

Uraemic toxins in chronic kidney disease

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■ ABSTRACT

The uraemic syndrome is a complex mixture of organ dysfunctions attributed to the retention of a myriad of compounds that under normal conditions are excreted by healthy kidneys. While recent years have seen major steps in the identification and characterisation of uraemic retention solutes and in the knowledge of their patho-physiological importance, this knowledge remains far from complete. This article discusses the general classification of uraemic solutes based on their molecular weight and protein binding characteristics, with reflection as to their removal. In addition, current knowledge on the main uraemic retention products and their clinical and biological effects is reviewed in detail.

Key-Words:

Biological effects; patho-physiology; removal; uraemic toxins.

■ INTRODUCTION

The uraemic syndrome is a complex of biological and biochemical alterations that result in a host of failing organs and disturbing symptoms. It originates from the retention of solutes that under normal conditions are cleared by the kidneys into the normal urine, although hormonal, metabolic and enzymatic axes disorders also play a role. The impact of retention is underscored by the clinical improvement resulting from dialysis and kidney transplantation.

The uraemic syndrome is characterised by a deterioration of biochemical and physiological functions (Table I) in parallel with the progression of renal failure. This results in a variable number of symptoms which mimic the picture of exogenous poisoning. Although the link between clinical deterioration and uraemia was first recognised several decades ago, and although the number of new patho-physiological elements in this area has risen exponentially over the last few years, our knowledge of the factors responsible remains incomplete.

Current knowledge of uraemic solute retention and its clinical and biological effects is reviewed below.

■ URAEMIC SOLUTE RETENTION

■ A) General classification of the uraemic solutes

A gradual retention of a large number of organic metabolites of proteins, fatty acids and carbohydrates characterises the progression of renal failure, whereby partial metabolisation and elimination by other than renal pathways may compensate for the loss of renal clearance. Some of the retained compounds are proven toxins. Toxicity is not a simple monofactorial process whereby only one or a few toxins affect many different metabolic processes at a time. Other retained substances are non-toxic but can be used as markers of retention.

A 2003 survey of the literature revealed the retention in uraemia of at least 90 compounds of which

Table 1

The uraemic syndrome – main clinical alterations

1. Cardiovascular system atheromatosis arteriosclerosis cardiomyopathy decreased diastolic compliance hyper-/hypotension pericarditis	6. Bone disease adynamic bone disease amyloidosis (β_2 -microglobulin) defective calcitriol metabolism osteitis fibrosa osteomalacia osteoporosis
2. Nervous system concentration disturbances cramps dementia depression fatigue headache motor weakness polyneuritis reduced sociability restless legs sleep disorders stupor, coma	7. Skin melanosis pruritus uraemic frost
3. Haematological system/ coagulation anaemia bleeding hypercoagulability	8. Gastro-intestinal system anorexia dyspepsia gastro-intestinal ulcers hiccup nausea, vomiting pancreatitis
4. Immunological system inadequate antibody formation stimulation of inflammation (baseline) susceptibility to cancer susceptibility to infection	9. Pulmonary system pleuritis pulmonary edema sleep apnoea syndrome
5. Endocrinology dyslipidemia glucose intolerance growth retardation hyperparathyroidism hypogonadism impotence, diminished libido	10. Miscellaneous hypothermia thirst uraemic foetor weight loss

the concentration had been reported¹. It is very likely that this is only the tip of the iceberg. While the 2003 survey described approximately 25 middle molecular weight peptides, a recent study by sophisticated proteome analysis revealed the presence of at least 1000 such compounds in ultrafiltrate from dialysed patients².

Under normal conditions, the glomerular filter clears molecules with a molecular weight up to $\pm 58,000$ Dalton. All these substances are supposed

to be retained in renal failure. An additional role can be attributed to changes in tubular secretion, reabsorption and metabolic breakdown, which are all altered when renal mass decreases. The molecules metabolised by the kidneys may have a higher molecular weight ($>58,000$ D) than those cleared. Renal and non-renal metabolism of solutes and non-renal clearance may in turn be inhibited following uraemic retention.

Uraemic retention products are arbitrarily subdivided according to their molecular weight (MW)^{3,4}. Low molecular weight molecules are characterised by an MW up to 500 D [e.g. urea (MW: 60), creatinine (MW: 113)]. They can further be subdivided into protein-bound and non-protein-bound molecules. Substances with an MW range above 500 D are called middle molecules [e.g. parathyroid hormone (PTH, MW: 9,424), β_2 -microglobulin (β_2 -M, MW: 11,818)]. Several clinical, metabolic and/or biochemical disturbances such as food intake, apolipoprotein (apo) A-I secretion, osteoblast mitogenesis, cell growth, lymphocyte proliferation and interleukin production are caused by uraemic compounds that conform to the middle molecular weight range⁵⁻¹⁰.

The number of clinical studies showing a benefit for middle molecule removal has been growing¹¹⁻¹⁴, culminating in the recent MPO study showing a clinical benefit in dialysis patients with the bleakest clinical perspectives, i.e. the malnourished and the diabetics (data presented by F. Locatelli at the 2007 ERA-EDTA congress in Barcelona). Of note, hypoalbuminaemic and diabetic patients constitute a substantial fraction of the current dialysis population^{15,16}.

