A Bench to Bedside View of Uremic Toxins

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ABSTRACT

Reviewing the current picture of uremic toxicity reveals its complexity. Focusing on cardiovascular damage as a model of uremic effects resulting in substantial morbidity and mortality, most molecules with potential to affect the function of a variety of cell types within the vascular system are difficult to remove by dialysis. Examples are the larger middle molecular weight molecules and protein-bound molecules. Recent clinical studies suggest that enhancing the removal of these compounds is beneficial for survival. Future therapeutic options are discussed, including improved removal of toxins and the search for pharmacologic strategies blocking responsible pathophysiologic pathways.


During the development of the uremic syndrome, losses of kidney function are accompanied by deteriorating organ function attributable to the accumulation of uremic retention solutes. Compounds that exert an adverse biologic impact are called uremic toxins.1 Recently, a complex picture has emerged of multiple compounds with different characteristics exerting divergent effects on organs.1,2 Does it make sense to seek out new uremic compounds or as yet undetected effects of known uremic toxins? In this review, we highlight how recent advances in our knowledge of uremic toxicity have led us to modify existing therapeutic concepts and discuss novel approaches that may be useful for future therapies of uremia.

In particular, we focus on the mechanisms potentially responsible for uremic cardiovascular damage, the major cause of morbidity and mortality in patients with chronic kidney disease (CKD).3,4 Because the treatment of traditional risk factors for cardiovascular disease in the CKD population only partially reduces the proportion of cardiovascular deaths in comparison with the effects observed in the general population,5 there is a need to elucidate these additional atherogenic mechanisms. This knowledge might, in turn, be applicable to the wider population, should the compounds that are pathophysiologically active in uremia also be elevated in people without CKD, as has already been observed for homocysteine and advanced glycation end products (AGE).

COMPLEXITY OF UREMIC TOXICITY AND UREMIC TOXIN RESEARCH

In 2003, the European Uremic Toxin Work Group (Eutox; http://EUTox.info) listed the 90 different uremic retention solutes known at that time.6 Since then, at least 25 additional retention solutes have been identified,6,7 creating a far more complex picture than was accepted a few years ago.

This highly diverse group of uremic retention solutes includes low molecular weight organic substances as well as peptides. As a result of differing hydrophobicity, low molecular weight organic compounds may either exist in free water-soluble forms or bind reversibly to serum proteins, thereby altering protein functions, such as reducing drug-binding capacity.8 In CKD, peptides may be found in their native form or, as a consequence of exposure to the uremic milieu, become irreversibly altered through posttranslational modifications, resulting in changes in structure and function. Examples include the heterogeneous group of AGE, advanced oxidation protein products, and carbamoylated proteins, which occur when amino groups are modified by cyanate, which is spontaneously transformed from urea. The molecular weight of most of these peptides belongs to the higher “middle molecular” range (10 to 30 kD). Importantly, both protein-bound solutes and peptides are particularly difficult to re-

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move by conventional dialysis treatments.

The identification and characterization of uremic retention solutes playing a main role in uremia-related complications is a prerequisite for the critical evaluation and systematic design of preventive and therapeutic interventions for patients with CKD. In vitro assays testing the biologic effects of individual solutes represent a straightforward tool to select rapidly candidates for further in-depth investigation; however, uniform approaches to the preparation of these compounds and the experimental techniques used are necessary to obtain reliable and comparable results. EUTox recently published basic protocols for the in vitro screening of uremic retention solutes, providing information about their availability, solubility, and the appropriate preparation of stock solutions. The use of the correct concentrations of solutes is a precondition to obtaining relevant conclusions, and it is recommended that the highest reported concentration in uremic plasma be used as a starting point, with evaluation of concentration dependence in cases in which a significant biologic effect is observed. The application of appropriate control conditions is necessary for the correct interpretation of the observed effects.

