

The intestine and the kidneys: a bad marriage can be hazardous

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Abstract

The concept that the intestine and chronic kidney disease influence each other, emerged only recently. The problem is multifaceted and bidirectional. On one hand, the composition of the intestinal microbiota impacts uraemic retention solute production, resulting in the generation of essentially protein-bound uraemic toxins with strong biological impact such as vascular damage and progression of kidney failure. On the other hand, the uraemic status affects the composition of intestinal microbiota, the generation of uraemic retention solutes and their precursors and causes disturbances in the protective epithelial barrier of the intestine and the translocation of intestinal microbiota into the body. All these elements together contribute to the disruption of the metabolic equilibrium and homeostasis typical to uraemia. Several measures with putative impact on intestinal status have recently been tested for their influence on the generation or concentration of uraemic toxins. These include dietary measures, prebiotics, probiotics, synbiotics and intestinal sorbents. Unfortunately, the quality and the evidence base of many of these studies are debatable, especially in uraemia, and often results within one study or among studies are contradictory. Nevertheless, intestinal uraemic metabolite generation remains an interesting target to obtain in the future as an alternative or additive to dialysis to decrease uraemic toxin generation. In the present review, we aim to summarize (i) the role of the intestine in uraemia by producing uraemic toxins and by generating pathophysiologically relevant changes, (ii) the role of uraemia in modifying intestinal physiology and (iii) the therapeutic options that could help to modify these effects and the studies that have assessed the impact of these therapies.

Keywords: uraemic toxins; CKD; haemodialysis; creatinine; systematic review

Introduction: the intestine in health and disease

The previous view that the gut is a largely inert organ has been challenged for more than a decade, as intestinal microbiota provide essential functions which the human body by itself is unable to supply [1], such as the harvesting of essential food products, energy saving, metabolism of xenobiotics, protection against invasion by alien microbes, renewal of the intestinal epithelium, generation of vitamins and balancing of immune function [1, 2].

The definition of the 'normal' microbiome with its striking inter-individual differences, sometimes due to day-by-day elements such as diet or exercise [3, 4], raised the obvious question about what happens during disease [5, 6]. Already in 2003, intestinal microbiome changes were described for multi-organ failure, colon cancer and inflammatory bowel disease [2]. In the last decade, this list was completed by a long series of highly prevalent chronic diseases. The relation between liver disease and gut microbiome could be expected by their juxtaposition [7]. However, also a link was demonstrated, as cause or consequence, with diabetes mellitus, obesity, insulin resistance, the metabolic syndrome, ageing, micro-inflammation and cardiovascular disease [6, 8, 9].

The awareness that also chronic kidney disease (CKD) is related to the intestinal status came relatively late [10] and stemmed in part from the insight that several toxins active in the uraemic syndrome originate from the intestine [11, 12].

Many of the above-mentioned chronic conditions linked to changes in the gut microbiome are also associated with renal failure or are considered as a cause of it. The question has been raised whether the gut microbiome might be one of the common denominators linking all these elements together [13, 14]. It could be possible that some of the solutes generated in the gut and currently called uraemic toxins exert their toxicity even with normally functioning kidneys [15] and thus are not only uraemic toxins but toxins in general. This hypothesis, however, remains to be confirmed.

Thus, there are good reasons to take a close look at the interaction between the gut and kidneys, especially since understanding the mechanisms might create opportunities to develop novel targeted therapeutic interventions [13, 16]. In this narrative review, we will consider the current state of the art on this topic, including the bidirectional interaction between gut and uraemic toxicity, a proof of concept of the role of the intestine in uraemic

toxin generation, the evidence of the toxicity of the generated molecules and a comprehensive review of the available therapeutic options, considering uraemic toxicity/toxins as key elements.

The bidirectional interference between the gut and uraemia

The gut is responsible for the generation of uraemic retention compounds with pathophysiologic impact, whereas uraemia as such causes structural and functional intestinal changes which indirectly cause morbidity and mortality [17] by inducing inflammation via diverse pathways [10]. Thus, the crosstalk between uraemia and the intestine is based on two different elements, but the consequences are the same, based on a double effect: induction of micro-inflammation going along with an incompetence of the pre-activated immune system to cope with stimuli [10, 18], resulting in an enhanced risk of infection which at the end in its turn again is pro-inflammatory. Inflammation remains one of the major causes of complications and death in CKD but also in non-CKD conditions.

