect of convective transport.

Compared to low-flux membranes, super-flux membranes have shown superior removal of IS, although this finding is at least partially explained by increased albumin removal[53]. Another method that may enhance the efficiency of removal of protein-bound uremic retention solutes, such as IS and PCS, is addition of a sorbent to the dialysate, which may achieve increases in clearance of more than two-fold[54].

Furthermore, a pilot study using two dialyzers in series showed an enhanced clearance of IS and PCS (by 57% and 66%, respectively) by increasing the dialyzer mass transfer area coefficient and dialysate flow compared to conventional practice[55]. A more recent study has shown similar benefits achieved with standard equipment, albeit requiring extended treatment[56]. These options appear to be making some headway in targeting clearance of toxins, such as IS and PCS. However, despite the significant advances in dialysis technology, the efficacy, safety and cost-effectiveness of achieving sustained decreases in serum concentrations of IS and PCS by these techniques require further investigation.

Charcoal adsorbent treatment

The oral charcoal adsorbent, AST-120 (Kremezin, Kureha Chemical Industry, Tokyo, Japan), is available in Asian countries, where it is approved for treatment of CKD[57]. The sorbent's mechanism of action has been proposed to be related to its role in intestinal absorption of indole and p-cresol. In fact, metabolomic research has identified IS as the principal serum metabolite to differentiate the effects of AST-120, and therefore, reduction in IS serum concentration has been used to demonstrate the effectiveness of AST-120[58]. Furthermore, Schulman et al.[57] illustrated a dose-dependent reduction in serum IS (approximately 40%, 20%, 2.5%), with ranging doses of AST-120 (9 g, 6.3 g, 2.7 g, respectively) in 163 CKD patients. Of note however, AST-120 absorbs a number of other uremic toxins, including hippuric acid, and its effect on PCS absorption is less apparent[58].

Apart from the effects of AST-120 on serum IS, there have been a number of intervention studies investigating clinical outcomes in response to this treatment. A 6-month controlled trial (n = 35) showed the administration of AST-120 significantly lowered the concentration of serum and urinary IS, and patients who showed a greater decrease in urinary IS also showed a more marked suppression of the progression of CKD (defined as 1/serum creatinine-time plot)[59]. Another study (n = 40) reported significant improvements in flow-mediated dilatation (3.24% vs. 4.01%, p = 0.009), a measure of endothelial dysfunction, and a 65% reduction in oxidized/reduced glutathione ratio, a marker of oxidative stress, with a 47% decrease in IS[60].

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However, a recent endpoint study suggested no benefit with AST-120 supplementation. The results of the Phase III trial (n = 2035), Evaluating Prevention of Progression In Chronic kidney disease (EPPIC), conducted in the US, demonstrated no difference between placebo and AST-120 in CKD progression (defined as time to initiation of dialysis, transplantation or doubled of serum creatinine)[61]. The authors have suggested that compliance may have been a contributing factor to the negative outcome.

Indeed, the compliance issues reported in EPPIC may have been related in part to the well-characterised side effects of AST-120. Being an oral adsorbent, it has the potential to bind to other beneficial nutrients along with uremic toxins and requires significant fluid intake to support its effect. Furthermore, the treatment has a high pill burden and is associated with constipation and gastrointestinal upset. These side effects could potentially contribute to the initiation of dialysis, therefore negating the theoretical benefit of this form of therapy.

OAT modulators

Animal models have suggested that the decreased renal excretion of uremic toxins in kidney disease may be related to the reduced expression of OAT. This decreased OAT expression in renal tubules has been confirmed by renal biopsies in CKD patients[62].

Statins have been shown *in vitro* to upregulate specific OAT transcription localised in the renal tubular cells, suggesting a possible therapeutic modality for enhancing IS and PCS excretion[63]. So far, however, there has been no report of serum or renal clearance benefits associated with statins, via OAT modulation. This is probably due to the overlap of substrate specificity where the effect of single-OAT gene modulation is likely to be minimal in overall transportation[64].

Other well-known OAT inhibitors, probenecid and cilastatin, have also been shown *in vitro* to dose-dependently decrease IS-stimulated cardiac myocyte hypotrophy[65]. More important, these OAT antagonists did not affect cardiac cell viability. Nonetheless, probenecid and cilastatin are also non-specific OAT inhibitors and therefore may concurrently decrease the renal excretion of IS through OAT in the proximal tubular cells. This limits the application of this mechanism, as it currently stands, for therapeutic intervention in the clinical setting.

Antioxidants and intracellular pathway inhibitors

The pathogenic actions of IS and PCS are thought to stem from the induction of reactive oxygen species (ROS), which activate the nuclear factor kappa B (NF-kB) pathway, resulting in both oxidative stress and stimulation of pro-inflammatory cytokines. An *in vitro* study in human renal proximal tubular cells has supported this through dose-dependent increases in oxidative stress and activation of NF-kB over a range of IS concentrations[19]. In addition, *in vitro* studies have found treatment with antioxidants and NF-kB inhibitors dose-dependently inhibit the fibrotic and oxidative effects of IS and PCS[19,20]. At present, these findings summarise only experimental data and, therefore, require translation and testing *in vivo* before consideration as a treatment for IS and PCS.

Conclusion

IS and PCS are important protein-bound uremic retention solutes that are found in increased concentrations in the serum of patients with CKD due to both enhanced colonic production, increased colonic substrate and reduced renal clearance. They have been shown to exert renal and cardiovascular toxicity *in vitro* and animal studies and have been associated with increased CVD, kidney disease progression and all-cause mortality in the CKD population. Overall evidence for treatments targeting the generation and clearance of IS and PCS is growing. In particular, pre- and pro-biotic therapy, although a relatively new concept in CKD, appears to be a novel and promising treatment strategy when considering a targeted, tolerable and cost-efficient therapy. Further clinical studies are warranted to determine the effectiveness of pre- and pro-biotics in achieving clinically important decreases in IS and PCS and, ultimately, improvements in patient-level outcomes.

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Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to the concept on, design, and preparation of the manuscript, as well as read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

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All authors contributed to the concept on, design, and preparation of the manuscript, as well as read and approved the final manuscript.
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