What Is Uremia? Retention versus Oxidation

R. Vanholder E. Schepers N. Meert N. Lameire

Nephrology Section, Department of Internal Medicine, University Hospital, Ghent, Belgium

Key Words

Uremic toxins · Retention solutes · Guanidino compounds

Abstract

The uremic syndrome is characterized by the retention of a host of compounds which in healthy subjects are secreted into the urine by the healthy kidneys. These compounds disturb many physiologic functions, resulting in toxicity. However, many of the retained compounds as well as many of the pathophysiologic actions of the known retention solutes remain unknown. In this publication, we review recent information on uremic toxicity. Especially the difficulty to remove compounds, such as protein-bound compounds and larger molecules, seems to play a crucial role. New strategies enhancing their removal might be highly useful. Part of the retained compounds are the result of oxidative processes due to the inflammatory status of uremic patients; however, other compounds are not, and even the concentration of oxidative compounds will be further increased by disturbances of urinary clearance.

Copyright © 2006 S. Karger AG, Basel

Introduction

The uremic syndrome is attributed to the retention of a host of compounds, which under normal circumstances are eliminated by the healthy kidneys. If those compounds

interfere with biological/biochemical functions, they are called uremic toxins. This toxicity results in the loss of a substantial number of biochemical and biological functions, which gradually deteriorate as uremic retention becomes more important.

Initial characterization and classification of relevant toxins depend to a large extent on in vitro studies, whereby potential toxins are brought into contact with cell systems as a reproduction of uremic functional changes. These studies help to identify new potential toxins and/or characterize new as yet unrecognized functional defects attributable to already known compounds. In this publication, we describe a number of newly defined retention compounds, as well as some already known, in which as yet unknown biological effects were recently depicted. Since patients with renal failure are often subjected to microinflammation resulting in oxidative processes, we will also focus on whether these toxic compounds are generated by oxidation or not.

In 2003, the European Uremic Toxin Work Group published an encyclopedic review of the retention solutes known at that time, together with their normal and uremic concentrations [1]. In the latter analysis, 90 different uremic solutes were identified and subdivided according to their physicochemical characteristics into (1) small water-soluble compounds (molecular weight <500 Da), (2) protein-bound solutes, and (3) larger middle molecules (molecular weight >500 Da). The physicochemical characteristics are important to explain the behavior of compounds during dialysis, the small water-soluble compounds being removed easily by all dialysis strategies, and

the larger middle molecules being difficult to remove unless dialyzers with a large pore size are applied (so-called high-flux membranes). The protein-bound compounds are difficult to remove in spite of low molecular weight because protein binding imposes resistance against transmembrane transfer.

Only a limited fraction of the molecules depicted in the above mentioned encyclopedic review are known end products of oxidative processes. In essence, these are the advanced glycation end products, such as pentosidine and carboxymethyllysine. All other compounds are retained as a result of inadequate clearance by the kidneys and/or defective metabolism as a result of kidney failure. First, peptidic compounds, which are essentially classified as middle molecules, may be present in their genuine form as well as after metabolic transformation; one of the pathways provoking these transformations is caused by oxidation [2, 3]. Second, also other compounds may be modified, at least in part, by oxidation. A third process with potential impact related to oxidation results in the structural alteration of albumin, possibly resulting in the liberation of protein-bound solutes from their binding sites [4], enhancing their toxic potential. However, in essence, it can be accepted that molecules are at least in part retained in their genuine, unmodified form, and that oxidation cannot explain the entire uremic process.

We will now describe a number of uremic solutes which have been shown to be retained in uremia to exert biological actions that could contribute to the uremic syndrome, and which are very likely not the result of oxidation. Some of these, in being pro-inflammatory, might induce oxidation themselves.

Exemplary Compounds

Dinucleoside Polyphosphates

Dinucleoside polyphosphates are newly detected protein-bound uremic solutes which are structurally constituted by two nucleosides at both ends of the structure, linked together by a variable number of phosphate moieties [5]. Diadenosine polyphosphates have previously been demonstrated to accumulate in the case of renal failure, especially in the platelets, and to induce smooth muscle cell proliferation [5, 6].