Removal of larger molecules is more efficient when the high flux membranes are used in a convective mode^{17,18} but there is no controlled data available on whether this affects mortality. Convective treatment modalities have a positive impact on the development of carpal tunnel syndrome¹⁹. On-line haemodiafiltration with large convective volumes results in increased erythrocyte counts and decreased erythropoietin needs²⁰. In two observational studies, on-line HDF resulted in a better outcome than diffusive strategies^{21,22}.

The high protein binding of small compounds such as hippuric acid or indoxyl sulfate makes

them behave like MM during dialysis. Their removal in diffusive mode by classical haemodialysis systems, even with large pore membranes, remains disappointingly low²³, something which may be attributed to these compounds' complex distribution and intra-dialytic kinetics. Therefore, alternative removal strategies to the classical ones should be considered, such as adsorption, changes in time-frames²⁴, use of protein leaking membranes and/or stimulation of metabolic pathways. Even small water soluble compounds, which in principle should show the same characteristics as urea, quite often show different kinetics, as has been demonstrated recently for the guanidines^{25,26}.

Peritoneal dialysate is a much richer source of protein-bound compounds than haemodialysate²⁷, since peritoneal pore size allows the transfer of substantial quantities of albumin together with its bound moieties, which is not the case with even the most open haemodialyser membranes. Also the continuous timeframe might enhance the removal of these compounds²⁸.

Until recently, no data had confirmed a potential clinical impact of protein-bound molecules, but at least four recent studies have pointed in that direction²⁹⁻³².

■ B) Main uraemic retention products

Several uraemic retention solutes influence biological functions. Other compounds have no proven direct toxicity, but may be useful markers of uraemic retention. An overview of the pathologically most relevant uraemic retention solutes with their MW is given in Table II. It should be acknowledged that anorganic compounds such as water and potassium exert toxicity as well. In what follows, we will concentrate on the organic retention compounds.

1) Advanced glycation end products (AGE)

As Maillard first described, glucose and other reducing sugars react nonenzymatically with free amino groups to form reversible Schiff base adducts (in days) and stable Amadori products (in weeks), which are then converted into AGE through chemical rearrangements and degradation reactions³³. Several AGE-compounds are peptide-linked degradation

products³⁴ (MW 2,000-6,000 D), although the baseline AGE-products such as pentosidine, 2-(2-fuoryl)-4(5)-(2-furanyl)-1H-imidazole (FFI), imidazolone, 3-deoxyglucosone, pyrrole aldehyde, and N^ε-(carboxymethyl)lysine have a substantially lower MW (table II).

AGE are retained not only in renal failure, but also in diabetes mellitus and ageing³⁵, where they are held responsible for tissular damage and functional disturbances. In the uraemic population, the level of glucose-modified proteins is higher than in diabetics without renal failure³⁶, and AGE-concentration does not depend on the glycaemic status^{37,38}. The

Table II

Major uraemic retention solutes and their molecular weight (Daltons)

Compound	MW	Compound	MW
ADMA/SDMA	202	Adrenomedullin	5729
<u>ANF</u>	<u>3080</u>	Benzylalcohol	108
β-endorphin	3465	β-guanidinopropionic acid	131
<u>β2-microglobulin</u>	<u>11818</u>	<u>CGRP</u>	<u>3789</u>
<u>Cholecystokinin</u>	<u>3866</u>	<u>CIP</u>	<u>8500</u>
<u>Clara cell protein</u>	<u>15800</u>	CML	188
CMPF	240	<u>Complement factor D</u>	<u>23750</u>
Creatine	131	Creatinine	113
<u>Cystatin C</u>	<u>13300</u>	Cytidine	234
<u>DIP I</u>	<u>14400</u>	<u>DIP II</u>	<u>24000</u>
3-deoxyglucosone	162	Dimethylarginine	202
<u>Endothelin</u>	<u>4283</u>	γ-guanidinobutyric acid	145
Glomerulopressin	500	<u>GIP I</u>	<u>28000</u>
<u>GIP II</u>	<u>25000</u>	Guanidine	59
Guanidinoacetic acid	117	Guanidinosuccinic acid	175
Hippuric acid	179	Homoarginine	188
Homocysteine	135	<u>Hyaluronic acid</u>	<u>25000</u>
Hypoxanthine	136	Imidazolone	203
Indole-3-acetic acid	175	Indoxyl sulfate	212
<u>Leptin</u>	<u>16000</u>	Melatonin	126
Methylguanidine	73	Myoinositol	180
<u>Neuropeptide Y</u>	<u>4272</u>	Orotic acid	156
Orotidine	288	o-OH-hippuric acid	195
Oxalate	90	p-cresylsulfate	188.2
p-OH-hippuric acid	195	<u>Parathyroid hormone</u>	<u>9225</u>
Pentosidine	135	Phenylacetylglutamine	264
Phenol	94	Phosphate	96
Pseudouridine	244	Putrescine	88
<u>Retinol binding protein</u>	<u>21200</u>	Spermine	202
Spermidine	145	Thymine	126
Trichloromethane	119	Tryptophan	202
Urea	60	Uric acid	168
Uridine	244	Xanthine	152

