The quest for a better understanding of chronic kidney disease complications: an update on uremic toxins

Em busca de uma melhor compreensão da doença renal crônica: uma atualização em toxinas urêmicas

Abstract

Chronic kidney disease is characterized by a progressive reduction of glomerular filtration rate and/or the appearance of proteinuria, and subsequently the progressive retention of organic waste compounds called uremic toxins (UT). Over the last decades, a large number of such compounds have been identified and their effects on organs and tissues, especially the cardiovascular system, has been demonstrated. In this review, we present the current classification of UT, as proposed by the EUTOx Group, and the effects of some of the probably most important UTs, such as phosphate, FGF-23, PTH, AGEs, indoxyl sulfate and para-cresyl sulfate. We provide an overview on therapeutic approaches aimed to increase their extracorporeal removal via convective and/or adsorptive strategies and to lower their intestinal production/absorption via dietetic and pharmacological interventions. The recognition that multiple toxins contribute to the uremia supports the need for new therapeutic targets, with a potentially positive impact on CKD progression and survival.

Keywords: cardiovascular diseases; dialysis; kidney failure, chronic; uremia.

Resumo

A doença renal crônica (DRC) caracteriza-se pela redução progressiva da filtração glomerular e/ou presença de proteinúria, e subsequente retenção progressiva de compostos orgânicos, denominados toxinas urêmicas. Nas últimas décadas, um grande número destes compostos foi identificado, assim como seus efeitos adversos no organismo, sobretudo no sistema cardiovascular. Nesta revisão, apresentamos a classificação das toxinas urêmicas, proposta pelo grupo europeu de estudo em toxinas urêmicas (EUTOx), e discutiremos os efeitos de algumas das principais toxinas, como ADMA, fosfato, FGF-23, PTH, AGEs, indoxil sulfato e para-cresil sulfato. Além disso, abordaremos as principais estratégias terapêuticas para aumentar a remoção das toxinas urêmicas por métodos convectivos e/ou adsorptivos; e para diminuir a produção e absorção intestinal dessas toxinas por meio de intervenções dietéticas e farmacológicas, respetivamente. A compreensão de que múltiplas toxinas contribuem para a uremia expõe a necessidade de novos alvos-terapêuticos, com potencial impacto positivo na progressão da DRC e na sobrevida dos pacientes.

Palavras-chave: diabetes; doenças cardiovasculares; falência renal crônica; uremia.

Introduction

In the last two decades, renewed interest has emerged about the uremic syndrome and its negative impact on chronic kidney disease (CKD) patient outcomes. The syndrome is primarily caused by the progressive decline in kidney function that leads to an accumulation of organic waste products.¹ These waste products, not all identified as yet, are called “uremic toxins” or “uremic retention solutes”. Under normal conditions, they are excreted by the kidneys. Thus, their concentrations increase progressively with the progression of CKD, interacting negatively with various biological functions. The clinical spectrum of this pathophysiological phenomenon is generally known as uremia (literally, “urine in the blood”) and at present more frequently designed as the uremic state.²,³

The first publication in this field is from 1877,⁴ and little was known regarding the nature of uremic toxins before the

¹ Pontifical Catholic University of Paraná.
² Federal University of Paraná.
³ University of São Paulo.
⁴ Université de Picardie Jules Verne.
⁵ Nove de Julho University, Uninove.
⁶ Faculty of Medical Sciences, State University of Campinas (UNICAMP).

Data de submissão: 18/02/2014.
Data de aprovação: 07/03/2014.

Correspondência para:
Fellype Carvalho Barreto.
School of Medicine, Pontificia Universidade Católica do Paraná.
Rua Imaculada Conceição, nº 1155, Curitiba, PR, Brazil.
CEP: 80215-901.
E-mail: fellype_barreto@hotmail.com
Tel: (41) 271-1657.
DOI: 10.5935/0101-2800.20140033
introduction of hemodialysis (HD) into clinical practice in the 1960s. The concept of uremic toxins has thus been developed at that time, and their identification and biological activities were actively investigated subsequently by a small number of research groups (Man and Funck-Brentano in France, Bergström in Sweden, etc). These research activities led to the “middle molecule” hypothesis. However, studies in this field ceased subsequently because no convincing demonstration of their role in the uremic syndrome could be made at that time. The topic was resuscitated later on, in the early 1990ies, by the nephrology group in Gent, Belgium (Ringoir, Vanholder). They were able to provide new, scientifically valid evidence in favor of the biological activity of several uremic toxins. Finally, in 1999 Vanholder’s group, together with other researchers in Europe, launched the European Uremic Toxins (EUTox) Work Group, which contributed substantially to the acceptance of the concept and the demonstration of the role of uremic toxins. Subsequently, the idea disseminated worldwide, as reflected by a remarkable increase in published reports devoted to this field.

According to EUTox, a compound can be considered as a uremic toxin if it fits a postulate, similar to Koch’s postulate, as modified by Massry et al. in 1977 (Table 1). At present, there are 152 solutes listed in the EUTox database (http://eutoxdb.odeesoft.com/index.php) and certainly an increase in this number in the following years can be expected.