In this group of compounds, a new molecule was recently detected: uridine adenosine tetraphosphate [7]. It was shown that this compound was present in endothelial cells and released from those cells when they were stimulated. This compound is a much stronger vasocon-

strictor than endothelin, a vasoconstrictive peptide which is released by the endothelium as well. The vasoconstrictive effect of uridine adenosine tetraphosphate could be neutralized by specific purine receptor antagonists. Uremic concentrations of the latter compound, either intra- or extracellular, are not yet known. If we extrapolate the data known for diadenosine polyphosphates, it might however be possible that we are confronted here with a new strong uremic toxin.

Guanidino Compounds

Guanidino compounds are small water-soluble solutes to which neurotoxic effects have been attributed [8]. Many guanidino compounds, especially guanidine, guanidinosuccinic acid and creatinine as a precursor of methylguanidine, as well as methylguanidine itself are highly increased in uremic biological fluids and tissues [9].

Until recently, no effects with cardiovascular damaging potential have been attributed to the guanidines, except for asymmetric dimethylarginine [10], which was shown to inhibit inducible nitric oxide synthase (iNOS) [11], a protective enzyme of endothelium. However, the concentrations of this compound as they are described in uremia [12] do not necessarily correspond to those inhibiting iNOS [11].

In a recent study, Glorieux et al. [13], demonstrated that several other guanidino compounds stimulated leukocytes. Methylguanidine and guanidine stimulated the proliferation of undifferentiated HL-60 cells, and the antiproliferative effect of calcitriol was neutralized in the presence of methylguanidine and guanidinosuccinic acid. The phorbol-myristate-acetate-stimulated chemiluminescence production of differentiated HL-60 cells was enhanced in the presence of guanidine. Methylguanidine and guanidinoacetic acid enhanced the lipopolysaccharide-stimulated intracellular production of tumor necrosis factor- α by normal human monocytes. Since atherosclerosis is nowadays considered as an inflammatory disorder whereby adhesion of activated leukocytes to the endothelium induces the first steps towards plaque formation [14], these compounds have thus a damaging potential on the vessels.

In another recent study, Perna et al. [15] evaluated the impact of a panel of uremic solutes on the structural modification of albumin by deamidation. Plasma proteins from hemodialysis patients showed an increase in deamidated/isomerized asparagine and aspartic acid moieties, and the incubation of normal plasma with various uremic toxins caused a similar deamidation reaction as well. Guanidine and guanidinopropionic acid showed the

highest degree of protein modification in a dose-responsive manner. Deamidated human albumin had a reduced binding capacity to homocysteine, hence increasing the toxic potential of this compound.

Guanidino compounds, like urea, are small water-soluble substances and could be expected to show a kinetic behavior similar to that of urea, i.e. an easy removal by any dialysis strategy. Eloit et al. [16] subjected several guanidino compounds such as guanidine, methylguanidine, creatinine, creatine, guanidinosuccinic acid and guanidinoacetic acid to intradialytic kinetic analysis, and compared those kinetics with that of urea. In spite of being water-soluble and small like urea, virtually all guanidino compounds were characterized by a markedly larger distribution volume as compared with urea. The distribution volumes of methylguanidine and guanidinoacetic acid were even more than twice as large as that of urea. This resulted in a dramatic decrease in effective removal which can probably be corrected only by changing the concept of dialysis, essentially by increasing dialysis duration and/or frequency.

In summary, recent data point to a role for guanidino compounds in vascular toxicity, next to their traditionally accepted neurotoxicity. In spite of their physicochemical characteristics which seemingly are comparable with those of urea, their intradialytic behavior is markedly different, so that they can be classified as molecules which are difficult to remove. A similar kinetic disparity versus urea has previously been described for other water-soluble compounds, such as phosphate and the purines xanthine and hypoxanthine [17].

Indoles and Phenols

Indoles and phenols belong to the group of protein-bound solutes which as individual compounds contain a heterogeneous set of molecules, such as *p*-cresol, indoxyl sulfate, hippuric acid and homocysteine, which are all hard to remove during dialysis [18].

Multiple toxic effects have been attributed to these compounds, but until recently, no data were available regarding a potential vascular impact, except for homocysteine.