The gut as a source of uraemic toxins

Uraemic toxins are generated in several ways (Figure 1): (i) some of them (e.g. peptides) are produced in the body without the contribution of the gut; (ii) others (e.g. the advanced glycation end products—AGEs) enter the body via unmodified absorption of nutritional elements by the intestine; (iii) finally, essentially protein-bound solutes such as the phenols, indoles or hippurates but also trimethylamine oxide (TMAO) are generated metabolically from precursors

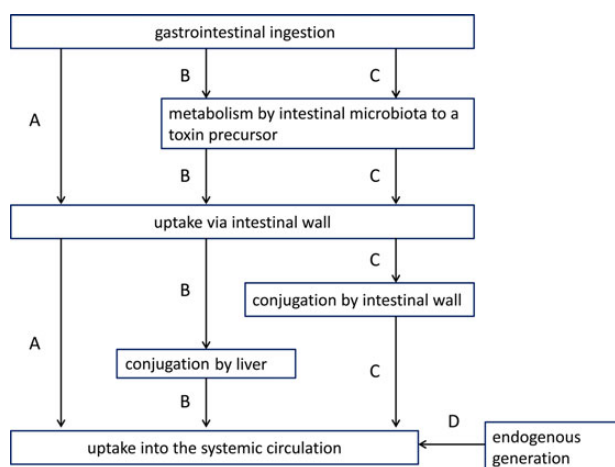


Fig. 1. Main metabolic pathways involved in uraemic toxin generation. (A) ingestion via the gastrointestinal tract and direct unmodified uptake via the intestinal wall into the systemic circulation (e.g. advanced glycation end products); (B) gastrointestinal uptake of a precursor (e.g. an amino acid), that is transformed by the intestinal microbiota into another precursor (e.g. indole); after its uptake via the intestinal wall and portal vein, this precursor is further conjugated by the liver (e.g. indoxyl sulphate) before its transfer into the systemic circulation; (C) gastrointestinal uptake of a precursor (e.g. an amino acid), that is transformed by the intestinal microbiota into another precursor (e.g. indole); the precursor is conjugated during its uptake via the intestinal wall (e.g. indoxyl sulphate) and then passes into the systemic circulation via the liver without further modification; (D) endogenous generation without contribution of the gastrointestinal tract (e.g. β_2 -microglobulin). Modified from Schepers *et al.* [12].

originating from microbial fermentation [12, 19]. A number of retention solutes (e.g. AGEs, uric acid) originate partially in the intestine and partially in the body.

The active role of the intestine is especially prominent in the generation of the protein-bound uraemic toxins (e.g. indoxyl sulphate and p-cresyl sulphate) (Figure 1, pathways B and C). At the origin are amino acids (*in casu* tryptophan and tyrosine) resulting from the digestion of proteins that are transformed by the intestinal microbiota into precursors of uraemic toxins like indole and p-cresol. Those are then conjugated during absorption in the intestinal wall or further downstream in the liver before their transfer into the systemic circulation [12]. The most abundant conjugates are the sulphates although many other structural variants are possible (Table 1). In addition, functional groups, such as methyl or hydroxyl, may be added in different positions. Some compounds remain unmodified (e.g. kynurenine).

Additional contributing factors

In uraemia, a number of further intestinal modifications involved in the so-called assimilation process cause an increase in the generation of uraemic toxins compared with the normal condition. First, amino acids, if less well absorbed along the gastrointestinal tract, present more substrate to the intestine for microbial metabolism [12]. Second, the balance of intestinal microbiota is shifted in favour of proteolytic microorganisms producing more toxin precursor by fermentation (see below) [20–22]. Finally, abnormal intestinal motility prolonging colon transit [23, 24] deprives the colon from carbohydrates inducing upstream expansion of proteolytic species increasing generation of bacterial toxins and increasing time for their absorption [23]. In an elegant study, Bammens *et al.* assessed the urinary excretion of p-cresol, the precursor of cresyl conjugates and a surrogate of their generation: approximately twice as much p-cresol was excreted per 24 h in the urine of advanced CKD patients than that of controls with normal kidney function, and urinary p-cresol excretion was negatively correlated with GFR [25].

It is an upside-down world: changes of the intestine due to uraemia

The intestine does not only play a role in the generation of uraemic toxins, but also the other way around uraemia

Table 1. Precursors and conjugates composing intestinally generated uraemic metabolites^a

Precursors
Indole
Phenol
Cresol
Hippurate
Methylamine
Conjugates
Sulphate
Lactate
Acetate
Glucuronide
Propionate
Acetylglycine
Propionylglycine
Oxide

^aThe list is not exhaustive.

Table 2. Fermenting intestinal bacterial species^a

Clostridia
<i>Clostridium bif fermentans</i>
<i>Clostridium sporogenes</i>
<i>Clostridium clostridiforme</i>
<i>Clostridium leptum</i>
<i>Peptostreptococcus asaccholyticus</i>
<i>Peptostreptococcus indolicus</i>
Bacteroidetes
<i>Bacteroides thelatoaomicron</i>
<i>Bacteroides putredinis</i>
Other
<i>Fusobacterium nucleatum</i>
<i>Actinomyces israelii</i>
<i>Megalosfaera elsdinii</i>
<i>Propionibacterium acnes</i>

^aBased on Smith and MacFarlane [20].

(and by extension uraemic toxins) may cause functional and structural modifications in the intestine with pathophysiological consequences.

First, bacterial species that are prone to generate uraemic toxins by fermentation are privileged in uraemia (Table 2) and outnumber protective species such as lactobacilli [20, 21]. Up till now, only three studies compared the composition of the intestinal microbiota of uraemic versus control patients by bacterial genomic analysis: all found a disbalance in the proportional distribution of intestinal bacteria with a predominance of species with a pathologic impact and/or a shortage of those having no deleterious effect [21, 26, 27], identical to what had been demonstrated before in uraemic rats [26]. Earlier studies using non-genomic techniques had also defined that a number of bacterial species prone to proteolytic fermentation, among which the clostridia species, were more abundant [20].