Table 1

<table>
<thead>
<tr>
<th>REQUIREMENTS FOR A GIVEN COMPOUND TO BE CONSIDERED AS A UREMIC TOXIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemically identified and accurately measured;</td>
</tr>
<tr>
<td>Total body/plasma levels should be higher than in non-uremic</td>
</tr>
<tr>
<td>subjects;</td>
</tr>
<tr>
<td>High concentrations should be related with specific</td>
</tr>
<tr>
<td>dysfunctions/symptoms, which disappear when concentrations</td>
</tr>
<tr>
<td>are reduced;</td>
</tr>
<tr>
<td>Biological activity of this compound should be proven in</td>
</tr>
<tr>
<td>ex vivo, in vivo or in vitro studies, and finally,</td>
</tr>
<tr>
<td>experimental concentrations of this molecule in those studies</td>
</tr>
<tr>
<td>should match those found in body fluids or tissue from</td>
</tr>
<tr>
<td>uremic patients.</td>
</tr>
</tbody>
</table>

The proper identification of new compounds that fulfill the criteria of uremic toxins has at least three main implications: firstly, to be able to explore the unknown pathophysiological mechanisms of the uremic syndrome; secondly, to use a specific uremic toxin as a biomarker of a specific pathophysiological process in CKD and thirdly, to explore clinical interventions directed to modulate the total body/plasma levels of uremic toxins.

Uremic toxins can be classified according to their physico-chemical characteristics and removal by dialysis into the following 3 classes:

I. Small water-soluble compounds with a maximum molecular weight (MW) of 500 Daltons (Da). The main molecules in this group include urea, creatinine and guanidines, which are easily removed by dialysis. They do not necessarily have toxic effects.

II. Middle molecules: Compounds of moderately elevated MW (> 500 Da), with β2-microglobulin and leptin as prototypes. They can only be removed by dialysis membranes with pores large enough to allow their passage. Many of these compounds are peptides. They affect a large number of organs and systems.

III. Protein-bound compounds: They are generally of low MW. The prototypes of this group are phenols and indoles. They exert a variety of toxic effects and are difficult to remove by dialysis.

A large number of uremic toxins have deleterious effects on various organs and tissues in the body, mainly the cardiovascular system (Figure 1). The pathophysiological mechanisms involved are complex and far from being completely understood. They include reactive oxidative stress, inflammation, protein glycation and cellular transdifferentiation. In this paper, we present the main toxin prototype groups, based on physico-chemical characteristics, clinical effects, and current or potential interventions aimed to modulate their concentration through extracorporeal removal methods and/or pharmacological approaches capable of opposing their toxic effects.

SMALL WATER-SOLUBLE COMPOUNDS

GUANIDINES

An important group of small water-soluble compounds consists of guanidine compounds, metabolites of L-arginine that have long been known for their neurotoxic effects. There are three types of methylated arginine residues: monomethyl arginine (MMA), symmetric dimethylarginine (SDMA) and asymmetric dimethylarginine (ADMA), the latter being the most abundant one. Several studies have shown an increase in serum ADMA levels in CKD, which may be particularly relevant to the presence of...
nephrotic proteinuria, although its correlation with glomerular filtration rate (GFR) has been found to be poor.\textsuperscript{9,10} Initially, it was assumed that this increase was the result of lower renal clearance. Later studies, however, suggested that the increase was due to both augmented synthesis and reduced catabolism of these compounds.\textsuperscript{11} Of note, even though guanidines and urea are structurally similar, the distribution volume of the former is significantly greater, which results in a decrease in removal efficiency by dialysis procedures.\textsuperscript{12}

ADMA has long been associated with endothelial dysfunction. In 1992, Vallence \textit{et al.}\textsuperscript{13} identified MMA and ADMA as endogenous inhibitors of nitric oxide synthase (eNOS). However, the intracellular concentration of MMA is very small, whereas ADMA is the most abundant eNOS inhibitor. ADMA has been further linked to cardiovascular disease (CVD) and mortality in both the general and CKD population ever since.\textsuperscript{14,15}

SDMA, the structural counterpart of ADMA, was considered inert until recently. However, it is currently recognized as a pro-inflammatory and a pro-oxidant agent.\textsuperscript{16,17} Unlike ADMA, SDMA was demonstrated to be capable of increasing TNF-\(\alpha\) and interleukine-6 (IL-6) expression, and enhancing NFkB activation, in human monocytic cell line THP-1. In line with this \textit{in vitro} data, SDMA has been associated with inflammatory markers, including TNF-\(\alpha\) and IL-6, in pre-dialysis patients at different stages of CKD.\textsuperscript{17}

\textbf{Uric Acid}

Uric acid (MW: 168 Da) is considered a uremic toxin since a considerable body of evidence suggests that supraphysiologic uric acid concentrations have deleterious effects on the kidney and on the cardiovascular system. In CKD, factors like reduced GFR, increased renal vascular resistance and co-existent insulin resistance as well as the use of diuretics can lead to hyperuricemia, defined as the accumulation of serum uric acid beyond its solubility point in water (6.8 mg/dL).\textsuperscript{18} Other factors such as high purine/protein diet, alcohol consumption and high cell turnover can also contribute to increase its serum concentration.