The underlined compounds conform with the definition of MM (MW above 500 D)

production of AGE in CKD stage 5 has been related to oxidative and carbonyl stress rather than to reactions with glucose³⁹. Not all AGE-generation is oxidative, however. AGE provoke monocyte activation⁴⁰, as well as the induction of interleukin-6, tumour necrosis factor- α , and interferon- γ generation⁴¹. AGE-modified β_2 -M may play a role in the generation of dialysis-associated amyloidosis⁴² (see below). Serum pentosidine levels are higher in patients with dialysis-related amyloidosis compared to their amyloid-free counterpart⁴³. AGE can react with and chemically inactivate nitric oxide (NO)⁴⁴, a potent endothelium-derived vasodilator, anti-aggregant and antiproliferative factor. AGE are also related to oxidative protein modification⁴⁵. 3-Deoxyglucosone inactivates glutathione peroxidase, a key enzyme in the neutralisation of hydrogen peroxide⁴⁶. AGE accumulate in atheromatous plaque of the aortic wall of subjects with CKD stage 5, where they may contribute to a more rapid progression of atherosclerosis⁴³. To our knowledge, there is no observational study in uraemia linking AGE directly to atherogenesis.

Late glycation products increase PMNL chemotaxis⁴⁷. Other recent data suggest that whereas AGE increase baseline leukocyte response, activated response to infectious stimuli is blunted⁴⁸. This suggests a dual response, related at the clinical level both to atherogenesis and susceptibility to infection⁴⁹.

Most of the biological actions of AGE that have been registered up to now have, however, not been obtained with AGE recovered from uraemic or diabetic serum, but with AGE artificially prepared in the laboratory⁴⁹. Recent data underscore as well the immune enhancing effect of genuine AGE, as they are found in renal failure⁵⁰.

Concentrations in CKD stage 5 patients might be attributed to increased uptake, production and/or retention. During industrial food processing, cooking procedures and storage of foods, food proteins are modified by carbohydrates^{51,53}, and those are absorbed via the gastro-intestinal tract³⁷. The healthy kidneys are responsible not only for glomerular filtration but also for tubular reabsorption and degradation of AGE^{54,55}. Specific receptors for AGE (RAGE) have been identified and their expression is enhanced during uraemia⁵⁶. A recent study in uraemic mice demonstrated that a blockade of RAGE reduces the proatherogenic effects of uraemia,

possibly through a systemic decrease in oxidative stress⁵⁷. AGE binding to RAGE has been shown to stimulate mesothelial cell activity, and results in overexpression of vascular cell adhesion molecule (VCAM-1), which activates human peritoneal cells and promotes local inflammation, implicating the development of tubular injury⁵⁸.

In spite of continuous contact with glucose via the dialysate, CAPD patients do not have higher serum AGE levels than haemodialysis patients³⁴. Nevertheless, protein glycation has been demonstrated in the peritoneal membrane⁵⁹. The heat sterilisation of glucose-containing peritoneal dialysate induces the formation of glucose degradation products (GDP), which are precursors of AGE⁶⁰. GDP inhibit leukocyte response, and this effect is attenuated when heat sterilisation is replaced by other procedures (e.g. filter sterilisation)⁶¹.

Removal of AGE is significantly more important with high flux haemodialysis than with conventional dialysis with low flux membranes⁶². AGE show a marked heterogeneity in removal pattern, even during high flux dialysis^{52,63}. It is unclear which compounds could be representative by their removal pattern in a way that they could serve as a marker for the overall group of AGE.

2) β_2 -microglobulin (β_2 -M)

β_2 -M (MW approximately 12,000 D) is a component of the major histocompatibility antigen. Uraemia-related amyloid is to a large extent composed of β_2 -M, and is essentially found in the osteo-articular system and in the carpal tunnel, although deposition can be systemic as well⁶⁴. Uraemia-related amyloidosis becomes most often clinically apparent after several years of chronic kidney disease (CKD) and/or in the aged⁶⁵. According to the most recent studies, its prevalence tends to decrease⁶⁶, probably due to modifications in dialysis strategies and improvements in dialysis water quality.

AGE-modified β_2 -M has been identified in amyloid of haemodialysed patients⁶⁷ and enhances monocytic migration and cytokine secretion⁶⁸, suggesting that foci containing AGE- β_2 -M may initiate inflammatory response, leading to bone and joint destruction. The lack of a higher clinical incidence of β_2 -M-amyloidosis in diabetic dialysis patients⁶⁹, who generate large

quantities of AGE in the presence of hyperglycaemia, casts a doubt on the patho-physiologic role of AGE in amyloid formation. Possibly the AGE-transformation plays a more important role in the inflammation surrounding β_2 -M-amyloid than in its generation.

Long-term haemodialysis with large pore membranes results in a progressive decrease of pre-dialysis β_2 -M concentrations although the levels remain far above normal, even after intensive removal therapy^{70,71}. Long-term dialysis with large-pore dialysers results in a lower prevalence of dialysis-related amyloidosis and/or carpal tunnel syndrome^{19,72-74}. Whether this benefit is attributable to a better removal of β_2 -M, lower complement and leukocyte activating capacity or to protection against the transfer of dialysate impurities into the blood stream (e.g. lipopolysaccharides)⁶⁶ is not evident, since most of the dialysers associated with a lower incidence of amyloidosis have all three abovementioned properties.

Because β_2 -M is only removed by dialysers with a large pore size, its kinetic behaviour might be representative for other large molecules. Behaviour of β_2 -M during dialysis is, however, not necessarily representative of that of other MM. Discrepancies in behaviour in the long run have been demonstrated in relation to other MM, such as complement factor D⁷⁵.

