

## Research Article

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# Role of Uremic Compounds in Organ Injury

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## Abstract

All substances with molecular weights up to 58 kDa retained in the blood as the results of renal dysfunction are potential uremic toxins. The search for endogenous toxic compounds seems to offer a novel approach to identifying and explaining any so far unexplored specific effects on the body organs and systems. In contemporary laboratory diagnostics there are no suitable markers for use in comprehensive evaluation of complex toxicity of uremic compounds accumulated in successive stages of developing renal dysfunction. To provide a sound basis for treatments which would effectively protect against or slow down multiple organ injury caused by uremic toxins novel parameters are needed, more specific than urea and creatinine. Identification of reliable biomarkers or their panels needs careful consideration of their concentrations in biological materials, biological activity and usefulness for effective diagnosis. Classification of uremic compounds, based on their chemical properties, role in pathophysiological processes and the organs where they are formed remains to be elucidated with meticulous observation of clearly formulated rules guiding the process.

**Keywords:** Uremic compounds; Renal dysfunction

## Background

In health, the renal glomerular filter cleanses the body of molecules with weights up to 58 kDa. In renal failure the reduced glomerular filtration or renal metabolism and the damage to non-renal organs comprise a variety of compounds specifically related to the metabolic processes and function of different cell types and organs. Inadequate removal of a large number of potentially toxic organic metabolites from the vascular bed into the urine in the course of acute kidney injury (AKI) and chronic kidney disease (CKD) is associated with various clinical symptoms which are often difficult to interpret [1-4].

It is of considerable importance to identify which of the uremic retention solutes are actually uremic toxins and what pathomechanisms are involved in their damaging effect on the kidneys and other organs. This would allow better documented confirmation of the suspected association between the clinical symptoms and uremic retention solute/toxin concentrations in biological materials, possible discovery of any missing pathophysiological links between progressive renal failure and loss of function in organs other than the kidneys, and identification of diagnosis-and organ-specific biomarkers for use in clinical practice. Uremic retention solutes are referred to as uremic toxins when they interact with normal biological functions. All substances retained in the body as a result of renal dysfunction are potential uremic toxins and are classified as uremic toxin according to strict criteria. The chemical structure and composition should be identifiable and the substances should be quantifiable in biological fluids using recognized methodology. Concentrations in the biological fluids or tissues of patients with renal dysfunction should significantly exceed those in non-uremic subjects. Increases in the concentration in the blood or tissue should correlate with the clinical manifestations. The association between the biological activity of the uremic toxins and the clinical manifestations should be demonstrable in *in vivo*, *ex vivo* and *in vitro* test systems [3-7].

So far only a few organic metabolites have been found with the properties which would allow their classification as uremic toxins. The most common biomarkers for assessment of renal dysfunction are urea and creatinine. Increased serum concentrations of urea have been routinely used to assess the effectiveness of dialysis. Creatinine, on the other hand, is in the current clinical practice, the only of all retained uremic solutes measured to evaluate the biochemical/biological and hence toxic effects of renal dysfunction. Paradoxically, unlike in the case of other potentially toxic compounds, there have been only a few

published studies confirming the ability of either urea or creatinine to induce adverse biochemical and physiological effects. There have been questions concerning the diagnostic value of the eGFR equations (estimated glomerular filtration rate) based on serum creatinine measurements and used to diagnose the stages and progression of renal dysfunction. The evidence so far collected confirms that the eGFR values show a very weak and diverse correlation with other proven uremic toxins. Hence, the current criteria based exclusively on eGFR seem inadequate to justify the decision to start dialysis [1-9].

Other retained compounds may be potentially toxic and hence useful as laboratory markers for evaluation of impaired glomerular filtration. In the last decade numerous previously unknown uremic compounds have been identified and their associations with specific pathophysiological mechanisms have been established. With the abundance of new information about potential toxicity of different uremic compounds, it becomes increasingly important to determine the principles guiding the comparison of data from different research centers [1-5,8,10-12,14-18].

## Identification, Characterization, Analytical Determination and Evaluation of Biological Activity of Uremic Retention Solutes

Uremic retention solutes present a great variety of properties which makes their accurate classification extremely difficult. They make up a group whose numerous members differ in their water solubility, protein-binding capacity, molecular weight, pattern of removal by dialysis, biological properties and potential to produce clinical symptoms [2,3,11,19,20].

The most common classification of uremic compounds into 3 groups proposed by European Uremic Toxin (EUTox) Work Group

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is by molecular weight, protein-binding capacity and removal pattern by dialysis. This group listed substances with presumed or proven biological activity whose accumulation in the body resulted from end-stage renal failure [2,3,11,12,19].

### Low molecular weight water soluble uremic toxins

Small molecules (molecular weight <500 Da), soluble in water and easily removed by any dialysis strategy. Low Molecular Weight organic compounds may occur in free water-soluble form or bound to plasma proteins, which alter the function of both the toxin and the transporter protein. The most common compounds being: ADMA (asymmetric dimethylarginine), creatine, creatinine, hyaluronic acid, guanidine, guanidinoacetate, guanidinosuccinate, oxalate, SDMA (symmetric dimethylarginine), urea and uric acid.

### Protein-bound solutes

Although molecular weight of most members of this group is less than 500 Da, because of their protein binding capacity they are recognized as “difficult to remove” by dialysis. The main protein-bound solutes include: AGEs (advanced glycation end products), carboxy methyl propyl furanpropionic acid, cytokines, interleukins, tumor necrosis factor- $\alpha$ , dimethylguanidines, hippuric acid, homocysteine, indole-3-acetic acid, indoxylglucuronide, IS (indoxylsulfate), kynurenic acid, kynurenine, leptin, phenolic compounds, p-CS (p-cresylsulfate), p-cresylglucuronide, phenolsulfate, phenolglucuronide, phenylacetic acid, quinolinic acid and retinol-binding protein.

