

## New insights in uremic toxins

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**New insights in uremic toxins.** The retention in the body of compounds, which normally are secreted into the urine results in a clinical picture, called the uremic syndrome. The retention compounds responsible for the uremic syndrome are called uremic toxins. Only a few of the uremic retention solutes fully conform to a true definition of uremic toxins. Uremic patients develop atheromatotic vascular disease more frequently and earlier than the general population. The classical risk factors seem to be less important. Other factors have been suggested to be at play, and among those uremic toxins are mentioned as potential culprits. The identification, classification and characterization of the solutes responsible for vascular problems seems of utmost importance but is far from complete due to a lack of standardization and organization. The European Uremic Toxin Work Group (EUTox) has as a primary aim to discuss, analyze and offer guidelines in matters related to the identification, characterization, analytical determination and evaluation of biological activity of uremic retention solutes. The final aim remains the development of new strategies to reduce the concentration of the most active uremic solutes. These activities will at first be concentrated on reducing factors influencing cardiovascular morbidity and mortality.

### DEFINITIONS

Since many decades, the problem of uremic toxicity has been a major area of concern for the entire nephrologic community. The retention in the body of compounds that normally are secreted into the urine by the healthy kidneys gives rise to a progressive deterioration of physiologic functions and of the clinical condition. The resulting clinical picture is the uremic syndrome.

If uremic retention solutes are considered as such without necessarily proven toxicity, at least 90 organic compounds have been retained in uremia [1]. The most important known organic uremic retention solutes are listed in Table 1. To this list should be added a number of inorganic substances, such as water, potassium, phosphate, and the trace elements [2–4]. This is probably only the tip of the iceberg, and many more still unidentified solutes are possibly retained and might exert toxicity.

<sup>1</sup>For the members of EUTox, see the Appendix.

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The retention compounds, which are considered responsible for the uremic syndrome, are uremic toxins. Before a retention solute can be accepted as a true uremic toxin, it should comply, however, with a number of conditions: (1) Such a compound should be chemically identified and accurate quantitative analysis in biological fluids should be possible; (2) the total body and plasma levels should be higher in uremic than in nonuremic subjects. (3) high concentrations should be related to specific uremic dysfunctions and/or symptoms that decrease or disappear when the concentration is reduced; (4) biological activity, conforming to clinical changes observed in conjunction with the uremic syndrome, should be proven in *in vivo*, *ex vivo*, or *in vitro* studies; and (5) concentrations in these studies should conform to those found in body fluids or tissue of uremic patients [5].

Obviously, only a few of the uremic retention solutes fully conform to the definition of a true uremic toxin, and even those might be a matter of debate (e.g., H<sub>2</sub>O, phosphate, potassium,  $\beta_2$ -microglobulin). One of the major problems arising in this area is that many of the compounds with a presumed or proven biologic potential are difficult to remove by conventional dialysis, either because of their molecular weight and/or as a consequence of their protein binding. As a result, number 3 of the above-mentioned conditions is especially difficult to corroborate. On the other hand, if clinical proof of benefit by removal remains absent, the impetus for researchers or industries to produce devices or other methodologies of potential help for the removal of these molecules usually remains minimal. This results in a vicious circle, whereby technologic/pharmacologic innovations to remove solutes other than the classic compounds, such as urea, are limited or even nonexistent.

### UREA AS A UREMIC RETENTION SOLUTE/ UREMIC TOXIN

One of the most typical examples of the sometimes-confusing state of the art in the area of uremic toxicity is the current knowledge about urea. Urea is a 60-dalton small water-soluble compound, which has among the