To evaluate the pathophysiologic impact of CKD, any biologic model system representative of the cellular dysfunction caused by uremia can be used, for example, by leukocytes for diminished immune defense or oxidative stress, endothelial cells for cardiovascular disease, smooth muscle cells for the progression of atherosclerosis, hepatocytes for disturbed metabolism, fibroblasts for fibrosis, and osteoblasts for renal osteodystrophy. Human cells should be used whenever possible, and if animal models are studied, a species for which the relevance to the human condition has already been proved should be chosen. In the following section, some recently described examples of uremic retention solutes are discussed with the potential to affect vascular damage.

**AFFECTED CELL SYSTEMS**

**Leukocytes**

For many years, studies have revealed dual effects of uremic retention solutes on leukocyte function: Blunting upon stimulation, which has been linked to infection, and basal activation linked to microinflammation, malnutrition, and atherosclerosis. The major leukocyte subtypes affected by uremic conditions are polymorphonuclear cells, specifically neutrophils and mononuclear cells of the monocyte/macrophage type. It is predominantly the latter cell type that is activated by uremic retention solutes, enhancing vascular damage.

Guanidino compounds are small water-soluble uremic retention solutes that have been implicated in neurotoxicity. Until recently, no potential for cardiovascular damage had been attributed to the guanidines except for asymmetric dimethylarginine, which inhibits inducible nitric oxide synthase (iNOS), an endothelial protective enzyme; however, guanidino compounds have now been shown to stimulate leukocytes, with methylguanidine and guanidino acetic acid significantly enhancing the LPS-stimulated production of TNF-α by normal monocytes.

AGE accumulate in the plasma of uremic patients and induce an increase in leukocyte oxidative stress. Until recently, the biologic effect of AGE had been studied mainly with artificially prepared AGE, which might not be representative of AGE compounds really present in uremia, such as fructoselysin, N-ε-carboxymethyllysine, pyrraline, or pentosidine. Glorieux et al. studied the proinflammatory effect of several AGE compounds that are retained in uremia, Arg I (arginine modified with glyoxal), carboxyethyllysine, and carboxymethyllysine, demonstrating increased production of free radicals by mononuclear cells. It is interesting that one of the studied AGE (Arg II) had no effect at all on leukocytes, showing that the behavior of a number of compounds belonging to a specific group cannot automatically be extrapolated to all solutes of this group.

Because it has been established that p-cresol in humans exists predominantly as the conjugate p-cresylsulfate (pCS), which is a protein-bound substance, the effect of pCS on leukocyte oxidative burst activity has been compared with that of the parent compound, p-cresol. Whereas p-cresol suppresses leukocyte activity, p-cresylsulfate enhances baseline leukocyte activity. This highlights the important point that conjugates do not necessarily have the same effects as the parent compound.

Homocysteine, another protein-bound uremic toxin, activates NF-κB in macrophages, which is associated with a significant increase in intracellular superoxide anion levels, an effect abolished by folic acid. Phenylacetic acid, also a protein-bound retention solute, inhibits its iNOS expression in a dosage-dependent manner. Inhibition of either endogenous NOS or iNOS may reinforce vascular damage. Furthermore, phenylacetic acid inhibits Ca^{2+} -ATPase activity, increasing intracellular Ca^{2+} concentrations.

Napoleone et al. demonstrated that leptin, a protein-bound peptide that accumulates in uremia, induces tissue factor expression by mononuclear cells. Tissue factor is a pivotal agonist in the clotting cascade and contributes to atherosclerosis by playing a key role in thrombosis and inflammation. When either a leptin antibody or leptin receptor antibody was added in these experiments, before leptin exposure, the observed effect was inhibited.