Classically, hyperuricemia could lead to hyperuricosuria and deposition of uric acid crystals in the kidney, increasing the risk of nephrolithiasis, renal inflammation and decrease of GFR by intraluminal obstruction and increased concentration of urate anion in renal tissue.\textsuperscript{19,20} On the other hand, non-crystal dependent effects of uric acid have been proposed, but this remains a matter of debate. Though urate anion, the predominant form of uric acid under physiologic pH, has long been considered a powerful antioxidant at physiologic concentrations, experimental data have shown that at higher concentrations this anion can lead to endothelial dysfunction, increased systemic cytokine production, activation of the renin-angiotensin-aldosterone system and last, not least, glomerular lesions.\textsuperscript{21}

In the clinical setting, observational studies have reported conflicting results. In the Cardiovascular Health Study, no association was observed between serum uric acid level and the incidence of CKD.\textsuperscript{22} Similarly, in the patient cohort of the MDRD Study, uric acid was not found to be an independent risk factor for CKD progression.\textsuperscript{23} In contrast, Hsu \textit{et al.} evaluated a cohort of 177,570 participants followed over 25-years and found that increased serum uric acid levels were independently associated with an increased risk of end-stage kidney disease (ESKD).\textsuperscript{24} A hypothesis to explain these apparent inconsistencies is that uric acid clearance is impaired in CKD, which in turn can act as a confounding factor.\textsuperscript{21} Taking into account interventional studies aimed to decrease serum uric acid levels in CKD, the results, although encouraging,\textsuperscript{25} are not strong enough to systematically recommend allopurinol as a therapy for halting or decreasing CKD progression.
INORGANIC PHOSPHATE (Pi)

The regulatory mechanisms responsible for Pi homeostasis, such as 1,25(OH)₂-vitamin D, parathyroid hormone (PTH) and fibroblast growth factor-23 (FGF23), together with Klotho, are disrupted in the CKD setting. Pi overload may occur early on in the course of CKD, while hyperphosphatemia appears only when GFR falls below 30 ml/min/1.73 m² of body surface. When CKD progresses and dialysis treatment is required, the positive balance of Pi becomes more pronounced due to (i) loss of kidney function and (ii) insufficient removal by conventional hemodialysis (HD) or peritoneal dialysis (PD) (Table 2). A detailed discussion of Pi homeostasis and hyperphosphatemia treatment can be found elsewhere.

TABLE 2

<table>
<thead>
<tr>
<th>Dialytic methods</th>
<th>Duration/frequency</th>
<th>Phosphate removal (mg/wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional HD*</td>
<td>4h; 3x/wk</td>
<td>2.356 ± 864</td>
</tr>
<tr>
<td>Short daily HD*</td>
<td>2-3h; 6x/wk</td>
<td>2.452 ± 720</td>
</tr>
<tr>
<td>Nocturnal HD*</td>
<td>6-8h; 6x/wk</td>
<td>8.000 ± 2.800</td>
</tr>
<tr>
<td>Hemodiafiltration</td>
<td>4h; 3x/wk</td>
<td>3.570 ± 270</td>
</tr>
</tbody>
</table>

Peritoneal dialysis

<table>
<thead>
<tr>
<th></th>
<th>Estimated weekly removal of phosphate by dialytic methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>APD</td>
<td>2.739 ± 1.042</td>
</tr>
<tr>
<td>CAPD</td>
<td>2.790 ± 1.022</td>
</tr>
</tbody>
</table>


Hyperphosphatemia has been classically linked to the pathogenesis of secondary hyperparathyroidism (sHPT). Furthermore, since the end of the 1990’s, the role of Pi in uremia-related cardiovascular complications and mortality has gained growing interest. Several observational studies showed an association of hyperphosphatemia with overall and cardiovascular mortality, both in dialysis and pre-dialysis patients. Vascular calcification (VC) has been proposed as one of the possible mechanisms linking hyperphosphatemia to mortality. In vitro studies have shown that Pi enters vascular smooth muscle cells (VSMCs) through sodium-phosphate co-transporter type III (PiT-1), activate Cbfa-1/Runx-2 genes and thereby promote VSMC transdifferentiation into osteoblast/osteochondrocyte-like cells. Even though animal studies have demonstrated very clearly that lowering serum Pi can decrease the progression of VC, this remains yet to be confirmed by appropriate clinical trials in patients with CKD.

More recently, the role of Pi as a potential cardiovascular toxin has received further support. Several observational studies have suggested that high Pi levels, even within the normal range, may be associated with an increased risk of incident CVD, left ventricular hypertrophy (LVH) and increased risk of heart failure, coronary disease, and higher mortality in the general population.