Second, colon bacteria in uraemia tend to move to sections of the body where they normally are not present unless in very low quantities (translocation). This includes migration of microbes into the jejunum [23], into the mesenteric lymph nodes and further downstream into the blood and the rest of the body [22, 28–31]. Bossola *et al.* found bacterial-derived DNA fragments in the blood of haemodialysis patients, but since bacteria were also detected in dialysate, an extra-intestinal source, i.e. contaminated dialysate, could not be excluded [28]. However, Wang *et al.* reported similar findings in non-dialysed CKD patients, pointing to the fact that also sources other than dialysate can be responsible for these DNA fragments in uraemic patients, and in this case, the intestine is a primary candidate culprit [22]. More convincingly even, all bacterial DNA found in the blood was the same as that from genera present in the gut [22]. In uraemic rats, bacterial DNA of enteric species was demonstrated in the mesenteric lymph nodes, blood and even in liver and spleen [30]. Both in humans and in animals, this bacterial overgrowth and translocation was associated to microinflammation [22, 30].

Third, compelling arguments favour a loss of the intestinal protective barrier in uraemia. After the first results in this direction published in 1991 [32], Vaziri and co-workers demonstrated *in vitro* and *ex vivo* that the uraemic milieu disrupted intestinal epithelial protection [21, 33] by depleting protein constituents of the tight junctions (Zona Occludens-1 (ZO-1), Claudin-1 and Occludin). The ensuing leakage could cause inflammation by propagating endotoxin transfer into the

bloodstream. Vaziri *et al.* could also demonstrate that urea created the same effect [34], with a further enhancement by adding urease, suggesting that urease producing bacteria generating ammonia amplify this effect [34]. The same authors also demonstrated that the administration of the sorbent AST-120 (Kremezin^R) protected the intestinal barrier in uraemic rats [35]. As AST-120 is essentially known for decreasing the concentration of protein-bound uraemic solutes rather than that of urea, it can reasonably be assumed that also other uraemic solutes than urea [36], or their precursors, disrupt the intestinal barrier.

Thus, in summary, uraemia causes a series of intestinal changes (bacterial translocation, leakiness of the intestinal barrier and the generation of protein-bound uraemic toxins) that all lead to inflammation, a major cause of morbidity and mortality, both in CKD and the non-CKD population.

Proof of concept

The theory that intestinal microbiota contribute to uraemic toxin generation by metabolizing breakdown products derived from dietary protein intake was confirmed in a number of proof-of-concept studies.

In 1965, Einheber and Carter demonstrated that germ-free rats made anephric survived longer and lost less weight than their counterparts with intestinal microorganisms [37]. In 1982, Yokoyama *et al.* demonstrated that sterilizing the intestine by a combination of antibiotics in weanling pigs with normal kidneys decreased the faecal and urinary excretion of phenolic and aromatic bacterial metabolites and increased their body weight [38]. A recent study demonstrated that antibiotic prophylaxis for episodes of neutropenia in patients having received an allogeneic stem cell transplantation resulted in a shift of intestinal microbiota and in a dramatic decrease of urinary indoxyl sulphate concentration [39].

Recently, Wikoff *et al.* compared by untargeted metabolomic mass spectrometry the plasma from germ-free mice with normal kidney function with that of animals with normal faecal microbiota and could indicate a number of discriminators, among which are several compounds known as protein-bound uraemic toxins such as indoxyl sulphate, hippuric acid and phenylacetic acid [40], which are also a substrate of renal tubular organic anion transporters (OATs) explaining their retention in kidney failure if the tubules are damaged [41].

Aronov *et al.* applied metabolomic analysis by mass spectrometry to compare concentration of plasma solutes of six haemodialysis patients without a colon, to that of nine patients with a colon [42], with as aim the identification of metabolites discriminating between these two groups. Among the 30 discriminators were several protein-bound uraemic retention products. In most cases, a ratio of plasma concentrations of <0.05 for those without versus with colon was found; for p-cresyl sulphate, the ratio was even 0.01 [42], indicating that concentration was ~100 times higher in patients with a colon.

Patel *et al.* found in healthy vegetarians a strikingly lower urinary excretion of indoxyl sulphate and p-cresyl sulphate, as equivalents of their intestinal generation, than that in subjects with a more conventional food intake [43]. A similar study of >20 years before had shown lower serum concentrations and faecal losses of phenol and p-cresol in veganists [44]. Increasing dietary fibre intake by administration of resistant starch for 6 weeks in a randomized controlled trial

(RCT) in haemodialysis patients decreased plasma-free indoxyl sulphate whereas there was a trend for a decrease of free p-cresyl sulphate [45].