**Table 1.** Main known uremic retention solutes

Small water soluble solutes	Protein-bound solutes	Middle molecules
Asymmetric dimethylarginine	3-Deoxyglucosone	Adrenomedullin
Benzylalcohol	CMPF	Atrial natriuretic peptide
$\beta$ -Guanidinopropionic acid	Fructoselysine	$\beta_2$ -Microglobulin
$\beta$ -Lipotropin	Glyoxal	$\beta$ -Endorphin
Creatinine	Hippuric acid	Cholecystokinin
Cytidine	Homocysteine	Clara cell protein
Guanidine	Hydroquinone	Complement factor D
Guanidinoacetic acid	Indole-3-acetic acid	Cystatin C
Guanidinosuccinic acid	Indoxyl sulfate	Degranulation inhibiting protein I
Hypoxanthine	Kinurenine	Delta-sleep-inducing peptide
Malondialdehyde	Kynurenic acid	Endothelin
Methylguanidine	Methylglyoxal	Hyaluronic acid
Myoinositol	N-carboxymethyllysine	Interleukin 1 $\beta$
Orotic acid	P-cresol	Interleukin 6
Orotidine	Pentosidine	Kappa-Ig light chain
Oxalate	Phenol	Lambda-Ig light chain
Pseudouridine	P-OHhippuric acid	Leptin
Symmetric dimethylarginine	Quinolinic acid	Methionine-enkephalin
Urea	Spermidine	Neuropeptide Y
Uric acid	Spermine	Parathyroid hormone
Xanthine		Retinol binding protein
		Tumor necrosis factor alpha

CMPF is carboxy-methyl-propyl-furanpropionic acid.

presently known uremic retention solutes the highest concentration in uremic serum. It has been used as a marker of uremic retention and removal for several years [6], and its removal is directly related to patient survival [7]. Nevertheless, there are very few studies demonstrating a direct biologic impact of urea at currently encountered uremic concentrations [8], and those studies show an impact that not necessarily concentrates on key organic functions in the biochemical/biologic status of the human body.

When urea was added to the dialysate during a period of several months at concentrations largely exceeding those currently encountered in dialyzed uremics, uremic symptomatology was not consistently altered over the entire study period [9], again suggesting that by itself, urea is not very important in the development of uremic toxicity.

It is difficult to explain the apparent paradox between the validity of urea as a marker and its presumed lack of toxicity. Of note, urea removal seems to be related as a surrogate marker only indirectly to survival, and not to quality of life. One possibility to consider is that urea removal by itself does not affect survival, but that it is representative for the removal of one or more other solutes with a more consistent impact. One such potential culprit is potassium, another small-water soluble compound known to substantially affect dialytic survival [4]. Another possibility is that, together with urea, other uremic solutes antagonizing its toxic impact are retained [10]. Finally, urea might be at the origin of other, more toxic moieties, such as some of the guanidines or carbamylation products [8, 11].

The global experience regarding urea teaches us that uremic toxicity is neither a question of retention of urea alone, nor of water-soluble compounds alone; in the treatment of the uremic syndrome, hence, more than the removal of urea alone should be pursued. It should be realized that urea removal is not representative for many other molecules, and that this is especially the case for hard to remove molecules, such as protein-bound solutes or the middle molecules (>500 daltons) [12]. Many of these molecules exert biologic and/or clinical activities [8]. Among the few small water-soluble uremic retention compounds that have been shown to exert biologic action, many again have an intradialytic kinetic behavior that is indisputably different from that of urea (e.g., the guanidines, phosphate, xanthine, and hypoxanthine [13]).

### Cardiovascular morbidity and mortality as a uremic problem

Uremic patients develop atheromatotic vascular disease more frequently and earlier than the general population [14, 15]. The classic risk factors affecting the general population, such as hypercholesterolemia or hypertension, seem to have less weight in the development of vascular problems in uremia [16, 17]. Other elements have been suggested to be at play, and among those, uremic toxins have been mentioned as potential culprits [17]. Dialytic treatment seems to have no major impact on the evolution of this process. The latter observation indicates that the current concept of dialysis, with major emphasis on removal of water-soluble compounds, is insufficient to prevent or slow down cardiovascular damage. In line with this observation, scattered studies based on different method-

ologies and evaluating different solutes show that most, if not all, compounds which up until now have been suggested to play a role in atherogenesis, show a dialytic behavior which is different from that of urea: advanced glycation end products (AGEs), advanced oxidation protein products (AOPP), homocysteine, phosphate, asymmetric dimethylarginine (ADMA), and cytokines [5].