Experimental studies have shed some light on the mechanisms by which Pi acts as a cardiovascular toxin, above and beyond the promotion of VC. Apolipoprotein E knockout mice fed an atherogenic diet with high (1.6%) Pi content had significantly more atheroma at the site of the aortic sinus than mice fed a low (0.2%) or standard (0.6%) Pi diet, while lipid profile and blood pressure were not affected. Furthermore, it has been demonstrated in two different mouse models of CKD that lowering serum Pi levels with sevelamer, a phosphate-binding agent, improved VC and atherosclerotic lesions, aortic stiffness, diastolic dysfunction, by inducing apoptosis, impaired acetylcholine-induced vasodilation, inhibition of nitric oxide production and downregulation of annexin II. Interestingly, lowering serum Pi levels (at least in animal models of CKD) by either dietary Pi restriction or sevelamer administration ameliorates endothelial dysfunction.

A major limitation in the interpretation of Pi effects in vivo is the difficulty to know whether they are the result of a direct action of Pi or rather due to indirect mechanisms, e.g. via an increase in serum PTH levels. In two studies using a rat model of CKD, in which the animals underwent parathyroidectomy and continuous controlled infusion of PTH, the authors were able to demonstrate a direct Pi effect on the heart, inducing myocardial hypertrophy, cardiomyocyte hyperplasia and interstitial fibrosis, and vessels in the absence of changes in serum PTH concentration. Recent clinical evidence attributing Pi an important role as a cardiovascular toxin has received further support. Several observational studies have suggested that high Pi levels, even within the normal range, may be associated with an increased risk of incident CVD, left ventricular hypertrophy (LVH) and increased risk of heart failure, coronary disease, and higher mortality in the general population.
Pi overload. Therefore, given the increased CVD and mortality risk associated with hyperphosphatemia, the proposition has been made to label food products with high Pi content in the interest of public health, similarly to what is currently recommended for food NaCl content.

**Middle molecules**

**Fibroblast growth factor-23**

FGF23 was described more than 10 years ago by two independent groups. This hormone, secreted by osteocytes and osteoblasts, is a protein constituted by 251-amino-acids (MW: 30,000 Da). Its major function is the control of mineral metabolism, particularly serum Pi and calcitriol levels, through inhibition of renal tubular Pi reabsorption and 1α-hydroxylase activity. FGF23 also acts on the parathyroid gland inhibiting PTH secretion. All these actions are mediated by FGF23 binding to FGFR receptors (FGFR) and its co-receptor Klotho, which greatly increases the binding affinity of FGF23 for FGFR.

The serum FGF23 level increases early in the course of CKD, probably due to a state of primary FGF23 excess, resulting from a combination of increased production and decreased degradation. As GFR declines, FGF23 increases and can reach levels > 1,000-fold above normal in patients with CKD stage 5D. Furthermore, residual clearance by the kidney and removal by dialysis do not appear to modify serum FGF23 to a significant extent.

Numerous reports showed a positive association between elevated FGF23 levels and adverse clinical outcomes, such as CKD progression, LVH, VC, and mortality in pre-dialysis and dialysis CKD populations. Considering the above effects of FGF23 on biological functions, one might expect to see evidence in support of a causal relationship in this respect. However, available experimental findings do not allow a clear conclusion. For example, complete neutralization of FGF23 action by a specific antibody has been shown to be harmful since it aggravated hyperphosphatemia, VC, and mortality in a rat model of CKD and mineral bone disorder (MBD). This observation suggests that FGF23, like PTH, exerts beneficial effects in early stages of CKD but that its excessive rise with the progression of CKD to ESKD is a maladaptation syndrome with highly deleterious consequences.

Regarding LVH, convincing experimental evidence has recently been provided by Faul et al. in favor of a direct noxious effect of FGF23. The authors showed that the intramyocardial or intravenous injection of FGF23 in wild-type mice induced LVH, independent of Klotho which is not expressed in the myocardium. In addition, the authors reported that elevated FGF23 levels were independently associated with LVH in a large, racially diverse CKD cohort, in line with findings by others in the general population. However, not all authors have been able to find an association of high FGF23 levels with vascular calcification or mortality. Apparent inconsistencies can be explained by small sample size, different approaches to adjust for confounding factors, imaging of different arterial beds, lack of prospective data, and finally, different FGF23 mechanisms of toxicity.

Therefore, it is not yet clear if FGF23 is only a surrogate marker of CKD-MBD, CKD progression and cardiovascular disease or whether it is also an active player and hence a potential therapeutic target. For a more detailed review of FGF23 the reader should refer to recent comprehensive reviews.

**Leptin**

The hormone leptin is a 167-amino-acid secreted protein (MW: 16,000 Da), expressed mainly in adipose tissue. It was originally proposed to be an antiobesity factor. However, the majority of obese healthy individuals have elevated serum leptin levels. Therefore, the hypothesis of leptin resistance in human obesity emerged. The classical actions of leptin include the control of feeding behavior, energy balance, fertility and immune function. Recently, other actions of leptin have been described, such as its influence on bone mass and the cardiovascular system. At the cellular level, leptin stimulates alkaline phosphatase activity, as well as the proliferation, migration and calcification of VSMCs.