Recently, several devices with strong adsorptive capacity for β_2 -M have been developed⁷⁶. In a sub-analysis of the haemodialysis (HEMO) Study, serum β_2 -M levels were directly related to patient outcome¹² and to infectious mortality⁷⁷. In a proteomic analysis for biomarkers of arteriosclerosis in the general population without renal dysfunction, β_2 -M was selected as the most appropriate index molecule⁷⁸.

The clinical expression of dialysis-related amyloidosis disappears after kidney transplantation, but the underlying pathologic processes such as bone cysts and tissular β_2 -M remain are preserved⁷⁹. It is possible that immunosuppressive therapy plays a role in the regression of the symptomatology.

3) Cytokines

In view of the strong associations between atherosclerosis, malnutrition and inflammation⁸⁰, it may be speculated that factors associated with malnutrition

and inflammation may contribute to the excess prevalence of cardio-vascular disease. The causes of inflammation in CKD stage 5 patients are probably multifactorial. All available evidence suggests that the pro-inflammatory cytokine system activity is elevated in CKD stage 5 patients⁸¹. It has been hypothesised that epoetin resistance is due to enhanced levels of immune activation since chronic inflammation can modify the process of erythropoiesis. The accumulation of TNF- α may contribute to the development of neurologic and haematologic complications in uraemia. Several lines of evidence suggest that decreased renal clearance might play an important role⁸². However, as the half-life of various cytokines is short and local tissue cytokine inactivation may be the most important pathway of cytokine degradation, more research is needed to determine the relative importance of the kidney in cytokine clearance.

4) Dinucleoside polyphosphates

Dinucleoside polyphosphates are a group of substances described to be involved in the direct regulation of the vascular tone as well as growth of vascular smooth muscle cells⁸³ and mesangial cells⁸⁴. Specific members of this group, the diadenosine polyphosphates, were detected in hepatocytes, human plasma⁸⁵ and platelets. In addition, concentrations of diadenosine polyphosphates were shown to be increased in platelets⁸⁶ from haemodialysis patients⁸⁷. Recently, uridine adenosine tetraphosphate (Up4A) was isolated and identified as a novel endothelium-derived vasoconstrictive factor. Its vasoconstrictive effects, its plasma concentration and its release upon endothelial stimulation strongly suggest that Up4A has a functional vasoregulatory role⁸⁸.

5) Guanidines

Guanidines are structural metabolites of arginine. Among them are well known uraemic retention solutes such as creatinine and guanidine, and newly detected moieties such as asymmetric and symmetric dimethylarginine (ADMA and SDMA).

Guanidine levels have been determined in serum, urine, cerebrospinal fluid and brains of uraemic patients^{89,90}. Four compounds, creatinine, guanidine, guanidinosuccinic acid (GSA) and methylguanidine (MG) are highly increased.

Several of the guanidino compounds modify key biological functions. GSA inhibits the production by 1α -hydroxylase of the active vitamin D metabolite, $1,25(\text{OH})_2\text{VitD}_3$ (calcitriol)⁹¹, and interferes with activation of ADP-induced platelet factor 3^{92} at concentrations currently found in haemodialysed uraemics^{93,94}. A mixture of guanidino compounds suppresses the natural killer cell response to interleukin-2⁹⁵ and free radical production by neutrophils⁹⁶. In recent studies, guanidine compounds have been shown to enhance baseline immune function, related to vascular damage⁹⁷. In addition, they also have been related to a decreased protein binding of homocysteine, another compound with vessel damaging potential⁹⁸.

GSA, γ -guanidinobutyric acid, methylguanidine, homoarginine and creatine induce seizures after systemic and/or cerebroventricular administration to animals^{99,100}. GSA plays an important role in the hyperexcitability of the uraemic brain¹⁰¹. GSA probably also acts as a selective agonist at the N-methyl-D-aspartate (NMDA) receptor^{102,103}. GSA displays *in vivo* and *in vitro* neuroexcitatory effects that are mediated by ligand- and voltage-gated Ca^{2+} channels, suggesting an involvement of the guanidines in the central nervous complications of uraemia¹⁰⁴.

Arginine enhances NO-production. Some of the other guanidines, such as arginine-analogues, are strong inhibitors of NO-synthase. The inhibition of NO-synthesis results in saphenous¹⁰⁵ and mesenteric vasoconstriction¹⁰⁶, hypertension¹⁰⁷, ischaemic glomerular injury¹⁰⁸, immune dysfunction¹⁰⁹ and neurological changes¹¹⁰. ADMA is the most specific endogenous compound which inhibits NO-synthesis. ADMA accumulates in the body during the development of renal failure^{111,112}, related to decreased renal excretion but possibly also to suppressed enzymatic degradation by dimethylarginine dimethylaminohydrolase¹¹³. The increase in SDMA is more pronounced. This structural variant of ADMA had been considered inert until recently, but has now equally been suggested to be related to vascular damage by inhibition of endothelial Nitric Oxide Synthase (eNOS)¹¹⁴. In the brain, ADMA causes vasoconstriction and inhibition of acetylcholine-induced vasorelaxation¹¹⁵. Also in thoracic and radial vessels, ADMA induces contractions¹¹⁶. Oestrogen has been shown to alter the metabolism of ADMA reducing the circulating concentration *in vivo*¹¹⁷. Methylguanidine, another endogenous guanidine, also shows a certain inhibitory activity on cytokine- and

endotoxin-inducible NO-synthesis, be it to a limited extent¹¹⁸.