Hence, the identification, classification, and characterization of the clinical importance of the solutes responsible for cardiovascular morbidity and mortality is of utmost importance to improve survival and quality of life of the uremic population before and after the start of dialysis. Only subsequent to this can the development of specific devices pursuing the removal of those compounds be launched. Finally, the possibility should be considered that the same factors might be at play in the general population.

This search for the responsible solutes is, however, flawed by a number of confounding factors, especially for in vitro studies: (1) the difficulty to define adequate measures to guarantee purity of samples (prevention of contamination with lipopolysaccharides or other contaminants); (2) the lack of a systematic coordinated approach (up until now, several research groups applied differently prepared compounds in different experimental set-ups at incorrect concentrations, not conforming to those encountered in clinical uremia); and (3) the lack of consideration of intermutual influences among various solutes when they are present together in the uremic milieu.

It is of note that those problems are not specific for uremic toxin research in the area of cardiovascular complications, but that they are equally relevant for any other aspect of the uremic syndrome (e.g., uremic anemia, progression of the loss of residual renal function, etc.).

### **The European Uremic Toxin Work Group (EUTox)**

While recognizing the problems related to an insufficiently coordinated approach in the area of uremic toxin research, the European Uremic Toxin Work Group (EUTox) started its activities in the autumn of 2000. This group is made up of several European researchers who have been active in this field for many years, together with representatives of the major industries active in dialysis treatment. This group was installed under the auspices of the European Society for Artificial Organs (ESAO).

The primary aim of this group is to discuss, analyze, and offer guidelines in matters related to the identification, characterization, analytical determination, and evaluation of biologic activity of uremic retention solutes. The Work Group has accomplished and is working on a number of projects. In October 2001, a common text endorsed by all members of the group presenting a state of the art in the area of uremic toxicity, especially in relation to cardiovascular events, was published [5]. A

review of the presently known uremic retention solutes was recently finalized and submitted; this publication contains a classification according to solute characteristics, and information about their concentration (see below) [1]. In June 2002, an Expression of Interest (EoI) was submitted to the European Community in the context of the 6th Framework Program (FP-6). Currently, the group is involved in the definition of standardized evaluation procedures for in vitro and in vivo research (inclusive high through-put and proteome analysis). More information on the intentions and structure can be obtained from the web site of the group: <http://www.uremic-toxins.org>.

The review of the existing retention solutes is based on a literature search of 857 publications, of which 141 deal with concentration [1]. Those publications appeared in the literature from 1968 to 2002. To compose the final lists, information from 55 publications covering 90 solutes was used, and the resulting tables display the mean (median) concentration in the normal population, the mean (median) concentration in the uremic population (highest reported value), the highest single concentration ever reported, and the molecular weight. This publication offers a guideline regarding the concentrations to be pursued for future in vitro or in vivo studies.

Solutes were subdivided into small water-soluble compounds (non-protein-bound), protein-bound compounds, and middle molecules. Sixty-eight molecules had a MW <500 daltons, whereas 22 compounds were classified as middle molecules (>500 daltons). Twelve molecules had a molecular weight in excess of 12,000 daltons. Twenty-five solutes were protein bound; these were mostly small compounds with a MW <500 daltons but included also the middle molecules leptin and retinol-binding protein. Concentrations range from ng/L (methionine-enkephalin) up to g/L (urea).

This review further contains reflections on the interindividual variability of these concentrations (e.g., the ratio between uremic and normal concentration, the scatter of concentrations in the uremic population, and the differences in concentration among various publications).

Indisputably, such a list will necessitate regular updating, with the introduction of newly detected compounds, as well as newly defined concentrations of already known compounds. The plan is to display the lists on the web site of the Work Group, where it will be made possible to contact the Work Group concerning newly identified solutes and/or concentrations which are aberrant from those reported in the Work Group's lists.