Although clinical studies in the general population have shown a link between leptin and CVD, leptin’s role in CKD seems to be more complex. A group of authors have suggested that dialysis patients with high, rather than low, serum leptin levels have better outcomes. However, there was no association between serum leptin and body mass index (BMI). Clearly, the relation between serum leptin and BMI is complex in ESKD, and other group did not find any survival advantage in obese HD patients as compared to lean HD patients.

Apart from the unsolved issue of the role of leptin in clinical outcomes of CKD patients, leptin appears to be related to the metabolic syndrome. In a clinical study of 142 patients with CKD stages 2-5D, followed for a minimum of 20 months, plasma leptin was an
independent predictor of metabolic syndrome but not of clinical outcomes. Interestingly, PTH was an independent predictor of plasma leptin levels. Taking into account these two findings, one could speculate that leptin is related to factors that exert a more close impact on the clinical outcomes of CKD patients, such as mineral disturbances and metabolic factors. This hypothesis remains to be proven in future clinical long-term studies.

**Parathyroid Hormone**

Secondary hyperparathyroidism, characterized by an increase in PTH (MW: 9.4 kDa) synthesis and secretion and by parathyroid gland hyperplasia, is a common complication of CKD. Elevated PTH levels are generally found since the early stages of CKD (GFR > 60 ml/min/1.73 m²), in any case much sooner than hyperphosphatemia. As one of the most important regulators of bone metabolism, PTH in excess induces significant changes in bone structure and function, leading to the development of high turnover bone disease which, in association with CKD, is called *osteitis fibrosa*. It is characterized by increased bone fragility, which may explain, at least in part, the association between sHPT and increased fracture risk. In addition, sHPT may cause bone marrow fibrosis and impair erythropoiesis. It has been suggested that very high PTH levels contribute to the polyneuropathy, glucose intolerance, dyslipidemia and inflammation of the uremic state.

It is important to recognize that toxic PTH levels may lead to deleterious effects on many other organs and tissues due to the ubiquitous expression of its main receptor, PTH1R, including the cardiovascular system. Observational studies have found associations between high PTH levels and CVD in the setting of CKD, such as VC, disturbed left ventricular function and mortality. Observational studies have pointed toward an association between PTH and cardiovascular mortality in the general population as well. CKD patients with severe sHPT often develop severe VC, which are particularly located in the arterial media such as the ones observed in digital arteries of the hands and the feet, and which can entirely regress after surgical parathyroidectomy.

The effect of PTH on the CV system is an issue under continuous investigation. Experimental studies using a rat model of CKD (5/6 nephrectomy) in which the animals underwent parathyroidectomy, the continuous infusion of supraphysiological rates of synthetic PTH was associated with the development of extensive VC - independently of serum Pi levels or the presence of uremia. In another study using the same CKD rat model, higher PTH levels were associated with myocardial hypertrophy and fibrosis along with higher myocardial expression of inflammation markers and oxidative stress. Furthermore, studies have reported the interplay between PTH on the one hand and aldosterone and norepinephrine release on the other, suggesting additional pathophysiologic pathways by which sHPT may lead to cardiovascular damage.

Finally, there is increasing evidence that circulating fragments of PTH, which are elevated in the uremic state, are hormonally active, exerting partially opposing effects to those of intact PTH. Thus, it has been suggested that 7-84 PTH fragments may be involved in the skeletal resistance to PTH observed in CKD.

There is an intricate relationship between PTH and other factors involved in the disturbances of mineral metabolism in CKD, which may fog the importance of PTH per se as a risk factor for uremia-related complications. The inconclusive, non-definitive results of the recent EVOLVE trial have further contributed to this uncertainty, since in intent-to-treat analysis there was no significant advantage of cinacalcet treatment over best presently available standard treatment in the combined primary endpoint (cardiovascular events plus death) despite a marked decrease in serum PTH. However, when performing prespecified corrections of major confounders such as age and study drug discontinuation the better control of hyperparathyroidism was associated with a nominally significant superiority in hard outcomes. Of note, numerous previous clinical and experimental studies support the hypothesis that PTH acts as a systemic uremic toxin, with direct and indirect effects on a variety of tissues and organs, and severe sHPT is a major threat to CKD patient outcomes. Therefore, PTH remains an important therapeutic target to avoid bone and cardiovascular complications in such patients.