Eloot *et al.* assessed the relationship between the pre-dialysis concentration of several essentially protein-bound uraemic toxins and a number of potential influencing factors such as Kt/V_{urea} as an index of dialysis adequacy, normalized protein catabolic rate (nPCR) as an index of dietary protein intake, residual renal function, age, diabetes, gender, body weight and dialysis vintage [46]. A correlation was found, essentially for residual renal function and nPCR, and not for the other factors, including Kt/V_{urea} , again suggesting a role for digestion of dietary proteins in uraemic toxin generation and concentration, even overruling the impact of dialysis adequacy.

Toxicity of the compounds of intestinal origin

The toxicity of the protein-bound solutes, which compose a large fraction of the intestinally generated compounds, remains a matter of debate, as in many experimental studies too high concentrations resulted in an overestimation of potential toxicity [47]. In analogy with drugs, such toxicity should reasonably be exerted by the free, unbound fraction. A recent systematic review, however, unravelled 27 studies where correct free concentrations of two prototypic protein-bound solutes, p-cresyl sulphate and indoxyl sulphate, had been applied [48]. Interference was shown with several key metabolic processes involved in the uraemic syndrome, such as reactive oxygen species generation, endothelial dysfunction, epithelial-to-mesenchymal transition, leukocyte-endothelial interaction, deterioration of cardiac cell functional capacity, renal tubular cell senescence, expression of tissue factor and sensitivity to insulin. These data refer to the core of the morbidity and mortality of uraemia, i.e. cardiovascular disease and progression of renal failure. Together with observational studies showing a highly significant association between concentration of protein-bound toxins with hard endpoints such as cardiovascular events, progression of renal failure and mortality [49–54], these data offer strong arguments in favour of a

key role of indoxyl sulphate and p-cresyl sulphate in the uraemic syndrome.

Without following the same rigid approach as applied in the systematic review mentioned [48], we found at least five more recent publications conforming the inclusion criteria of the latter study, covering inhibition of drug metabolism [55]; increased crosstalk between leukocytes and endothelium, glycocalyx degradation and vascular leakage [56]; apoptosis of osteoblasts [57]; induction of tubular endothelial growth factor receptor leading to tissue remodelling [58] and inhibition of breakdown of angiotensin II [59].

Similar effects were also described for other protein-bound toxins [60] such as hippuric acid [61–63], indole acetic acid [61, 62, 64–67], phenyl acetic acid [61, 67, 68]; p-cresylglucuronide [56, 67, 69] and kynurenic acid [61, 67] (Table 3).

The link of AGEs with inflammation has been emphasized before [70]. Nutritional AGEs contribute to this effect since AGE-rich food administered to diabetics increased endothelial free radical production and decreased arterial responsiveness to vasodilatory stimuli [71].

Several arguments thus plead in favour of a broad array of biological effects exerted by uraemic solutes of intestinal origin, and it seems rational to explore novel strategies to decrease their concentration, especially since removal even with the most efficient dialysis strategies is deceiving.

Therapeutic options

Several approaches have the potential to decrease the capacity of intestinal microbiota to generate uraemic solutes and their precursors (Table 4) [12, 72]. Following hypothesis-generating experimental studies, several clinical studies have recently resulted in a few reviews expressing an affirmative opinion about such therapies [13, 14, 16]. However, those clinical studies are to be checked critically with regard to their evidence base, with an increasing order of importance given to (i) uncontrolled concentration studies in the normal population, (ii) uncontrolled concentration studies in uraemics, (iii) uncontrolled hard outcome trials, (iv) RCTs with toxin concentration as outcomes and (v) hard outcome RCTs (Table 4).

Diet

With protein as the source of amino acids, decreasing protein intake should diminish generation of uraemic retention solutes. A shift from a vegetable to meat diet in healthy subjects indeed impacts the intestinal balance of microbiota [4] and increases urinary excretion of phenols and ammonia [109]. Veganistic and vegetarian diets in non-uraemic populations decrease the concentration and generation of protein-bound uraemic solutes or their precursors [43, 44] but remain a matter of concern in the malnourished uraemic population. Although low-protein diet has a nephroprotective effect, it should be taken into account that a large section of CKD patients, not even on dialysis, suffer from protein malnourishment. On the other hand, what is currently a standard diet in Western countries often contains excessive quantities of protein.

Selective diets excluding the sources of specific toxins are an alternative. When AGE-rich food was administered to both non-uraemic and uraemic diabetics, AGE concentration and cross-linking in plasma increased compared

Table 3. Protein-bound uraemic toxins other than indoxyl sulphate and p-cresyl sulphate with toxic impact^a

Affected biological function	Toxic solute
Protein binding of drugs [63]	Hippuric acid
Expression of drug transporters [62]	Hippuric acid Indole acetic acid
Renal tubular efflux pumps [61]	Hippuric acid Indole acetic acid Phenyl acetic acid Kynurenic acid
Tissue factor expression [64, 66]	Indole acetic acid
Glucuronidation [67]	Indole acetic acid Phenyl acetic acid Kynurenic acid
Mitochondrial metabolism [67]	Indole acetic acid Phenyl acetic acid P-cresyl glucuronide
Nitric oxide synthase [68]	Phenyl acetic acid
Leukocyte-free radical production [69]	Phenyl acetic acid
Endothelial albumin leakage [56]	P-cresyl glucuronide ^b
Endothelial Cox-2 mRNA expression [65]	P-cresyl glucuronide ^b Indole acetic acid

^aList is not exhaustive.