In contradiction to the hypothesis of inhibition of NO-synthesis in uraemia, Noris *et al.* described an enhanced NO-production, in patients susceptible to uraemic bleeding tendency¹¹⁹. This effect is possibly limited to a subgroup of the uraemic population.

In the renal proximal convoluted tubule of rats with renal failure, the generation out of arginine of guanidinoacetic acid and creatine is depressed¹²⁰, whereas the synthesis of GSA, guanidine and methylguanidine is markedly increased, due to urea recycling.

Dialytic removal of guanidino compounds is subjected to a substantial variability⁹⁴. It is possible that tissular distribution or protein binding play a role. In spite of a low MW, removal by haemodialysis of ADMA is only in the range of 20-30%¹¹². Several of the guanidines have a substantially larger distribution volume than the standard marker urea, resulting in a decreased dialytic effective removal and substantial post-dialysis rebound^{25,26}

6) Homocysteine

Homocysteine (Hcy), a sulphur-containing amino acid, is produced by the demethylation of dietary methionine. Retention results in the cellular accumulation of S-adenosyl homocysteine (AdoHcy), an extremely toxic compound, which competes with S-adenosyl-methionine (AdoMet) and inhibits methyltransferase¹²¹. Moderate hyperhomocysteinaemia, caused by a heterozygous deficiency of Hcy breakdown or by vitamin B₆, B₁₂ or folate deficiency, is an independent risk factor for cardiovascular disease in the general population^{122,123}. Reduced and oxidised forms of Hcy are present in the plasma, and total fasting levels are a reflection of intracellular metabolism and cellular excretion of Hcy¹²⁴.

Hcy increases the proliferation of vascular smooth muscle cells, one of the most prominent hallmarks of atherosclerosis¹²⁵. Moderate hyperhomocysteinaemia may involve endothelial dysfunction and generate reactive oxygen species¹²⁶. The administration of excessive quantities of the Hcy precursor methionine to rats induces atherosclerosis-like alterations in the aorta¹²⁷. Hcy also disrupts several anticoagulant functions in the vessel wall, which results in enhanced

thrombogenicity¹²⁸. Guanidines have been related to release of homocysteine from its protein binding sites, by induction of structural modifications of albumin⁹⁸.

Patients with CKD have total serum Hcy levels two- to fourfold above normal. The serum concentration depends not only on the degree of kidney failure, but also on nutritional intake (e.g. of methionine)¹²⁹, vitamin status (e.g. of folate)^{130,131}, genetic factors¹³²⁻¹³⁴ and decreased renal metabolism¹²¹. Almost all filtered Hcy is reabsorbed in the tubular system so that urinary excretion is minimal¹³⁵. Detoxification by remethylation of homocysteine to methionine is inhibited in haemodialysis patients^{136,137}.

Hyperhomocysteinaemia is the most prevalent cardiovascular risk factor in CKD stage 5^{134,138}. Plasma homocysteine and cardiac mass correlate to each other¹³⁹. In a study by Suliman *et al.*, total plasma Hcy was lower in haemodialysis patients with cardiovascular disease than in those without, however¹²⁹. This study found a correlation between total Hcy and serum albumin, pointing to a negative impact of malnutrition on Hcy concentrations. Also, more recent data point to an inverse relation between homocysteine levels and mortality¹⁴⁰. Hcy is partly bound to albumin, which hampers removal by haemodialysis. Hyperhomocysteinaemia is more pronounced in haemodialysis patients, than in PD¹³¹. In haemodialysed patients, homocysteine levels correlate with plasma folate^{130,131}, and with the activity of enzymes that are at play in Hcy-metabolism. Even with peritoneal dialysis, it is impossible to reduce total Hcy plasma levels to normal¹⁴¹.

Dialysis with extremely leaky haemodialyser membranes with large pore size (so-called super-flux membranes) results in a progressive decline of pre-dialysis plasma homocysteine concentrations¹⁴². This effect has at least in part been attributed to changes in homocysteine metabolism, induced by enhanced middle molecule removal through these highly efficient membranes.

Hcy levels can be reduced by folic acid, vitamin B₆ and vitamin B₁₂¹⁴³. The population with CKD stage 5 might require high quantities of vitamins¹⁴⁴.

Possibly, the disappointing efficiency of folic acid might be related to an impairment of the metabolism of folic acid to 5-methyltetrahydrofolate (MTHF), which

is the active compound in the remethylation pathway¹⁴⁵. In an attempt to obviate such a deficiency, Bostom *et al.* directly administered oral MTHF (17 mg/d) to haemodialysed patients¹⁴⁶. No benefit was found, however. Touam *et al.*, on the other hand, could reduce total Hcy to normal in approximately 80% of the studied population, by the administration of folinic acid, a precursor of MTHF¹⁴⁵. Since the supplementation with folate is inexpensive and relatively harmless, there is no formal objection against its therapeutic use.

Direct clinical proof of the benefit of a lower Hcy concentration in uraemia is to our knowledge not available. Even when it was possible to decrease Hcy levels therapeutically, carotid artery stiffness was not altered¹⁴⁷.