**Advanced Glycation End Products (AGEs) and Advanced Oxidized Protein Products (AOPPs)**

AGEs are a heterogeneous group of molecules formed by non-enzymatic glycosylation reactions with sugars, lipids and nucleic acids. Humans are exposed to two
main sources of AGEs. The exogenous forms come from diet whereas the endogenous ones are formed in the body. AGE transformation of nutrients occurs when foods are processed at high temperature. In contrast, endogenous transformation results from exposure to high glucose levels such as in diabetic patients, from aging, and from uremia.91,92 AGEs result in a first step from non-enzymatic glycation of proteins by aldehyde and ketones to form a Schiff base. After rearrangement of the these structures, the intermediary products are formed (Amadori products), and finally, AGEs in a second step.93 There are more than 20 AGE compounds, including 1,2-dicarbonyl precursor compounds glyoxal, methylglyoxal, and the end products, N-carboxymethyl-lysine, pentosidine, and hydroimidazolone as the best characterized compounds, which serve as markers of AGE accumulation in a range of tissues.94 AGEs main pathway of elimination from the body is through the urine, or through dialysis in case of renal function replacement.95 Patients under PD are more susceptible than HD patients to systemic and local formation of AGEs, not only as a result of the uremic state but also of the constant exposure of the peritoneum to high levels of glucose and glucose degradation products generated during sterilization of dialysis fluid by heat.95 Moreover, AGEs accumulate progressively in the peritoneal mesothelial layer with increasing dialysate dwell time.96 Another cause of increased AGE formation in the uremic state is oxidative stress, generated by an imbalance between pro-oxidant forces (such as an increase in the ratio of oxidized glutathione to reduced glutathione) and anti-oxidant defense system (such as reduced superoxide dismutase/ peroxidase activity).

AGEs exert several potentially deleterious effects in the body. In the cardiovascular system, their accumulation contributes to myocardial changes, endothelial dysfunction, arterial stiffness, and atherosclerotic plaque formation. When these molecules bind to collagen and elastin, they accumulate in the matrix of blood vessels in a non functional and disorderly way, changing endothelial vasomotor tone modulation, platelet adhesion, and cell proliferation.97 AGEs exert their actions in the vascular system by binding to a specific receptor, called RAGE. Activation of RAGE induces an inflammatory response, leading to the effects described above and also increasing the production of adhesion molecules, increasing proliferation of the vessel intimal layer, angiogenesis and oxidative stress.98 RAGE is expressed in all cells involved in atherogenesis, including monocytes, macrophages, endothelial cells and VSMC. Interestingly, these cells do not express significant amounts of RAGE under physiological conditions, but can be induced to express RAGE more vigorously in conditions where their ligands and/or transcription factors accumulate, like in uremia.99

Advanced oxidation protein products (AOPPs) are a class of dityrosine-containing protein products, which arise from the reaction between chlorinated oxidants and plasma proteins. Increased levels of AOPPs are detected in uremic serum since predialysis stage. AOPPs have been suggested to be not only reliable markers of oxidative stress but also mediators of inflammation, by triggering monocyte activation.100 They have been associated with podocyte injury,101 incident thrombo-occlusive atherosclerotic CV events in the pre-dialysis setting102 and carotid atherosclerosis in ESKD patients.103 These findings support the hypothesis that AOPPs should be considered as uremic toxins.

**Protein-bound uremic toxins**

**Indoxyl sulfate**

Indoxyl sulfate (IS) is a low-MW (213.21 Da) uremic toxin derivated from dietary protein. It is an indol derived from the amino acid tryptophan by action of intestinal bacteria.104 It is normally excreted into the urine, with its mean urinary excretion rate ranging from 50-70 mg/day in healthy persons. In CKD patients, as renal function declines it accumulates in serum due to its reduced renal clearance.105 The main part of IS in the blood of CKD patients is bound to serum albumin, which means that its urinary excretion occurs mainly by tubular secretion, mainly by proximal tubular cells, and secondarily by glomerular filtration. Organic anion transporter (OAT) 1, the prototypic para-amino hippurate transporter, is localized in renal tubular cells and interacts with a wide range of small endogenous substances, including IS. IS is taken up from the blood by OAT1 and OAT3 at the basolateral membrane of tubular cells and accumulates in the cells at high concentrations.106 Several studies have shown an impact of IS accumulation in CKD patients. Increased IS concentrations compete for protein binding or excretion with other molecules. IS probably mediates its toxicity by direct induction of a host of genes involved in inflammation and fibrosis.107 IS plays a key role...
in endothelial damage and triggers the production of pro-inflammatory molecules, inhibition of endothelial regeneration and repair, and endothelial free radical production.\textsuperscript{108} In addition, the participation of IS in VC, endothelial microparticle release, disruption of adherent junctions of endothelial cells, proliferation of VSMCs, renal and cardiac fibrosis, and impairment of osteocalc differentiation and function has been reported as well.\textsuperscript{109-112} It has been proposed that IS may affect the remnant nephrons, especially proximal tubular cells, and stimulate tubulointerstitial fibrosis, glomerular sclerosis, and the progression of CKD by increasing the gene expression of TGF-β1, TIMP-1, and pro-α1collagen, leading to a further loss of nephrons, completing the vicious circle of progressive renal injury.\textsuperscript{104} Several clinical studies have shown that high IS levels are associated with high IL-6 levels, coronary artery disease, vascular damage, progression of CKD and mortality (Figure 2).\textsuperscript{112-116}

Figure 2. Kaplan-Meier estimates of several survival probability for CKD patients as a function of tertiles of serum indoxyl sulfate levels. Footnote: reprinted with permission from Barreto FC (ref.115).