^bIn combination with p-cresyl sulphate.

Table 4. Summary of studies on therapeutic options affecting uraemic toxin concentration/generation via intestinal pathways in humans

Intervention	Target molecule	Uncontrolled			RCT		
		Concentration		Outcomes Uraemia	Concentration		Outcomes Uraemia
		Healthy	Uraemia		Healthy	Uraemia	
Diet							
Vegetable/vegetarian [43, 44]	PBUT	+					
	Phenols	+					
	Phosphate	+					
AGE-free diet [19]	AGE	+	+				
Oxalate-controlling diet [73]	Oxalate	+					
Prebiotics							
Resistant starch [45, 74]	Phenols	+					
	Ammonia	+					
	IS					+	
	pCS					-	
Inulin [75]	p-Cresol ^a	+					
	Phenol	-					
Lactulose [76]	p-Cresol	+					
OF-IN [76-79]	p-Cresol	+			+		
	pCS		+				
	IS		-				
AXOS [80]	p-Cresol				+/- ^b		
	Phenol				-		
Arabinogalactan [81]	Creatinine		-				
	Urea		+				
Isphagula husk [81]	Creatinine		-				
	Urea		+				
Gum Arabic supplement [82]	Creatinine					-	
	Urea					+	
Fermentable carbohydrate [83]	Urea		+				
probiotics							
<i>L. casei</i> Shirota [77, 78, 84]	Urea		+/- ^c				
	Creatinine		-				
	p-Cresol				+		
<i>L. acidophilus</i> , <i>B. longum</i> , <i>S. thermophilus</i> , enteric coated [85]	Urea					+	
	Creatinine					-	
	Uric acid					-	
<i>B. breve</i> [77, 78]	p-Cresol				+		
Lebenin [®] [86]	IS		+				
	Phenol		-				
	p-Cresol		-				
Bifidobacteria, gastro-resistant capsule [87-89]	IS		+ ^d		-/+ ^e		
	Hcy		+				
Renadyt [®] [90]	IS					-	- ^f
	IG					-	
	pCS					-	
	pCG					-	
	Pentosidine					-	
	B ₂ -M					-	
<i>Oxalobacter formigenes</i> [91]	Oxalate		+				
Lactic acid bacteria [73, 92-95]	Oxalate	+/-				-	

(continued)

Table 4. Continued

Intervention	Target molecule	Uncontrolled		Outcomes Uraemia	RCT		
		Concentration			Healthy	Uraemia	Outcomes Uraemia
		Healthy	Uraemia		Healthy	Uraemia	
Synbiotics							
<i>L. casei</i> , <i>B. breve</i> , galacto-oligosachharides [96]	p-Cresol		+				
	phenol		–				
	IS		–				
<i>L. casei</i> Shirota, OF-IN [78]	p-Cresol				+		
Probinul-neutro [®] [97]	p-Cresol					+	
Prebiotic–probiotic mixture [98]							+ ^g
AKSB [73]	oxalate				–		
Sorbents							
Sevelamer [99, 100]	Oxalate	–					
	PBUT					–	
AST-120 [101–106]	PBUT			+		+	+/ ^h
Chitosan [107]	IS		+	+ ⁱ			
	Phosphate		+				
Others							
Acarbose [108]	p-Cresol	+					

The plus symbols indicate that the study has a positive impact; thus in general a decrease in concentration or generation or a protective effect against morbidity; the negative symbols indicate that there was no impact; plus or minus symbols indicate contradictory results; blank spaces indicate that no valid data were found to include in the table.

PBUT, protein-bound uraemic toxins; AGEs, advanced glycation end products; pCS, p-cresyl sulphate; pCG, p-cresyl glucuronide; IS, indoxyl sulphate; IG, indoxyl glucuronide; β_2 -M, β_2 -microglobulin; Hcy, homocysteine; OF-IN, oligofructose-enriched inulin; AXOS, arabinoxylan-oligosaccharides; Lebenin[®], antibiotic-resistant *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Enterococcus faecalis*; Renady[®], *Streptococcus thermophilus*, *L. acidophilus*, *B. longum*; Probinul-neutro[®], mixture of several Lactobacilli and Bifidobacteria, *S. thermophilus*, inulin and tapioca-resistant starch; AKSB, fructo-oligosaccharide, *E. faecium*, *S. cerevisiae*.

^ap-cresol in all studies surrogate for p-cresyl sulphate + p-cresyl glucuronide.

^bPositive after 2 weeks but negative after 3 weeks.

^cPositive at high dose, negative at low dose, no statistical comparison between high and low dose.

^dOnly if included in gastro-resistant capsule—not in powder formulation.

^eOverall negative; only an inhibitory effect on CKD progression in the subgroup with the worst kidney function.

^fInflammation and quality of life.

^gDecline of eGFR.

^hSmall Japanese randomized trials showed a benefit on progression of CKD, which could, however, not be confirmed in a large European–American RCT; one RCT showed an improved response to ESAs.