**Endothelium**

Endothelial dysfunction plays an important role in the development of atherosclerotic vascular disease. Besides the classical causes of endothelial dysfunction, such as hypertension, diabetes, and dyslipidemia, CKD per se also plays a role. Patients with CKD have alterations in endothelial properties with increases in both plasminogen activator inhibitor-1 and von Willebrand factor, whereas tissue plasminogen activator decreases, suggesting a procoagulant state at the endothelial surface. Regulation of vascular tone is also impaired with de-
creased endothelium-dependent vasodila-
tion\(^{39}\) associated with the inhibition of
endothelial NOS by uremic solutes such as
asymmetric dimethylarginine,\(^{17}\)
AGE,\(^{31}\) and homocysteine.\(^{32}\) CKD also
induces oxidant stress and inflammation
in endothelial cells and production of re-
atve oxygen species in cultured endo-
thelial cells by the protein-bound uremic
toxin indoxyl sulfate.\(^{33}\) TNF synthesis is
also enhanced by AGE.\(^{31}\)

A new insight into endothelial dys-
function is also provided by the obser-
vation of circulating endothelial mi-
croparticles. These are intact vesicles
derived from cell membranes that arise
from two processes, cell membrane ac-
tivation and apoptosis.\(^{34}\) Micropar-
ticles can originate from endothelial
cells and also from other cells, such as
platelets, monocytes, granulocytes,
and erythrocytes. Microparticles are
involved in the regulation of coagula-
tion and apoptosis, and pathologic
conditions associated with micropar-
ticles have been described. A defect in
microparticle generation is responsible
for Scott syndrome, a bleeding disor-
der,\(^{34}\) whereas increased microparticle
formation is observed in cardiovascu-
lar disease, diabetes, and both undia-
lized and hemodialyzed patients with
CKD.\(^{35,36}\) The generation of endo-
thelial microparticles is elicited \textit{in vitro}
by the presence of indoxyl sulfate.\(^{35}\) Pa-
tients who had CKD and were treated
with high-efficiency hemodiafiltration
during 4 mo showed a decrease in the
number of endothelial microparticles
when compared with patients who
were treated with conventional high-
flux hemodialysis.\(^{37}\)

A remarkable characteristic of the
endothelium is its capacity for contin-
uous regeneration and repair.\(^{38}\) This
involves two mechanisms: The classi-
cally described proliferation of adja-
cent endothelial cells and the more re-
cently described homing of circulating
endothelial progenitor cells (EPC).\(^{38}\)
These latter cells may be mobilized
from bone marrow in response to cyto-
kines or ischemia or derive from circu-
lating leukocytes.\(^{39}\) In CKD, endo-
thelial repair mechanisms are altered,
representing a possible threat to vascu-
lar integrity. Some uremic toxins such as
indoxyl sulfate reduce endothelial
proliferation,\(^{40}\) and serum from ure-
mic patients decreases the ability of
EPC to migrate.\(^{41}\) In addition, patients
with CKD generally have a decrease in
the number of circulating EPC,\(^{42}\) al-
though contrary observations have
been described, possibly as a result of
inflammation or ischemia.\(^{41}\)

\textbf{Other Effects}
Besides leukocytes and endothelial cells,
platelets also play a central role in vas-
cular damage by inducing hemostasis and
arterial thrombosis.\(^{43}\) Platelets interact
with coagulation factors, in particular
thrombin, a potent platelet-activating
agonist,\(^{44}\) and during thrombin-induced
aggregation, almost the entire content of
platelet granules is released.\(^{45,46}\)

Platelets from patients with renal fail-
ure have increased intracellular concen-
tration of the diadenosine polyphos-
phates. Diadenosine pentaphosphate
(Ap\(_5\)A) and diadenosine hexaphosphate
(Ap\(_6\)A) act as strong growth factors for
vascular smooth muscle cells (VSMC)
via P2Y receptors.\(^{47}\) Because enhanced
VSMC growth is a hallmark of athero-
sclerosis in renal failure, the increased
amount of diadenosine polyphosphates
in platelets may play an important role in
causing increased cardiovascular dam-
age. Furthermore, diadenosine polyphos-
phates are strong vasoconstrictors with
direct effects on vascular tone mediated
by P2X receptors.\(^{48,49}\) Thus, diadenosine
polyphosphates may be one as yet un-
identified cause of hypertension in renal
failure.