\textbf{Para-cresol (p-cresol) and p-cresyl sulfate (PCS)}

P-cresol (4-methylphenol) is another uremic toxin (MW: 108 Da) linked to serum proteins, with various deleterious effects. This molecule originates from tyrosine and phenylalanine metabolism by bacterial microbiota fermentation in the large intestine. During its passage through the colonic mucosa and the liver, it is metabolized by conjugation processes (sulfation and glucuronidation) forming two compounds, PCS and p-cresylglucoronidate. In patients with CKD it is possible to find these two derivatives of p-cresol, both in conjugated and unconjugated form.\textsuperscript{117} The earliest studies with phenol compounds involved p-cresol; however, it was discovered subsequently that this compound is found in only tiny concentrations in the body since it is rapidly metabolized to its conjugates by the intestinal microbiota. Of note, it has been shown that the biochemical impact of p-cresol is not necessarily the same as the impact of its conjugates.\textsuperscript{118} Previous studies have demonstrated that these uremic toxins are first taken up by the kidneys, blood vessels, bones, and across the blood-brain barrier via OATs and then induce the production of oxygen free radicals and inflammatory cytokines in the respective organs.\textsuperscript{119-122} p-cresol affects the inflammatory response, interfering with the activation of polymorphonuclear leukocytes and endothelial response to cytokines.\textsuperscript{123} However, neither in uremic patients nor in normal people this molecule can be detected in the circulation in its unconjugated form, in contrast to PCS.\textsuperscript{124} Meijers et al. demonstrated that PCS induces the release of endothelial microparticles, even in the absence of endothelial injury, suggesting that this toxin is involved in endothelial dysfunction.\textsuperscript{125} Furthermore, Schepers et al. observed a pro-inflammatory effect of PCS, measured by increased formation of free radicals produced by leukocytes, contributing to vascular damage in patients with CKD.\textsuperscript{126} Importantly, high PCS levels have been associated with mortality in CKD.\textsuperscript{124} In addition, Koppe et al. observed that normal mice treated with PCS for 4 weeks, developed insulin resistance, loss of fat mass, and ectopic redistribution of lipid in muscle and liver, mimicking features associated with CKD.\textsuperscript{127}

\textbf{Therapeutic strategies}

As discussed above, the uremic syndrome is a complex condition resulting from the effects of wide variety of toxins. Current strategies aimed to decrease their serum concentration are dietary and pharmacological interventions, mainly via modulation of intestinal absorption capacity through binding effects and/or reduction of ingested amounts of the toxins or their precursors, and extra-corporeal removal via PD and HD. Notably, removal of middle and protein-bound uremic toxins by conventional HD and PD is insufficient. The main therapeutic strategies aimed to reduce the level of these compounds and possible advantages are briefly discussed below.

\textbf{Dietary intervention}

Protein-bound uremic toxins are generally produced from the metabolism of amino acids in the intestine. Thus, low-protein diet is often considered as a possible
dietary approach to reduce the serum concentration of these toxins. Animal experiments have shown that rats fed a low-protein diet had lower concentration of protein-bound solutes. Recently, it was demonstrated that a reduced protein and Pi intakes associated with a very low protein diet, supplemented with ketoanalogues and essential amino acids, significantly lowered IS in CKD patients. However, nephrologists should be aware of the fact that reducing protein intake excessively may deteriorate the nutritional status of CKD patients and that continuous nutritional evaluations by an expert dietitian are required. Another interesting issue that needs further investigation is the influence of vegetarian diets on the production of uremic toxins. The urinary excretion of PCS and IS was 62% and 59% lower, respectively, in vegetarians than in participants consuming an unrestricted diet, which was associated with higher fiber intake and lower protein intake. Finally, the use of pre and probiotics has been suggested as interesting approach to reduce IS and PCS serum levels as well.

**Pharmacological Intervention**

The rationale for using oral sorbents to achieve a reduction of protein-bound toxins in the circulation is that they are derived from the intestinal metabolism of amino acids. Clinical studies have demonstrated that the oral sorbent AST-120 (Kremezin®) is associated with lower levels of IS, slower progression of CKD and improved survival after dialysis was started. Experimental studies had also shown potentially useful therapeutic effects of AST-120 in preventing IS-induced pathological alterations, such as VC. Results of two EPPIC trials, that evaluated the effectiveness of AST-120 added to standard-of-care therapy in moderate to severe CKD, did not support efficacy of this drug in slowing CKD progression. However, a subgroup analysis indicated a trend for AST-120 associated reduction of CKD progression in compliant patients with rapid decline of renal function. Further trials are required to confirm the reality of this trend.

The use of specific types of phosphate binders has also been proposed as a possible means to decrease the concentrations of several uremic toxins, apart from their main therapeutic utilization in the control of hyperphosphatemia. Recently, two small clinical studies pointed to the possibility of achieving a pharmacologic modulation of leptin and FGF23 levels in a cohort of early stage CKD patients via the use of sevelamer. Moreover, Vlassara et al. demonstrated in patients with diabetes and early CKD that sevelamer reduced markers of inflammation and oxidative stress, independently of changes in Pi, possibly due to sevelamer’s capacity to bind AGEs in the intestinal lumen. In a recent observational cross-sectional study, in PD patients, sevelamer use was associated with lower levels of p-cresol as well. In a pilot study in healthy volunteers treatment with acarbose, an alpha-glucosidase inhibitor, decreased the generation and serum concentrations of the protein bound uremic solute p-cresol. Whether these actions may lead to an improvement of specific uremia-related complications and overall outcome of patients with CKD has yet to be shown.