7) Indoxyl sulfate

Indoxyl sulfate is metabolised by the liver from indole, which is produced by the intestinal flora as a metabolite of tryptophan. It enhances drug toxicity by competition with acidic drugs at the protein binding sites¹⁴⁸, inhibits the active tubular secretion of these compounds¹⁴⁹, and inhibits deiodination of thyroxine 4 by cultured hepatocytes¹⁵⁰.

The oral administration of indole or indoxyl sulfate to uraemic rats causes a faster progression of glomerular sclerosis and of renal failure¹⁵¹. This effect is possibly mediated by the renal gene expression of transforming growth factor β (TGF β), tissue inhibitor of metalloproteinase-1 (TIMP-1) and pro- α 1(I)collagen¹⁵². In animals, progression of renal failure is refrained by adsorbant administration, together with a diminished expression of the above mentioned factors¹⁵². Indoxyl sulfate refrains endothelial repair upon trauma¹⁵³ and induces endothelial free radical production¹⁵⁴.

Reduction of serum indoxyl sulfate concentration, by intra-intestinal absorption of the precursor indole, reduces uraemic itching¹⁵⁵. AST-120 retards the development of acquired renal cystic disease and aortic calcification¹⁵⁶ and ameliorates tubulo-interstitial injury by reducing the expression in the kidneys of ICAM-1, osteopontin, TGF- β 1 and clusterin in uninephrectomised rats¹⁵⁷. A large prospective clinical study in humans with AST-120 demonstrated a decrease of plasma concentration of indoxyl sulfate, but showed no clinical benefit¹⁵⁸, possibly because of a too short running time

Because of protein binding (approximately 100% in normal subjects and 90% in uraemics), the intradialytic behavior of indoxyl sulfate diverges from that of other small compounds such as creatinine. Removal by CAPD is more effective¹⁵⁹. Recent data show better dialysis removal with haemodialysis than with PD, which is however not translated in a proportional difference in indoxyl sulfate serum concentration¹⁶⁰. High-flux haemodialysis does not enhance removal²³. Alternative extracorporeal removal procedures such as haemoperfusion might be considered. Dialysis against albumin-containing dialysate removes albumin-bound uraemic toxins such as indoxyl sulfate more efficiently than conventional dialysis and may be useful for reducing these compounds¹⁶¹.

8) Oxidation products

Oxidative capacity is increased in uraemia¹⁶²⁻¹⁶⁴ both before and after the start of dialysis⁴⁵. Uraemic patients also show an impaired anti-oxidant response, partly related to plasma glutathione deficiency¹⁶⁵.

Oxidatively modified proteins act as mediators of oxidative stress and monocyte respiratory burst⁴⁵. Albumin seems to be one of the target proteins of these oxidative reactions^{45,166}. Structural modification of albumin may alter its binding capacity for drugs and other solutes¹⁶⁷. Modification of haemoglobin to glutathionylhaemoglobin has been proposed as another marker of oxidative stress¹⁶⁸.

Low-density lipoprotein (LDL) from uraemic patients is more susceptible to oxidation than that from control subjects¹⁶⁹ (oxidised LDL – oxLDL). This chemically modified LDL is more readily accumulated in macrophages, which results in the development of foam cells, an early event in atherogenesis. LDL autoantibodies against oxLDL have been demonstrated in CKD stage 5, especially in haemodialysed patients¹⁷⁰. Oxidative modification of the protein moiety of LDL is a trigger of macrophage respiratory burst^{171,172}.

Malondialdehyde levels are increased in CKD stage 5¹⁷³. The capacity of malondialdehyde to form DNA adducts¹⁷⁴, may play a patho-physiologic role in carcinogenesis. Low dose IV folinic acid given to dialysis patients reduced the levels of serum malondialdehyde and thus improved the cardiovascular risk profile¹⁷⁵.

Several small molecular compounds might also be modified by oxidation. Organic chloramines are generated by the chemical binding of hypochlorite, a free radical produced by activated leukocytes, to retained organic compounds¹⁷⁶.

9) Peptides

Peptides constitute a heterogeneous group of molecules. In general, peptides can be considered as typical MM. β_2 -M has been discussed previously.

Granulocyte inhibiting protein I (GIP I – 28 kD), recovered from uraemic sera or ultrafiltrate, suppresses the killing of invading bacteria by polymorphonuclear cells¹⁷⁷. The compound has structural analogy with the variable part of kappa light chains. Another peptide with granulocyte inhibitory effect (GIP II – 9.5 kD) is partially homologous with β_2 -M, and inhibits granulocyte glucose uptake and respiratory burst activity¹⁷⁸. A degranulation inhibiting protein (DIP – 24 kD), identical to angiogenin, was isolated from plasma ultrafiltrate of uraemic patients¹⁷⁹. The structure responsible for the inhibition of degranulation is different from the sites that are responsible for the angiogenic or ribonucleic activity of angiogenin. A structural variant of ubiquitin inhibits polymorphonuclear chemotaxis (chemotaxis inhibiting protein – CIP – 8.5 kD)¹⁸⁰.