In addition to platelets, renal tissue is
a source of diadenosine polyphosphates,
and renal tubular cells release Ap\(_5\)A
and Ap\(_6\)A.\(^{50}\) Because of the close prox-
imity of tubules and peritubular vessels
in the kidney, these diadenosine polyphosphates may act in a paracrine
manner to promote vascular disease by
inducing VSMC proliferation. Diade-
osine polyphosphates are predomin-
antly protein bound and character-
ized by a middle molecular weight,
factors that hamper their removal from
the plasma by conventional hemodial-
ysis.\(^{51}\)

Another evolving area in uremia re-
search is the role of structural variants of
angiotensin, with a novel angiotensin
peptide, angiotensin-A (Ang-A), re-
cently identified in human plasma.\(^{52}\) The
affinity of Ang-A to the AT\(_1\) receptor is
nearly equal to that of Ang II; however,
its vasoconstrictive effect is lower. Thus,
Ang-A is a less potent and only partial
AT\(_1\) agonist. It is interesting that the af-
finity of Ang-A to the AT\(_2\) receptor is
higher than that of Ang II. Whether the
impact of Ang-A at the AT\(_2\) receptor also
translates into an increase in intrinsic ac-
tivity will require the development of a
suitable model to study AT\(_2\)-mediated
signaling events.

Plasma Ang-A is increased in renal
failure. The Ang-A/Ang II plasma ratio
of healthy individuals is <0.2, but in re-
nal failure, this ratio increases to up to
0.7. This may indicate increased activity
of decarboxylase in mononuclear cells,
decreased enzymatic degradation, or im-
paired renal removal. Increases in the
half-life of other low molecular weight
peptides have also been described in re-
nal failure. Currently, conventional en-
zyme immunoassays do not distinguish
between Ang II and Ang-A, because these
assays quantify the sum of Ang II and
Ang-A.\(^{52}\)

\textbf{Summary}
As research continues, more and more
uremic toxins are uncovered with the
potential to have significant impacts
on a variety of cell types and functions
within the vascular system. The afore-
mentioned uremic toxins can be added
to the list published in 2001, summa-
rizing the compounds known at that
time to have the potential to affect vas-
cular quality (Table 1).\(^{3}\) These recent
data confirm that most pathophysi-
ologically relevant compounds are
molecules that are “difficult to remove
dially,” such as the larger “middle
molecules,” protein-bound molecules,
and molecules such as guanidines,
which show a kinetic behavior that dif-
fers markedly from our current marker
urea.\(^{53,54}\)
Table 1. Compounds with the potential to provoke vascular damage