ⁱOxidative stress parameters.

with AGE-poor diet [19]. In the run-in phase of an RCT on oxalate decreasing intervention in hyperoxaluria, a diet controlling oxalate intake decreased urinary oxalate compared with a free-choice diet [73].

Prebiotics

Prebiotics are non-digestible compounds improving the composition and/or function of the intestinal microbiota [110].

An animal study by Koppe *et al.* showed that CKD as well as p-cresyl sulphate caused insulin resistance, disturbances of adipose tissue metabolism and reallocation of fat throughout the body [111]. When arabinoxylan-oligosaccharides (AXOS) were administered to CKD animals, p-cresyl sulphate concentration decreased while insulin sensitivity was restored [111]. In 5/6 nephrectomized rats, galacto-oligosaccharides decreased caecal indoles and serum indoxyl sulphate and improved renal injury, stress markers and apoptosis [112].

In healthy humans, resistant starch decreased faecal phenol and ammonia [74]. When assessing solute generation out of radiolabelled tyrosine, urinary excretion of p-cresol was decreased by inulin [75]. A similar effect on p-cresol was seen also for oligofructose-enriched inulin (OF-IN) and lactulose [76], a finding confirmed for OF-IN in two RCTs [77, 78]. In another RCT assessing AXOS, urinary p-cresol was decreased after 2 but not 3 weeks, and no change was shown for phenol [80].

In uraemia, a few early studies only focused on the concentration of the water-soluble compounds creatinine and/or urea [81–83, 113]. In 1984, Rampton *et al.* studied the effect of arabinogalactan and of Isphagula husk on blood urea and creatinine [81]. The study contained no control group, and no effect was seen on creatinine. The therapeutic decrease in urea claimed by the authors is doubtful in view of the far from stable baseline [81]. Bliss *et al.* randomized non-dialysed CKD patients on low-protein diet to gum Arabic supplement or placebo and found a decrease in urea concentration but not of creatinine, while also nitrogen in stools increased [82]. In 2006, Younes *et al.* confirmed their earlier findings in animals [113] in men, indicating a decrease of urea after administration of fermentable carbohydrate supplements. The study, however, contained no controls [83].

More recently, in a single-centre, non-randomized, open-label study with an escalating dose of prebiotic over 4 weeks, followed by a 4-week washout, OF-IN [79] has been shown in maintenance haemodialysis patients to impact plasma concentration of p-cresyl sulphate, but not of indoxyl sulphate [79]. In an RCT in haemodialysis patients, the administration of resistant starch decreased plasma-free indoxyl sulphate [45]. The study was, however, characterized by a substantial number of dropouts, both in the control and the verum group (29%) [45].

Probiotics

Probiotics are living organisms, administered as food components or supplements, which provide specific benefits by themselves, essentially by creating a more favourable balance in the composition of intestinal microbiota [114].

Urea. Urease-expressing bacteria may decrease blood urea. Most studies in this context are experimental, either *in vivo* in rats after 5/6 nephrectomy or acetaminophen-induced kidney failure or *in vitro* [115–117]. The probiotics

not only decreased urea but also other markers of kidney failure like creatinine or oxidative stress, and they prolonged life span [116, 117]. In a rat study assessing five different probiotic combinations, only two (one containing *Bacillus pasteurii* and one *Lactobacillus sporogenes*) decreased BUN and serum creatinine [118]. A matter of concern is that degradation of urea by urease probably generates ammonia, which is now considered a uraemic toxin by itself [119].

In an uncontrolled study in CKD 3–4, high-concentration *Lactobacillus casei* Shirota (16×10^9 CFU/mL) but not low-dose (8×10^9) decreased blood urea only while creatinine remained unmodified [84]. The decrease of urea was only by 11%.

In an RCT assessing in CKD patients, the effect of an enteric-coated probiotic combination of *Lactobacillus acidophilus*, *Bifidobacterium longum* and *Streptococcus thermophilus* on the evolution of BUN, serum creatinine and uric acid, only a borderline significant decrease in BUN could be observed, which remained absent in close to 40% of subjects [85].

Protein-bound uraemic toxins. Essentially lactic acid bacteria including Lactobacilli and Bifidobacteria have been studied.

In two placebo-controlled RCTs in healthy subjects, *Lactobacillus casei* Shirota and *Bifidobacterium breve* both reduced urinary p-cresol in the urine after 4 weeks [77, 78].

In haemodialysis patients, Lebenin[®], a mixture of antibiotic-resistant lactic acid bacteria, resulted in a decrease in plasma indoxyl sulphate but not in phenol and p-cresol [86]. In a study by Takayama *et al.*, *Bifidobacterium* in a gastro-resistant capsule but not in powder formulation decreased indoxyl sulphate in haemodialysis patients [87]. The same encapsulated variant also decreased homocysteinemia and increased serum folate [88]. None of these studies, however, checked hard endpoints or were even placebo controlled. The study by Takayama *et al.* contained a non-randomly selected group, treated with powdered *Bifidobacterium* without showing any effect [87]. This might be considered as a sort of control, but, if true, these data cast doubts on other studies with unencapsulated lactic acid bacteria that were successful.