Dou et al. [19] recently investigated the *in vitro* effect of a large panel of uremic retention solutes on endothelial proliferation and endothelial repair in human umbilical vein endothelial cells. Both these functions were significantly inhibited by indoxyl sulfate. The presence of albumin did not affect the inhibitory effect. Hence, these solutes could play a role in endothelial dysfunction observed in uremic patients.

To these data should be added the studies by D'Hooge et al. [20], showing neurotoxic effects for indoxyl sulfate, spermine and the phenols, and by Canalejo et al. [21], showing that HPLC fractions containing protein-bound compounds, and phenol as individual compound, inhibited the regulatory impact of calcitriol on parathyroid gland cells, hence inducing calcitriol resistance.

Of note, two recent clinical studies pointed to a relationship between *p*-cresol and the clinical condition in uremia [22, 23].

The potential impact of guanidino compounds on the liberation of homocysteine from its albumin binding sites has already been pointed out above [15]. Further, homocysteine has been linked to altered DNA methylation and altered gene expression patterns, and treatment with folic acid not only decreases plasma homocysteine levels, but also restores DNA and gene expression back to normal [24].

Recent data indicate that *p*-cresol, and by extension probably other phenolic compounds as well, do not circulate in the body, but as conjugates, e.g., *p*-cresol sulfate and *p*-cresol glucuronide [25].

Phenylacetic Acid

Phenylacetic acid is a protein-bound compound which is present at increased concentrations in uremic plasma and was shown to inhibit Ca^{2+} ATPase [26].

More recently, Jankowski et al. [27] tested the hypothesis that uremic toxins are responsible for reduced iNOS expression. NO prevents atherogenesis and inflammation in vessel walls by inhibition of cell proliferation and cytokine-induced endothelial expression of adhesion molecules and proinflammatory cytokines. Reduced NO production due to inhibition of either endogenous NOS or iNOS may therefore reinforce vascular damage. In the above mentioned study, lipopolysaccharide-induced iNOS expression in mononuclear leukocytes was examined using real-time polymerase chain reaction [27]. The iNOS expression was blocked by addition of plasma from patients with stage 5 renal disease, whereas plasma from healthy controls had no effect. Chromatographic fractionation of hemofiltrate obtained from patients with stage 5 renal disease revealed a homogenous fraction inhibiting iNOS expression. Using gas chromatography/mass spectrometry, this inhibitor was identified as phenylacetic acid. Authentic phenylacetic acid inhibited iNOS expression dose-dependently. Patients with stage 5 renal failure had a substantial increase in phenylacetic acid serum concentration. Since accumulation of phenylacetic acid in these patients inhibited iNOS expression, this mechanism

may contribute to increased atherosclerosis and cardiovascular morbidity in the presence of renal failure.

Unidentified Peptides

Reviewing the uremic retention compounds which have been identified reveals that only close to 100 uremic retention solutes have been described [1]. Very likely, much more compounds are retained in uremia. To examine unknown uremic substances thoroughly, the identification of as many compounds as possible in the ultrafiltrate and/or plasma of patients would lead to a less biased definition of the uremic retention process compared with what is proposed today. Proteomic analysis is a novel tool for the identification of a large number of molecules present in biological fluids. In a study on ultrafiltrate from uremic and normal plasma obtained with high- or low-flux dialysis membranes, Weissinger et al. [28] used separation by capillary electrophoresis coupled with on-line mass spectrometry, yielding identification of as yet unknown polypeptides based on their molecular weight. Between 500 and >1,000 different polypeptides with a molecular weight ranging from 800 to 10,000 Da could be detected in individual samples and were identified via their mass and their particular migration time in capillary electrophoresis. In the ultrafiltrate from uremic plasma, 1,394 polypeptides were detected in the high-flux versus 1,046 in the low-flux samples, while 544 versus 490 polypeptides were found in the ultrafiltrate from normal plasma obtained from membranes with comparable cut-off. In addition, polypeptides >5 kDa were virtually only detected in the uremic ultrafiltrate from the high-flux membrane ($n = 28$ vs. $n = 5$ with the low-flux membrane). To demonstrate the feasibility of further characterizing the detected molecules, polypeptides exclusively present in the uremic ultrafiltrate were chosen for sequencing analyses. A 950.6-Da polypeptide was identified as a fragment of the salivary proline-rich protein and a 1,291.8-Da fragment was a derivative from α -fibrinogen. These data strongly suggest that the application of proteomic approaches such as capillary electrophoresis and mass spectrometry will result in the identification of many more uremic solutes than those known at present.

