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Uraemic toxins and cardiovascular disease

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Definitions

When renal failure develops, uraemic retention solutes are retained which are normally excreted by the healthy kidneys. If these retention solutes exert biochemical/biological activity, they are called uraemic toxins. According to their physico-chemical characteristics, they can be subdivided into [1]: (i) small water-soluble compounds (<500 Da, prototype urea); (ii) the larger so-called middle molecules (>500 Da, prototype β_2-microglobulin); and (iii) the protein bound solutes.

The retention of these uraemic solutes results in the progressive failure of virtually every organ system, in parallel with the failing function of the kidneys. The resulting clinical picture is the uraemic syndrome.

Cardiovascular disease as an integral part of the uraemic syndrome

The incidence of vascular disease and the morbidity and mortality related to it are extremely high in the population of uraemic patients [2]. Atheromatosis frequently causes ischaemic problems such as angina pectoris, myocardial infarction, cerebrovascular accidents and ischaemia of the lower limbs. Vascular disease occurs much earlier than in the general population [3] and affects subjects who are normally at low risk, such as women and young adults [2,3]. In a recent survey of adolescents and young adults with a long-term history of end-stage renal disease, coronary calcifications were present even at an age of 20 years [4].

In uraemia, classical risk factors (hypertension, dyslipidaemia, obesity, smoking) may be less important than in the general population [5]. Presumably, other factors are at play, and among these, uraemic toxins are considered of prime importance [6]. The same compounds might even be relevant in the general population, but their effect may be masked by the classical risk factors.

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Cardiovascular disease as an inflammatory disorder

Traditionally, atherosclerosis has been considered as a degenerative disease, but more recently it has been recognized that it is, at least in part, an inflammatory disorder [7]. A key role in this process is played by the adhesion of activated leukocytes to the vascular endothelium, causing vascular damage by products of inflammation, such as free radicals.

A large proportion of uraemic patients suffer from inflammation [8]. Several authors demonstrated a correlation between such inflammation and vascular disease [8–10], and a third clinical feature, malnutrition [8,9].

Uraemic toxins as potential culprits

The role of uraemic solutes in the development of the uraemic syndrome has recently been reviewed [1,11]. In this concise editorial we summarize the available evidence concerning the most important toxins, which play a potential role in the genesis of vascular disease. We also point to some pitfalls that may interfere with scientific progress in this area, and draw attention to therapeutic possibilities, which may result from better understanding of this condition.

Space limitations prevent discussion of a number of compounds, which are potentially important, such as angiogenin, immunoglobulin light chains, β_2-microglobulin, leptin, guanidines and oxalate. The reader is referred to more extensive reviews [1,11].

The calcium–phosphate–parathyroid hormone axis

Phosphate

In a cohort study using multivariate analysis with adjustments for comorbid conditions, Block *et al.* [12,13] demonstrated a progressive increase in overall and cardiovascular mortality once serum phosphate and calcium–phosphorus product exceeded 5.5 mg/dl and 55 mg^2/dl^2, respectively. These findings were interpreted to suggest a role for the deposition of calcium in the vessel walls. The role of vascular calcium deposition was further corroborated by the demonstration of pathophysiologically significant amounts of calcium in the coronaries of young adults, after prolonged dialysis treatment, from the age of 20 on [4].

Parathyroid hormone

Not only the calcium–phosphorus axis, but also secondary hyperparathyroidism plays a relevant role in the deposition of calcium in the vessel walls [14]. Hyperphosphataemia, either directly or by inducing hypocalcaemia, is one of the main causes of hyperparathyroidism. After parathyroidectomy, calcium deposits may disappear from the small muscular arteries [15], but not from large arteries.

Vitamin D

A fourth player in the field is the active vitamin D metabolite, calcitriol [16], which suppresses parathyroid hormone production, but also favours deposition of calcium in the vessel wall, at least in part because of its hypercalcaemic effects.

The uraemic state is characterized not only by an inadequate production of calcitriol due to reduced renal mass, but also by calcitriol resistance related to retained uraemic 'toxins' [17]. Hence, calcitriol repletion *per se* might not suffice to control hyperparathyroidism.

A role of vitamin D is also suggested by recent findings, showing that overall mortality and particularly cardiovascular mortality are influenced by vitamin D receptor genotype [18].

Homocysteine

In the general population, a correlation is found between homocysteine concentration and cardiovascular mortality [19]. The concentration of total homocysteine is elevated in the sera of uraemic patients. Instead of being generated from methionine, homocysteine can be transformed into methionine after administration of folic acid and/or vitamin B_{12} [19]. This is an example illustrating that in the evaluation of a concentration of uraemic metabolites, one has to consider not only impaired renal excretion and extracorporeal removal, but also generation via intermediate metabolism.