In an uncontrolled Japanese hard-endpoint study evaluating an enteric capsule preparation of *Bifidobacterium longum*, no effect was seen on progression of renal failure [89]. An RCT in end-stage CKD patients studying Renady[®] showed no differences in protein-bound uraemic toxin concentration nor in surrogate endpoints such as inflammatory and quality-of-life parameters [90].

Oxalate. A specific area of interest is the research on oxalate degrading bacteria, mostly in the context of oxalate urolithiasis and of hyperoxaluria, either because of genetic reasons or of medical predisposition (bowel disease). Of note, apart from being a reason for CKD in extreme forms of hyperoxaluria or because of kidney stones, oxalate is also a uraemic retention compound by itself.

A number of uncontrolled clinical trials in subjects without manifest renal failure with *Oxalobacter formigenes* or with lactic acid bacteria showed a decrease in oxalate plasma concentration and/or generation [91, 92], but another uncontrolled trial with a mixture of lactic acid bacteria gave contradictory results, with a decrease for the lower but not for the higher dose [93]. Another

uncontrolled study showed no effect at all [94], which was also the case for two RCTs [73, 95].

Synbiotics

Synbiotics contain a mixture of prebiotics and probiotics.

In an uncontrolled study in HD patients, a preparation combining *Lactobacillus casei*, *Bifidobacterium breve* and galacto-oligosaccharides decreased p-cresol but not indoxyl sulphate or phenol [96]. Patients with high p-cresol tended to have defaecation difficulties, which were normalized together with the decrease in p-cresol after 2 weeks of symbiotic treatment.

In a crossover RCT in healthy subjects, co-administration of *Lactobacillus casei* Shirota and OF-IN resulted in a reduction of urinary p-cresol excretion [78]. An RCT applying Probinul-neutro[®] in CKD 3-4 showed a decrease of plasma p-cresol versus baseline and placebo [97]. In placebo controls, however, plasma p-cresol spontaneously rose by >50% during a short follow-up of only 1 month [97], suggesting unidentified confounders forcing the results to a difference favouring synbiotics. A randomized open-label trial allotting CKD 3-4 patients to either protein restrictive diet alone or a combination of protein restriction, prebiotics and probiotics, showed a slower decline of eGFR in patients on the combined regimen [98]. A placebo-controlled RCT with crossover after 4-week washout has recently been developed to assess the effect on indoxyl sulphate and p-cresyl sulphate next to cardiovascular risk parameters and indices of renal damage, quality of life and gastrointestinal status [120].

Finally, in an RCT assessing next to diet and two prebiotic formulations, also the symbiotic AKSB (fructo-oligosaccharide, *Enterococcus faecium* and *Saccharomyces cerevisiae*) for their effect on oxalate levels could show no effect [73].

Absorption

The possibility that orally administered sorbents could remove uraemic solutes or their precursor by binding them in the intestine has long since been recognized, but not extensively explored. In fact, all phosphate binders are a classical example of formulations capturing in the intestine an actor associated with negative outcomes of uraemia.

One of the novel phosphate binders, sevelamer, absorbs also other compounds such as cholesterol [121] and antibiotics [122]. It has been hypothesized to absorb oxalate, but this could not be confirmed in an open-label non-randomized clinical trial [99]. Studies exploring the capacity of sevelamer to absorb protein-bound uraemic solutes or their precursors turned out negative, both in animals [123] and in an RCT in haemodialysis patients [100].

Probably the most successful results in the context of intestinal absorption have been obtained with the sorbent AST-120, which in a randomized trial dose-dependently decreased plasma indoxyl sulphate [101], while also other protein-bound compounds are likely to be removed by AST-120 [124]. Protein-bound compounds have been associated in both experimental [48] and clinical studies [53] to progression of CKD. Animal studies and retrospective clinical studies have suggested a nephroprotective effect for AST-120 [125, 126], which was confirmed in a number of small Japanese RCTs [102-104]. A subsequent large RCT in the USA and Europe could, however, not confirm those findings [105]. In a recent animal study, AST-120 protected against cardiorenal fibrosis subsequent to myocardial infarction [127]. In an RCT, AST-120 potentiated the activity of

erythropoiesis-stimulating agents (ESAs) in stage 5 CKD patients [106].

In an uncontrolled longitudinal follow-up study in haemodialysis patients, a decrease of indoxyl sulphate, phosphate and oxidative stress parameters versus baseline was seen with the sorbent chitosan [107].

Other interventions

Oral administration of sulfotransferase inhibitors to rats with acute renal failure suppressed indoxyl sulphate accumulation and had a positive effect on kidney function [128]. This study, however, raises the question of what happens to the mother compound p-cresol if it is not sulphated. Either it may exert its own toxicity [129], or it may be metabolized into the other main conjugate p-cresylglucuronide, which also has biological effects on its own [69]. Also meclofenamate strongly inhibited hepatic generation of indoxyl sulphate and had a nephroprotective effect [130]. In human volunteers, acarbose, an α -glucosidase inhibitor increasing colonic availability of carbohydrates, decreased urinary p-cresol excretion [108].