It is conceivable that similar strategies might also be useful for the analysis of smaller, non-peptidic compounds. Part of these yet unknown variants might be the result of oxidative modification, as suggested by the presence of several structures which elute close to each other; however, this does not exclude that the genuine mother compounds should be present as well and exert biological activity per se.

Conclusions

Uremic retention corresponds to the accumulation of a host of compounds in the body. Some of these are relatively innocuous, such as urea, whereas others exert a myriad of biological and biochemical activities, resulting, at the clinical level, in the uremic syndrome. Unfortunately, our knowledge about the true culprits as well as the spectrum of activities of individual compounds remains largely incomplete. There is an urgent need for a complete mapping and classification of uremic retention compounds according to importance, before the development of therapeutic strategies will exceed the stage of empiricism. To reach this aim, an unbiased approach is needed, avoiding a search for elements whose role is already known to a large extent, if not completely. New research tools such as genomic, functional genomic and proteome analysis are promising strategies to accomplish these aims.

Undoubtedly, part of the retention compounds accumulate because of decreased renal excretion and/or deficient metabolism in the presence of renal failure. On the other hand, these compounds are often present in a pro-inflammatory milieu, which might result in the oxidative modification of retained molecules. Oxidative modifications in turn change the properties of retained molecules which might themselves become pro-inflammatory [29]. It remains difficult to perceive whether all noxious substances are the result of oxidation, and even if this would be the case, the harmful effect of such a sequence is largely aggravated by the increased concentrations, merely due to the retention process per se.

It might be interesting to transfer these ideas to the clinical problem which is as of today one of the greatest concerns for the nephrological community: the enhanced cardiovascular risk in uremia. Patients with renal failure are prone to develop cardiovascular problems and these start long before the dialysis stage has been reached. Moreover, the weight of kidney failure in its relation to cardiovascular risk remains, even if corrections are applied for traditional as well as less traditional risk factors. Subsequently, it is acceptable that renal failure per se is also a cause of vascular lesions, and if this is the case, it is conceivable that vascular damage is provoked by the factors retained in renal failure.

Some of these factors, as well as their potential role in cardiovascular damage, are known and might be related to functional changes in white blood cells, platelets, smooth muscle cells and endothelium [30]. While reviewing these factors in 2001 [30], it became already clear that

most factors involved were either larger 'middle' molecules or protein-bound molecules. The data reviewed at present add to this perception. The only solutes which do not conform with this definition are the guanidino compounds, but according to kinetic studies, these substances show a kinetic behavior that is divergent from the standard small water-soluble compound, which is urea. Hence, most or at least a substantial part of molecules involved in uremic toxicity and vascular damage seem to be difficult to remove, so that standard dialytic approaches might be less relevant. Therefore, alternative approaches such as convective strategies, treatment with extremely open membranes which are permeable for proteins, adsorption, alternative timeframes (long slow dialysis, daily di-

alysis) and medicamentous approaches aiming at blockage of receptors and activation pathways induced by toxins as well as at modification of the metabolic generation or breakdown of those toxins are needed.

Part of these molecules are the result of oxidation. A typical example are the advanced glycation end products, which have been shown to increase the baseline activity of white blood cells [29, 31, 32]. The reported data show that such effects cannot be expected from oxidation alone, and at least part of the damage is due to mere retention.

In parallel to these efforts, a more complete mapping of responsible toxins and a classification of their importance should be pursued as well.