Unfortunately, so far evidence is not available that reduction of homocysteine concentration has a beneficial effect on cardiovascular endpoints [20,21]. It is possible that reduction of homocysteine concentration in the available series had been attempted too late to affect vascular damage.

Advanced glycation end products (AGEs)

AGEs result from the irreversible modification of amino acids, proteins or peptides by carbohydrates and other metabolites. Whereas in diabetes these compounds predominantly result from excess glucose concentration, in uraemia oxidative stress and carbamoylation seem to be important alternative sources. In many constellations, AGEs are the result of inflammation [22].

Conversely, however, AGEs may also induce inflammation *in vitro* [23,24]. In a recent study, leukocytes were activated at baseline, but the response to stimulation was attenuated [24], presumably as the result of such inflammation-related stimuli. Such leukocyte dysfunction may contribute to the susceptibility of uraemic patients to infection.

In most studies, AGE-modified proteins were prepared artificially *in vitro*. This raises the question whether such artificial AGEs are representative for the AGEs retained *in vivo* [25]. Indeed it has not yet been resolved which of the AGEs identified in uraemia actually have toxic actions [11]. Remarkably enough, an inverse correlation was observed in a recent study between the concentration of certain AGEs and the development of cardiovascular disease in haemodialysed patients [26].

Advanced oxidation protein products (AOPP)

In analogy with the AGEs, oxidative metabolites of albumin with inflammatory potential have been demonstrated by the group of Witko-Sarsat *et al.* [23] in the plasma of uraemic patients. Of note, plasma concentration of AOPP has been shown recently to be correlated with common carotid artery intima-media thickness [27].

Cytokines

High concentrations of cytokines with an immune activating potential are present in the plasma of uraemic patients [11]. The concentration of interleukin-6 has been related to carotid artery stenosis [28]. Moreover, several pro-inflammatory cytokines were correlated with overall mortality in the dialysed population [29]. It is still an unanswered question whether they activate leukocyte function at the concentrations found in uraemia, and whether such cytokines are culprits or only markers.

Asymmetric dimethylarginine (ADMA)

ADMA is an arginine analogue with a guanidine structure, which blocks the arginine-induced vasoprotective and vasodilatory effects of nitric oxide. In elegant multivariate analyses, Zoccali *et al.* [30] demonstrated that in uraemic patients a relation exists between ADMA on the one hand, and cardiovascular events on the other hand. Furthermore, a correlation was found between ADMA concentration and carotid artery thickness [31].

Pitfalls of research

Research efforts trying to unravel the mechanisms underlying vascular disease in uraemia have often focused on a single solute. Data comparing the effect of a hypothetical toxin with known toxins have usually not been presented. Several studies examined only one organ or cell system. Frequently, experiments were performed at concentrations, which did not correspond to what is found in uraemia. One example

of the current confusion is the discrepancy between the lowest and the highest ever reported concentrations for solutes such as ADMA [32,33] interleukin-6 [29, 34] and 3-deoxyglucosone [35,36]. A broad initiative is needed, involving cooperation between several laboratories, to work out at a standardized approach.

In an attempt to enable such a standardized approach and to reduce methodological bias, the European Uremic Toxin Work Group (EUTox) has recruited members from several European groups that are involved in toxin research. More information on the structure, aims and activities can be found on the website (www.uremic-toxins.org).

Therapeutic/preventive options

Clinical studies attempting to remove solutes, especially middle molecules, should be performed. While obviously parathyroid hormone concentrations and Ca × P product should be optimized, most of the currently available methods, such as the administration of calcium salts and classical vitamin D analogues, induce hypercalcaemia. Calcimimetics and new vitamin D analogues might offer new solutions. Measures to combat inflammation such as use of ultrapure dialysate and biocompatible membranes, as well as administration of vitamin C, vitamin E, statins, ACE-inhibitors, aspirin or radical scavengers might prove useful and should be studied in controlled studies. Some interventions, e.g. administration of folic/folinic acid, might remove unwanted substances such as homocysteine by promoting removal via intermediary metabolism. It should also be examined whether it is not possible to inhibit uptake of toxins and their precursors from the intestine by appropriate measures, e.g. sorption.

A concerted multicentric and multifocused research programme in conjunction with pharmaceutical and extracorporeal removal industries will be necessary to make further progress in this area. New approaches should be tried such as proteome/genome analysis, high throughput analysis, adsorption technologies and regenerative medicine.

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