Atrial natriuretic peptide (ANP – 3.1 kD) and endothelin (3.5 kD) are elevated in dialysis patients, and may play a role in the regulation of the blood pressure¹⁸¹. ANP levels correlate with left atrial size, fluid overload, decreased systemic clearance¹⁸² and cardio-vascular mortality¹⁸³, although the compound seems a marker of volume status rather than a toxic mediator. Endothelin causes peripheral insulin resistance, even at concentrations that induce no blood flow changes¹⁸⁴, and may play a role in uraemic hypertension¹⁸⁵. Endothelin-1 contributes to arterial stiffness, oxidative stress and inflammation¹⁸⁶.

The opioid peptides β -endorphin (3.5 kD), methionine-enkephalin (0.6 kD) and β -lipotropin (1.9 kD) are elevated in dialysed patients¹⁸⁷. Delta sleep-inducing peptide (0.9 kD) may modulate sleep-wakefulness¹⁸⁸.

Neuropeptide Y (NPY – 4.3 kD) is increased in uraemia¹⁸⁹, and tends to increase further during

haemodialysis¹⁹⁰. It is a 36-amino acid peptide with renal vasoconstrictive activity¹⁹¹. Recently, plasma NPY was found to predict incident cardiovascular complications in CKD stage 5¹⁹². NPY also acts as an orexigen¹⁹³. Uraemic patients with anorexia have lower NPY levels^{193,194}. However, constantly elevated NPY levels in CKD irrespective of food intake suggest a continuous tonic hypersecretion, and no key role in anorexia¹⁹⁵. The concentration of the anorexigen cholecystokinin (CCK) is increased in most patients with CKD¹⁹³. CCK is related to fullness and hunger perception in PD patients¹⁹⁶.

Adrenomedullin, a 52-amino acid and potent hypotensive peptide, is found at markedly increased concentrations in CKD patients¹⁹⁷, and activates inducible nitric oxide synthase¹⁹⁸. In CKD, plasma adrenomedullin concentration reflects cardiac dysfunction and hypervolaemia¹⁹⁹.

Cystatin C (13.3 kD), Clara cell protein (CC16) (15.8 kD) and retinol binding protein (RBP) (21.2 kD) are elevated in renal failure²⁰⁰. Cystatin C is an inhibitor of proteinases and cathepsins²⁰¹. CC16 is an immunosuppressive α -microprotein²⁰².

Leptin, a 16 kD plasma protein decreases appetite of uraemic patients²⁰³. The rise in serum leptin is mostly attributed to decreased renal elimination²⁰⁴, and is almost entirely limited to the free fraction²⁰⁴. Increased leptin is associated with low protein intake and loss of lean tissue in CKD²⁰³. Recent data suggest an inverted correlation between leptin and nutritional status²⁰⁵, and a direct correlation with C-reactive protein (CRP)²⁰⁶. In CAPD-patients, serum leptin showed a progressive rise only in patients with body weight loss²⁰⁷. Erythropoietin treatment results in a decline of leptinaemia and an improvement of nutritional status²⁰⁸. Leptin-receptor deficient mice resist uraemic cachexia²⁰⁹.

However, leptin levels are also elevated in obese people and are hence not necessarily related to reduced appetite. Body fat and serum leptin also correlate in uraemia²⁰⁶. Female gender and obesity are important factors that affect serum leptin also in CKD stage 5-patients²¹⁰. Don *et al.* suggest that in CKD stage 5-patients leptin may be depressed during inflammation and may actually act as a negative acute phase reactant²¹¹. Therefore, the biochemical role of leptin in renal failure remains inadequately defined.

Ghrelin is a recently described polypeptide hormone produced mainly by the stomach but also synthesised in various tissues including the kidney²¹². Ghrelin has been shown to stimulate a variety of nutrition related effects, such as Growth Hormone (GH) release from the pituitary gland²¹³, increase in food intake²¹⁴, fat accumulation, and body weight gain²¹⁵. A recent study described that plasma ghrelin was significantly increased in CKD patients compared with that in patients with normal renal function, and that plasma ghrelin was significantly correlated with both serum creatinine and GH. Moreover, hemi-nephrectomy in mice caused a marked increase in the plasma ghrelin without significant changes in ghrelin mRNA levels in the stomach, suggesting that the kidneys are important in ghrelin clearance²¹².

The status of ghrelin as uraemic toxin can be doubted. Recent data point at the favourable effects of subcutaneously administered ghrelin on the nutritional condition of malnourished patients on peritoneal dialysis²¹⁶ and on the vascular status of rats *in vivo* and *in vitro*²¹⁷.

10) Phenols

Phenol depresses various functional parameters of enzymatic activity in polymorphonuclear leukocytes²¹⁸. A depressive effect was demonstrated on the 3':5'-cyclic monophosphate response of the neostriatum to dopamine²¹⁹. This effect was abolished after conjugation of phenol to phenylglucuronide. These findings may be relevant to hepatic and uraemic coma. Phenol prevents *in vitro* the inhibition of parathyroid cell proliferation induced by calcitriol²²⁰.