References

- 1 Vanholder R, De Smet R, Glorieux G, Argiles A, Baurmeister U, Brunet P, Clark W, Cohen G, De Deyn PP, Deppisch R, Descamps-Latscha B, Henle T, Jorres A, Lemke HD, Massy ZA, Passlick-Deetjen J, Rodriguez M, Stegmayr B, Stenvinkel P, Tetta C, Wanner C, Zidek W: Review on uremic toxins: classification, concentration, and interindividual variability. *Kidney Int* 2003;63:1934–1943.
- 2 Ward RA, Brinkley KA: A proteomic analysis of proteins removed by ultrafiltration during extracorporeal renal replacement therapy. *Contrib Nephrol* 2004;141:280–291.
- 3 Ward RA, Ouseph R, McLeish KR: Effects of high-flux hemodialysis on oxidant stress. *Kidney Int* 2003;63:353–359.
- 4 Sarnatskaya VV, Ivanov AI, Nikolaev VG, Rottel E, von Appen K, Haspar M, Maslenny VN, Klinkmann H: Structure and binding properties of serum albumin in uremic patients at different periods of hemodialysis. *Artif Organs* 1998;22:107–115.
- 5 Jankowski J, Hagemann J, Yoon MS, van der Giet M, Stephan N, Zidek W, Schluter H, Tepel M: Increased vascular growth in hemodialysis patients induced by platelet-derived adenosine polyphosphates. *Kidney Int* 2001;59:1134–1141.
- 6 Jankowski V, Vanholder R, Henning L, Karadogan S, Zidek W, Schluter H, Jankowski J: Isolation and quantification of dinucleoside polyphosphates by using monolithic reversed phase chromatography columns. *J Chromatogr* 2005;819:131–139.
- 7 Jankowski V, Tolle M, Vanholder R, Schonfelder G, van der Giet M, Henning L, Schluter H, Paul M, Zidek W, Jankowski J: Uridine adenosine tetraphosphate: a novel endothelium-derived vasoconstrictive factor. *Nat Med* 2005;11:223–227.
- 8 De Deyn PP, D'Hooge R, Van Bogaert PP, Marescau B: Endogenous guanidino compounds as uremic neurotoxins. *Kidney Int* 2001;59(suppl 78):S77–S83.
- 9 De Deyn PP, Marescau B, Cuykens JJ, Van Gorp L, Lowenthal A, De Potter WP: Guanidino compounds in serum and cerebrospinal fluid of non-dialyzed patients with renal insufficiency. *Clin Chim Acta* 1987;167:81–88.
- 10 Zoccali C, Benedetto FA, Maas R, Mallamaci F, Tripepi G, Malatino LS, Boger R: Asymmetric dimethylarginine, C-reactive protein, and carotid intima-media thickness in end-stage renal disease. *J Am Soc Nephrol* 2002;13:490–496.
- 11 Vallance P, Leone A, Calver A, Collier J, Moncada S: Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 1992;339:572–575.
- 12 Marescau B, Nagels G, Possemiers I, De Broe ME, Becaus I, Billioud JM, Lornoy W, De Deyn PP: Guanidino compounds in serum and urine of nondialyzed patients with chronic renal insufficiency. *Metabolism* 1997;46:1024–1031.
- 13 Glorieux GL, Dhondt AW, Jacobs P, Van Langeraert J, Lameire NH, De Deyn PP, Vanholder RC: In vitro study of the potential role of guanidines in leukocyte functions related to atherogenesis and infection. *Kidney Int* 2004;65:2184–2192.
- 14 Ross R: Atherosclerosis – An inflammatory disease. *N Engl J Med* 1999;340:115–126.
- 15 Perna AF, Ingrosso D, Satta E, Lombardi C, Galletti P, D'Aniello A, De Santo NG: Plasma protein aspartyl damage is increased in hemodialysis patients: studies on causes and consequences. *J Am Soc Nephrol* 2004;15:2747–2754.
- 16 Elout S, Torremans A, De Smet R, Marescau B, De Wachter D, De Deyn PP, Lameire N, Verdonck P, Vanholder R: Kinetic behavior of urea is different from that of other water-soluble compounds: the case of the guanidino compounds. *Kidney Int* 2005;67:1566–1575.
- 17 Vanholder RC, De Smet RV, Ringoir SM: Assessment of urea and other uremic markers for quantification of dialysis efficacy. *Clin Chem* 1992;38:1429–1436.
- 18 Lesaffer G, De Smet R, Lameire N, Dhondt A, Duym P, Vanholder R: Intradialytic removal of protein-bound uraemic toxins: role of solute characteristics and of dialyser membrane. *Nephrol Dial Transplant* 2000;15:50–57.
- 19 Dou L, Bertrand E, Cerini C, Faure V, Sampol J, Vanholder R, Berland Y, Brunet P: The uremic solutes *p*-cresol and indoxyl sulfate inhibit endothelial proliferation and wound repair. *Kidney Int* 2004;65:442–451.
- 20 D'Hooge R, Van De Vijver G, Van Bogaert PP, Marescau B, Vanholder R, De Deyn PP: Involvement of voltage- and ligand-gated Ca^{2+} channels in the neuroexcitatory and synergistic effects of putative uremic neurotoxins. *Kidney Int* 2003;63:1764–1775.
- 21 Canalejo A, Almaden Y, De Smet R, Glorieux G, Garfia B, Luque F, Vanholder R, Rodriguez M: Effects of uremic ultrafiltrate on the regulation of the parathyroid cell cycle by calcitriol. *Kidney Int* 2003;63:732–737.
- 22 Bammens B, Evenepoel P, Verbeke K, Vanrenterghem Y: Removal of middle molecules and protein-bound solutes by peritoneal dialysis and relation with uremic symptoms. *Kidney Int* 2003;64:2238–2243.
- 23 De Smet R, Van Kaer J, Van Vlem B, De Cubber A, Brunet P, Lameire N, Vanholder R: Toxicity of free *p*-cresol: a prospective and cross-sectional analysis. *Clin Chem* 2003;49:470–478.