**Hemodialysis with High-Flux Membranes**

Several randomized controlled trials (RCT) aimed to demonstrate that an increase in the clearance of higher weight molecules via the use of high-flux dialysis membranes led to a survival benefit as compared to standard low-flux membranes. The first large-scale RCT was the HEMO study. It failed to find a difference in mortality between the two groups. It also was unable to demonstrate a benefit of high dialysis dose as compared to standard dialysis dose. Post hoc secondary analyses of the HEMO study pointed to a benefit of using high-flux membranes in terms of cardiac outcomes, and decreased cerebro-vascular disease mortality in patient subcategories, possibly as the result of better middle molecule removal due to the larger pore size of this type of membrane. It is worth mentioning that high-flux HD has no effect on protein-bound uremic toxin levels. Similarly to the HEMO trial, the subsequently done MPO trial also failed to demonstrate a survival advantage with the use of high-flux as compared to low-flux dialysis membranes. It must be pointed out that positive results obtained by secondary analyses may strengthen, but do not prove, the hypothesis that high-flux treatment may improve cardiovascular events and survival in certain subpopulations of HD patients.

**Convective Strategies (Hemofiltration or Hemodiafiltration)**

It has been demonstrated that convective strategies improve the removal of middle molecules and
protein-bound uremic toxins. By comparing three main convective strategies in parallel, named pre-dilution hemodiafiltration (HDF), post-dilution HDF and pre-hemofiltration (HF), Meert et al. reported that under similar convective volumes, post- and pre-dilution HDF had similar effects on protein-bound molecules removal, though the former appeared to be better for small water soluble compounds and β2-microglobulin. Pre-HF was superior to pre-HDF only for β2-microglobulin removal and not at all superior to post-HDF. In summary, post-HDF was found to be the most effective convective strategy for solute removal. Mid-dilution HDF, a new strategy that allows simultaneous infusion in a pre- and post-dilution fashion, appears to be as efficient as post-dilution HDF for small water-soluble and protein-bound solutes removal.

A beneficial impact of convective therapies on hard clinical outcomes is however far from being firmly established. Although benefit has been suggested by observational studies, the results obtained in recent RCTs are less clear. Thus, Grooteman et al. found in a recent multicenter RCT that dialysis patients assigned to high-efficiency post-dilution on-line HDF had less cardiovascular events but there was no difference between the two groups in the primary endpoint, namely all-cause mortality. Ok et al. set up an RCT comparing the effect of on-line HDF to that of high-flux HD. The primary outcome (composite of death from any cause and nonfatal cardiovascular events) was not different between the two groups. However, in adjusted Cox-regression analysis treatment with high-efficiency on-line HD was associated with a 46% risk reduction for overall mortality. In a very recent RCT, Asci et al. failed to find an advantage in fatal or non-fatal cardiovascular event-free survival with high flux HD as compared to standard HD treatment. Only Maduell et al. were able to demonstrate that dialysis patients randomized to on-line HDF had a better survival than those randomized to standard HD. Whether the superiority of HDF was due to a better clearance of uremic toxins in the middle-to-large MW range remains to be demonstrated.

**Adsorption**

Other possible therapeutic strategies that could be used to improve dialysis removal of protein-bound uremic toxins are: addition of activated charcoal or 5% albumin to the dialysate; use of highly permeable dialysis membranes to promote albumin leakage; fractionated plasma separation and adsorption. They have not yet been used in large clinical trials, though. The risk of aggravating malnutrition, limited effectiveness due to saturation of adsorption sites and thrombotic complications are potential limitations of these therapies. Additional studies are required before recommending these strategies in routine clinical use.

**Peritoneal dialysis**

Data on uremic toxins in PD is scarce. In spite of the serum concentration of protein-bound uremic toxins being lower in PD compared to HD patients, their removal is worse in the former group. These observations suggest that factors beyond the dialysis method, such as intestinal generation and/or metabolism, may play a role to determine their serum levels.

Although several studies have suggested a benefit of therapeutic intervention on middle and protein-bound uremic toxins levels, most of these data came from post hoc analysis or small studies. Larger, prospective, well-designed RCT aimed to identify the impact of reducing the levels of different uremic toxins on hard outcomes, such as overall and cardiovascular mortality, are required.

**General conclusions**

The study of uremic toxins is of growing interest in nephrology. Evidence from clinical and experimental studies have demonstrated the impact of these compounds on a variety of organs and systems. Notably, uremic toxins have recently been claimed as new cardiovascular risk factors, attracting interest from other fields of medicine. Understanding the effects of uremic toxins may provide a more comprehensive view of CKD and its complications and, most likely, new therapeutic targets to retard the progression of CKD and to counteract its cardiovascular complications.

**References**


52. ADHR Consortium. Autosomal dominant hypophosphataemia is associated with mutations in FGF23. Nat Genet 2000;24:441-6. DOI: http://dx.doi.org/10.1038/70866