Summary of interventional studies

A dietary approach by restricting protein intake helps reducing the generation of uraemic toxins. Of note, protein restriction has been considered for a long time as nephroprotective [131], while at the same time reducing the generation of protein-bound uraemic toxins which are likely to play a role in progression of kidney failure. Nevertheless, in view of the possible link to malnutrition, such regimes should be tested in controlled studies, before they really can be considered of clinical value in uraemia. Keto-analogues may neutralize the negative impact of protein intake, but their effect on uraemic toxins has not yet been studied.

Prebiotics, probiotics and synbiotics could play a role in reducing the generation of uraemic toxins, but the results of clinical studies have been deceiving, with sometimes contradictory results [45, 73, 75, 79, 80, 84, 86, 95, 96, 118], e.g. if several uraemic toxins of similar origin were studied or if the same solute was assessed in several different studies. The two hard outcome studies that we retrieved assessing progression of kidney failure were negative at primary analysis [89, 90]. Of note, our literature search was not systematic, but a systematic review and meta-analysis on the topic a few years ago came to the same conclusions and classified the quality of the retrieved papers from moderate to very low [72, 89].

In view of the concern that probiotics might lose their effect if they do not survive the acidic milieu of the stomach, encapsulated formulations have been considered to offer new perspectives [16]. A few studies showed a decrease in uraemic toxin concentration but were non-randomized [85, 87, 88]. The only hard outcome study on encapsulated probiotics was not randomized either and, as mentioned earlier, the results were negative [89].

The studies in uraemia are scarce, and mostly not randomized, or if randomized, results are often inconsistent. The number of RCTs in healthy subjects is larger, but here only urinary excretion and no plasma levels were checked.

Consistent data have been reported on AST-120 as far as reduction of protein-bound solutes is concerned. A few small Japanese RCTs of variable quality suggested that this sorbent could refrain progression of CKD [102-104]. A large RCT could, however, not confirm these promising results [105].

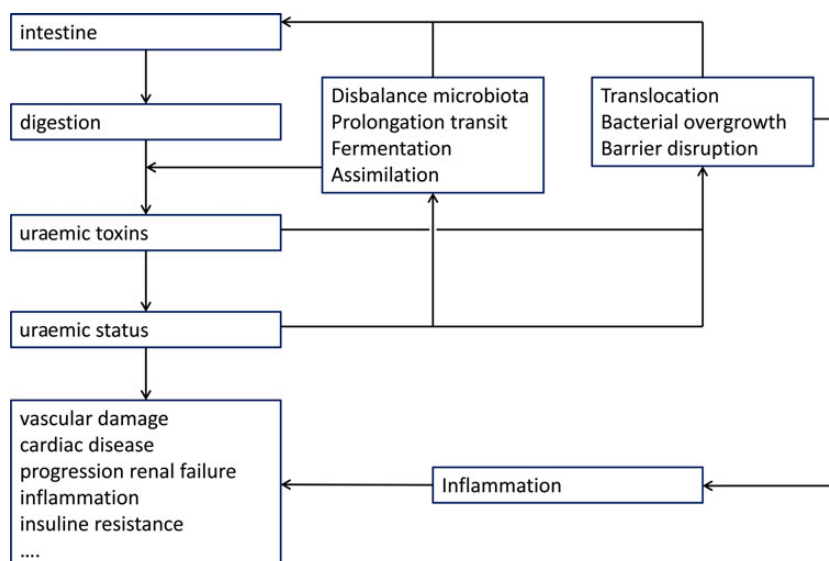


Fig. 2. The dual interference between the intestine and uraemia. The intestine is responsible for generating and absorbing uraemic toxins with a deleterious impact on body functions. Uraemia in its turn modifies intestinal functions with a further increase in uraemic toxin generation and pro-inflammatory modifications which again are deleterious to body functions.

In brief, what is needed in this area is well conceived, sufficiently powered, randomized placebo-controlled trials, performed in comparable groups (e.g. in regard to kidney function) and under standardized conditions of food intake, adherence and other influencing factors. If promising data are obtained with regard to toxin concentration, these should be followed by hard outcome studies.

Conclusions

A host of data that have been acquired lately pointing to a strong bidirectional link between renal failure and the intestine, whereby in the intestine, uraemic toxins are generated which cause inflammation and are deleterious to body functions, while uraemia affects intestinal functions and protective barriers, which in their turn lead to inflammation (Figure 2). Several recent studies deliver proof of concept of the crosstalk between the intestine and the uraemic condition. In spite of the availability of a number of therapeutic options to interfere with this process, results of clinical trials remain unconvincing, partly due to the design of the studies and partly to the lack of a hard outcome evidence base.

Future therapeutic clinical studies in this context should focus on a more rigid controlled setting, with hard outcomes and a decrease of target uraemic solutes as primary endpoints, including a control of compliance and if possible of dietary elements with potential influence. However, first we need more insight in the basic mechanisms and bacteriological underpinning of the gut–kidney crosstalk. Maybe single interventions are not efficient enough, and combination therapies should be considered.

Conflict of interest statement. None declared.

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