- 24 Ingrosso D, Cimmino A, Perna AF, Masella L, De Santo NG, De Bonis ML, Vacca M, D'Esposito M, D'Urso M, Galletti P, Zappia V: Folate treatment and unbalanced methylation and changes of allelic expression induced by hyperhomocysteinaemia in patients with uraemia. *Lancet* 2003;361:1693–1699.
- 25 De Loor H, Bammens B, Evenepoel P, De Preter V, Verbeke K: Gas chromatographic-mass spectrometric analysis for measurement of *p*-cresol and its conjugated metabolites in uremic and normal serum. *Clin Chem* 2005; 51:1535–1538.
- 26 Jankowski J, Luftmann H, Tepel M, Leibfritz D, Zidek W, Schluter H: Characterization of dimethylguanosine, phenylethylamine, and phenylacetic acid as inhibitors of Ca²⁺ ATPase in end-stage renal failure. *J Am Soc Nephrol* 1998;9:1249–1257.
- 27 Jankowski J, van der Giet M, Jankowski V, Schmidt S, Hemeier M, Mahn B, Giebing G, Tolle M, Luftmann H, Schluter H, Zidek W, Tepel M: Increased plasma phenylacetic acid in patients with end-stage renal failure inhibits iNOS expression. *J Clin Invest* 2003;112:256–264.
- 28 Weissinger EM, Kaiser T, Meert N, De Smet R, Walden M, Mischak H, Vanholder RC: Proteomics: a novel tool to unravel the pathophysiology of uraemia. *Nephrol Dial Transplant* 2004;19:3068–3077.
- 29 Witko-Sarsat V, Friedlander M, Nguyen KT, Capeillere-Blandin C, Nguyen AT, Canteloup S, Dayer JM, Jungers P, Drueke T, Descamps-Latscha B: Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. *J Immunol* 1998;161:2524–2532.
- 30 Vanholder R, Argiles A, Baurmeister U, Brunet P, Clark W, Cohen G, De Deyn PP, Depisch R, Descamps-Latscha B, Henle T, Jorres A, Massy ZA, Rodriguez M, Stegmayr B, Stenvinkel P, Wratten ML: Uremic toxicity: present state of the art. *Int J Artif Organs* 2001;24: 695–725.
- 31 Glorieux G, Vanholder R, Lameire N: Advanced glycation and the immune system: stimulation, inhibition or both? *Eur J Clin Invest* 2001;31:1015–1018.
- 32 Bernheim J, Rashid G, Gavrieli R, Korzets Z, Wolach B: In vitro effect of advanced glycation end-products on human polymorphonuclear superoxide production. *Eur J Clin Invest* 2001; 31:1064–1069.