<table>
<thead>
<tr>
<th>Middle molecules$^b$</th>
<th>Protein-bound molecules$^b$</th>
<th>Small water-soluble compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE</td>
<td>AGE$^c$</td>
<td>ADMA$^d$</td>
</tr>
<tr>
<td>Ang-1</td>
<td>dinucleotide polyphosphates</td>
<td>guanidino acetic acid$^d$</td>
</tr>
<tr>
<td>dinucleotide polyphosphates</td>
<td>Ap$_3$A</td>
<td>methylguanidine$^d$</td>
</tr>
<tr>
<td>Ap$_3$A</td>
<td>homocysteine</td>
<td></td>
</tr>
<tr>
<td>leptin</td>
<td>indoxyl sulfate</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>pCS</td>
<td></td>
</tr>
<tr>
<td>Protein-bound molecules$^b$</td>
<td>Protein-bound molecules$^b$</td>
<td>Protein-bound molecules$^b$</td>
</tr>
<tr>
<td>AGE$^c$</td>
<td>dinucleotide polyphosphates</td>
<td>protein-bound molecules and/or larger compounds</td>
</tr>
<tr>
<td>Ap$_3$A</td>
<td>homocysteine</td>
<td></td>
</tr>
<tr>
<td>Ap$_3$A</td>
<td>indoxyl sulfate</td>
<td></td>
</tr>
<tr>
<td>leptin</td>
<td>pCS</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>phenylacetic acid</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ADMA, asymmetric dimethylarginine.  
$^b$Protein-bound molecules and middle molecules can be defined as molecules that are “difficult to remove by standard dialysis strategies.” Middle molecules might at the same time be protein bound; in that case, these are mentioned under the heading of protein-bound molecules as well as under that of middle molecules.  
$^c$AGE exist in their free form or are incorporated into amino acids, peptides, and proteins by irreversible linking; this is a different way of protein binding as compared with the relatively reversible links for most other protein-bound compounds.  
$^d$Although these guanidino compounds are small water-soluble solutes, they nevertheless have a different kinetic behavior compared with the prototypic water-soluble compound urea.

TRANSLATION INTO THERAPEUTICS

Current Situation

Most in vitro knowledge of uremic toxins implicated in vascular damage has pointed to a critical role for solutes that are difficult to remove by dialysis (Table 1). This knowledge has stimulated the development of randomized, controlled trials that have suggested superior cardiovascular outcomes for large-pore dialyzer membranes in secondary55–58 or primary analyses (Membrane Permeability Outcome [MPO] study; data presented at the 2007 meeting of the European Renal Association–Renal Dialysis and Transplantation Association in Barcelona and at the 2007 American Society of Nephrology in San Francisco).59,60 Whether further enhancing the convective removal of solutes will improve outcomes, as suggested by the relationship between β2-microglobulin and survival61 and additional observational studies,62,63 will need to be confirmed by controlled trials.

The finding of a potential role for the guanidines in vascular damage is interesting because of their extended volume of distribution53,54; this results in poor clearance from the extravascular compartment during hemodialysis with substantial rebound occurring at the end of dialysis.55 This could be countered by increasing dialysis time and/or frequency.64,65 Problems of intercompartmental transfer might represent a major limitation to the removal of other molecules as well, such as β2-microglobulin.66 As many toxins have been shown to be generated by inflammation,2,67 it seems prudent that dialysis conditions should be minimally proinflammatory, by avoiding dialysate impurities,68 central venous catheters,69 and membrane bioincompatibility.70

The Future

Two principal therapeutic options exist to improve further the treatment of uremia: The first is to enhance the removal of uremic toxins and the second is to develop pharmacologic approaches to interfere with their toxic effects. Although the maximal removal capacity of currently available diffusive and especially convective strategies has probably not yet been achieved, the question arises as to how much additional improvement is achievable. With regard to convection, technical refinements are still possible, but these must be friendly to patients and users. Importantly, nonspecific strategies to increase the removal of uremic toxins might also eliminate essential solutes that are beneficial (e.g., trace metals) or medications, and these unwanted effects will need to be assessed and compensated for in the future. With both conventional diffusive and convective therapies, increasing treatment time and/or frequency64,65,71 or the molecular weight cutoff of membranes72 might offer another option to improve uremic toxin removal without the need for new technologies.

Partly as a result of the use of liver supportive therapies, several sophisticated techniques have recently been developed to enhance the removal of protein-bound molecules and/or larger compounds through convective strategies, adsorption from whole blood, or combinations of adsorption and convection/diffusion. The manipulation of convection is based on large-pore filtration, which purposely leaks large solutes and even albumin. The albumin loss may range up to 50 g per treatment, which must then be replaced, together with other plasma components, as proposed for selective plasma exchange therapy.73

Direct adsorption from blood by hemoperfusion with bead columns74 has probably not yet reached its full potential. One interesting possibility is the targeted elimination of selected molecules responsible for uremic complications, and the technology required to do this is currently available, as shown for β2-microglobulin75; however, a classification of uremic solutes according to their importance is needed to permit a clinically and economically justified choice of target molecules.