Phenols are lipophilic and protein-bound, and their removal by haemodialysis is markedly less than that of urea and creatinine²³. Although most of the pioneering research on the phenolic compounds has focussed on the concentration and the toxicity of the mother compound p-cresol, later work revealed that genuine p-cresol was present at very low concentrations in patients with renal failure and that most of the p-cresol, generated by the intestinal flora, was conjugated to p-cresylsulfate in the intestinal wall and to p-cresylglucuronide in the liver^{221,222}. The reason for the incorrect previous emphasis on p-cresol was due to the fact that most determination

methods were based on deproteinisation by acidification, causing disintegration of the conjugates by hydrolysis. Application of deproteinisation methods without acidification revealed the presence of the conjugate p-cresylsulfate²²². Further studies indicated that the biochemical impact of the mother compound p-cresol is not necessarily the same as that of the conjugate. Whereas p-cresol suppresses activity of leukocytes, especially after their activation, p-cresylsulfate essentially appeared to be linked to baseline leukocyte activation²²³. Nevertheless, since there is very likely a correlation between former p-cresol estimations and current p-cresylsulfate measurements, previously held conclusions about protein binding and relationship with clinical outcome parameters of p-cresol^{30,31,224} are probably still valid.

Daily haemodialysis results in lower pre-dialysis serum levels compared to conventional alternate day dialysis²²⁵. In a haemodialysis setting, the removal of p-cresol and that of urea and creatinine are not correlated²³, demonstrating that the latter markers are not representative for the intra-dialytic behaviour of protein-bound compounds. Levels are markedly lower in PD, compared to haemodialysis^{29,160, 226}.

Hypoalbuminaemia and a rise in total concentration are correlated to an increase of the free active fraction³¹. A correlation between free p-cresol and rate was demonstrated³¹. Patients hospitalised for infection also had a higher free p-cresol³¹. P-cresol also correlates with clinical uraemic symptoms²²⁷ and with outcome parameters³².

11) Phenylacetic acid

Phenylacetic acid (PAA) is a degradation product of the amino acid, phenylalanine. Plasma concentrations of PAA in patients with CKD stage 5 strongly exceed those in healthy controls. PAA was shown to inhibit iNOS expression and consequently, NO production²²⁸, was identified as an inhibitor of Ca²⁺ ATPase activity in CKD stage 5²²⁹. PAA was recently shown to increase formation of ROS in VSMCs²³⁰ and to have inhibitory effects on macrophage-killing function²³¹.

12) Purines

Uric acid, xanthine and hypoxanthine are the most important purines retained in uraemia. The purines disturb calcitriol production and metabolism²³².

Administration of purines to animals results in a net decrease of serum calcitriol²³² and a decrease of uric acid in response to allopurinol administration results in a rise of plasma calcitriol levels²³³. Purines are involved in the resistance to calcitriol of immune competent cells²³⁴. Xanthine and hypoxanthine have been implicated as modulators of neurotransmission, poor appetite and weight loss²³⁵. Both xanthine and hypoxanthine induce vasoconstriction²³⁶ and disturb endothelial barriers²³⁷.

Uric acid is a small water soluble compound that is removed by haemodialysis from the plasma in a similar way as urea²³⁸, but removal from the intracellular compartment is by far not as efficient²³⁹. Dialytic removal of xanthine and hypoxanthine shows no correlation with that of urea and creatinine²³⁸.

■ CONCLUSIONS

The uraemic syndrome is the result of a complex set of biochemical and patho-physiologic disturbances, leading to a state of generalised malaise and dysfunction. This condition is related to the retention of a host of compounds, many of which exert a negative impact on key functions of the body and which have consequently been identified as uraemic toxins. Up to now, the toxic action of single solutes has repeatedly been studied, but the intermutual interference between compounds has rarely been considered. Although solute retention is one of the major patho-physiologic events, deficiencies are functionally important as well.

Removal and generation of many compounds with proven biological or biochemical impact, especially toxins which are hydrophobic and/or not generated from protein break-down, can hardly be predicted by the intra-dialytic behaviour of urea, a current marker but a small water soluble compound generated from protein, with relatively little biological impact.

Solute clearance eventually reaches a plateau as dialyser blood flow and/or dialysate flow are increased and this plateau is reached much sooner for molecules with a higher molecular weight. As a result, clearance of MM *stricto sensu* is relatively blood and dialysate flow independent. Only an increase of dialysis time, dialyser surface area, ultrafiltration rates and/or dialyser pore size can enhance their removal.

Removal of solutes that behave like larger molecules due to their protein binding, multicompartmental distribution and/or lipophilicity, will be less affected by the use of high flux dialysers and/or dialysers with a larger pore size. To improve the clearances of these “new definition MM”, it may be necessary to develop renal replacement systems with different characteristics, e.g. specific adsorption systems and/or procedures that allow a slower exchange of solutes.

Earlier concepts of adsorption, eventually largely abandoned, should perhaps be reconsidered, especially for the removal of organic acids. More specific and/or more efficient adsorptive systems may be needed, however. As an alternative, adsorption of toxins or of their precursors may be pursued at the intestinal level.

In addition to improving removal, a second option is to neutralise the toxic effects of uraemic retention solutes by drug administration. A number of such measures have already been taken, mostly based on empirical experience or from evidence collected in the general population. This approach has the advantage of impacting not only upon the $\pm 0.1\%$ of the global population with CKD stage 5, but as well on the $\pm 10\%$ of the population with CKD stages 3 and 4, who are also affected by the major consequences of uraemia such as cardiovascular disease²⁴⁰. A further option might be the modification of the intestinal flora to affect the generation of uraemic toxins or their precursors²⁴¹.

Conflict of interest statement. None declared.

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