### **MODIFICATIONS IN THE THERAPEUTIC/PREVENTIVE APPROACH**

While extending our knowledge about solutes responsible for the uremic syndrome, the final aim remains the

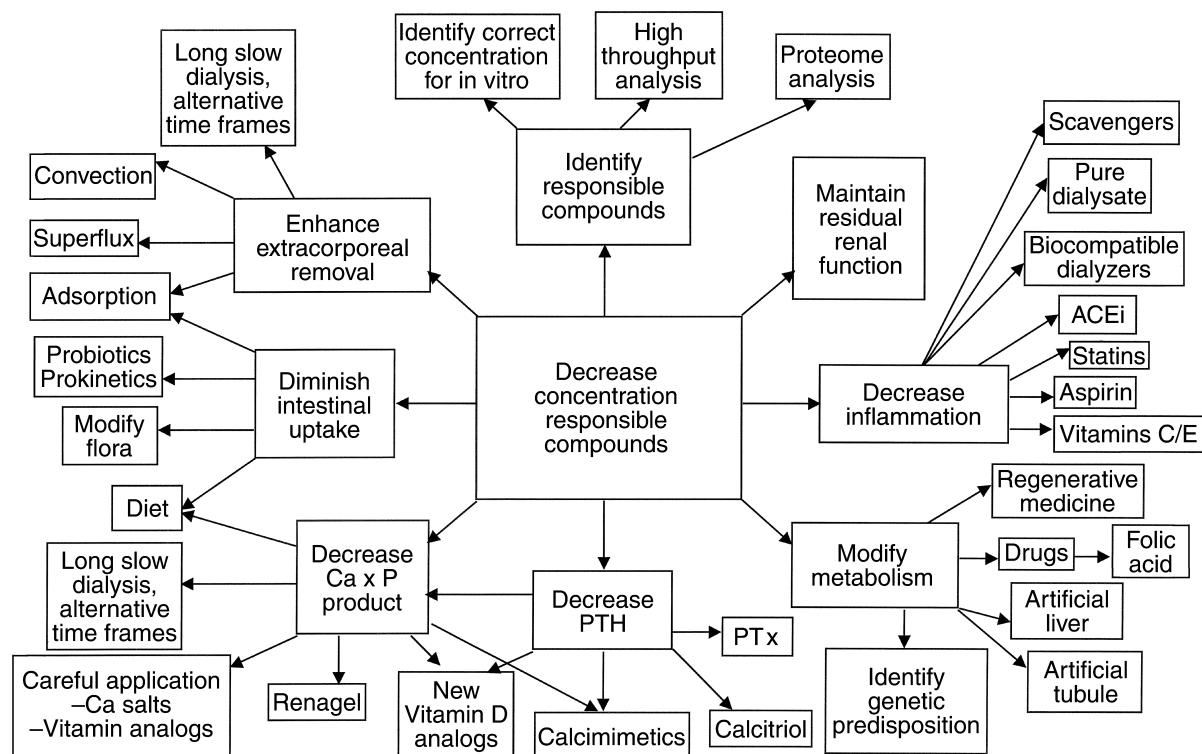


Fig. 1. Possible preventive or therapeutic measures in the context of uremic cardiovascular disease.

development of new strategies to reduce the concentration of the most active uremic retention solutes.

In what follows we will depict the therapeutic/preventive options for cardiovascular disease (Fig. 1, diagrammatic summary), but similar flow charts could be composed for any aspect of the uremic problem. Some of the solutions will overlap with those presented here, but some will be specific for these alternative problems and, hence, different. The complexity of the final solutions for the uremic problem that can be proposed or thought of is striking. In what follows and in the Fig. 1, only measures which are directly or indirectly related to a decrease of solute concentration and/or a decrease of inflammation are mentioned, such as the correct identification of the responsible toxins, so that more direct removal strategies can be developed, the enhancement of solute removal, the development of devices for extracorporeal adsorption of solutes, the application of strategies to reduce the input of solutes or solute precursors via the gastrointestinal system, the decrease of the  $\text{Ca} \times \text{P}$ -product and hyperparathyroidism, the modification of toxin metabolism, and the maintenance of residual renal function.

These preventive measures will come to pass only through the application of new research and therapeutic options, such as high through-put, proteome and genome analysis, development of new extracorporeal removal systems such as adsorptive devices and hybrid artificial

organs, application of alternative time frames for extracorporeal solute removal, development of alternative diets, and development of new drugs and/or regenerative medicine.

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## APPENDIX

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