Adsorption when combined with convective therapies, such as large-pore filtration with subsequent adsorption of filtrate and its reinfusion, has shown some utility.76,77 Similarly, adsorption can be combined with diffusion when used for dialysis against dialysate containing lipophilic elements78 or albumin.79 Adsorption of spent peritoneal dialysate, with reinfusion into the peritoneal cavity, may be another diffusive/adsorptive approach that has the advantage of eliminating biocompatibility reactions with blood constituents.

In addition to improving removal, a second option is to neutralize the toxic effects of uremic retention solutes by drug administration. A number of such measures are already in practice, mostly based on empirical experience or from evidence collected in the general popula-
Table 2. Currently applied pharmacologic strategies to prevent uremic complications

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Effect</th>
</tr>
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<tbody>
<tr>
<td>Aspirin</td>
<td>Decrease procoagulant and proinflammatory effects</td>
</tr>
<tr>
<td>AST-120</td>
<td>Adsorb indoxyl sulfate</td>
</tr>
<tr>
<td>Antihypertensives</td>
<td>Decrease hypertension and preserve kidney function</td>
</tr>
<tr>
<td>ACEi, ARB, and/or renin inhibitors</td>
<td>Decrease hypertension</td>
</tr>
<tr>
<td>β blockers</td>
<td>Decrease hypertension</td>
</tr>
<tr>
<td>Diuretics</td>
<td>Decrease hypertension</td>
</tr>
<tr>
<td>Statins</td>
<td>Combat fluid overload</td>
</tr>
<tr>
<td>Phosphate binders</td>
<td>Decrease P, PTH</td>
</tr>
<tr>
<td>calcium containing</td>
<td>Decrease Ca × P, P, PTH</td>
</tr>
<tr>
<td>non–calcium containing</td>
<td>Decrease homocysteine</td>
</tr>
<tr>
<td>ESA</td>
<td>Combat dyslipidemia</td>
</tr>
<tr>
<td>Folic acid</td>
<td>Decrease PTH</td>
</tr>
<tr>
<td>Resins (e.g., kayexalate)</td>
<td>Decrease potassium</td>
</tr>
</tbody>
</table>

*aACEi, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; Ca × P, calcium-phosphate product; ESA, erythropoiesis-stimulating agents; P, phosphate; PTH, parathyroid hormone.

Table 3. Potential targets for the neutralization of uremic effects

| Activity of receptors | Changes in intracellular calcium level | in flux of intracellular calcium release of calcium from intracellular stores activity of Ca2+-ATPase Activation/inhibition of MAPK ERK1/2 JNK P38 Activation/inhibition of transcription factors NF-κB AP-1 Activation/inhibition of PKC Generation of ROS inhibition of production neutralization Effects at the transcriptional level (mRNA) Effects at the posttranscriptional level |
|-----------------------|---------------------------------------|-------------------------------------------------------------------------------------------------|-----------------------------------|--------------------------|-------------------|--------------------------|--------------------------|----------------------------|
| Generation of ROS     |                                       |                                                                                                 |                                   |                          |                   |                          |                          |                            |

CONCLUSIONS

The current picture of uremic toxicity is complex because of the groups of compounds that are retained and pathways affected. Molecules that are difficult to remove by dialysis, such as the larger middle molecular weight molecules and protein-bound molecules, play significant roles in uremic toxicity, and recent clinical studies suggest that enhancing the removal of these compounds has a beneficial effect on survival. Future therapeutic options include improved or novel removal of toxins and/or the search for pharmacologic inhibitors of the relevant pathophysiologic pathways and the opportunity to improve the quality of life for patients with kidney failure.

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DISCLOSURES

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