### Middle molecular weight molecules (molecular weight > 500 Da)

So far more than 50 such compounds have been found to have a cause-and-effect relationship with the origin and development of many pathophysiological processes. This group includes adiponectin, cystatin C, leptin, motilin,  $\alpha$ 1-acid glycoprotein,  $\alpha$ 1-microglobulin, endothelin, ghrelin, osteocalcin, atrial natriuretic peptide, prolactin, retinol-binding protein,  $\beta$ 2-microglobulin, cholecystokinin and vasoactive intestinal peptide.

The EUTox classification does not describe the toxicity of the compounds listed and so far there has been no effective method of such characterization. The search for new uremic compounds and combining them into panels of substances involved in the same pathophysiological processes seems to offer a novel approach to identifying and explaining so far unexplored specific effects of endogenous compounds on the body organs and systems (Table 1).

It has been suggested that further more precise classification of the compounds should take into consideration any similarities between their chemical structures, common biological or organ function and the anatomical site of their origin.

### Investigative Methods to Determine Toxicity of Retained Compounds

Studies *in vitro* of the biological effects of candidate compounds are a basis for their further identification in epidemiological and clinical studies. The need for the use of specific cells for the purposes of disease modelling has been underlined, such as leukocytes to study compromised immune defense or oxidative stress, endothelial cells for cardiovascular disease, smooth muscle cells for progression of atherosclerosis, hepatocytes for disturbed metabolism, fibroblasts for fibrosis or osteoblasts for renal osteodystrophy. When possible, human cells should be used, and the animal cell models should be restricted to

the species for which the relevance to conditions in humans has already been proved [2,5,6].

Analytical methods should be reproducible and the ranges of quantitative measurements should be precisely defined and carefully analyzed when applied to different patient populations. The method chosen depends of the sensitivity of detection of particular compounds. A number of isolation and detection techniques have been used for the quantification of retained uremic compounds, including: chromatographic methods (ion exchange chromatography, gas chromatography, HPLC), spectrophotometry, fluorometry, chemiluminescence, nephelometry, radioimmunoassay, nuclear magnetic resonance and mass spectrometry [5-7].

Proteomic and genomic studies are valuable research tools used both to evaluate known uremic compounds and to search for and identify new substances potentially affecting selected conditions and organ functions. These techniques allow differentiation of retained uremic solutes as they occur in particular conditions and patient populations [6,7,11].

Analysis of metabolites of potential uremic compounds which is a cause altered initial biological activity. An example of this phenomenon is p-cresol generated by intestinal bacteria as a metabolite from the amino acids tyrosine and phenylalanine, which is an inhibitor of leukocyte function while p-CS demonstrates pro-inflammatory effects by activating leukocyte free-radical production [12,13].

Analysis of protein-binding capacity and the proportions of the free fraction and the protein-bound fraction, which may modify the toxicity of uremic compounds. In patients with chronic kidney disease the albumin-binding capacity is decreased and associated with the severity of their renal disease and accumulation of uremic albumin-bound retention solutes. Mechanisms which may be responsible have been taken into consideration, including hypoalbuminemia, accumulation of endogenous substances competing for binding on serum albumin and conformational changes in the albumin molecule [15].

### Analysis of the interaction of retained uremic compounds

Examples of interactions between retained uremic toxins in the course of renal dysfunction:

-Soluble guanidines are responsible for the production of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL-6).

-IS induces the synthesis of free radicals in the cells of renal tubular and mesangial cells activating the N- kappaB pathway.

-ADMA increases may result from the inhibition of the enzyme dimethylarginine dimethylaminohydrolase (DDAH) by hyperhomocysteinemia. Decreases in blood homocysteine levels may affect the levels of both DDAH and ADMA.

-SDMA and its structural analogue ADMA are members of the same group of water-soluble compounds and have a similar biological effect of inhibiting NO synthesis although their mechanism of action differ.

-*In vitro*, p-CS induces leukocyte free radical production, which was enhanced when p-cresyl-glucuronide was added but p-cresylglucuronide alone had no effect on leukocyte oxidative burst.

### Uremic compounds and the development of renal failure

It has been suggested that uremic toxins promote progression of renal failure by damaging tubular cells and their overload accelerates

Similarities between uremic compounds	Examples of the most common compounds	References
Chemical structure	- Guanidine ( $\alpha$ -keto- $\delta$ -guanidinovaleic acid, $\alpha$ -N-acetylarginine, ADMA, argininic acid, $\beta$ -guanidinopropionic acid, creatine, creatinine, $\gamma$ -guanidinobutyric acid, guanidine, guanidino acetic acid, guanidinosuccinic acid, methylguanidine, SDMA and taurocyamine.	3, 5, 12
	- Purine (cytidine, hypoxanthine, xanthine and uric acid)	
	- Pyrimidine (thymine, orotic acid, orotidine and uridine)	
	- Methyl amine (methylamine, dimethylamine, trimethylamine)	
	- Phenyl (2-methoxyresorcinol, phenol, hydroquinone, p-cresol)	
Association with the endothelial dysfunction	- Indole (kinurenine, indole-3-acetate, kinurenic acid, melatonin, IS, quinolinic acid)	3, 9, 20
	- Manifestations of atherosclerosis (guanidine derivatives, AGEs, p-Cs, platelet diadenosine polyphosphates, IS, vascular cell adhesion molecule-1, von Willebrand factor, thrombomodulin, plasminogen activator inhibitor 1, matrix metalloproteinases, (ADMA, AGEs, circulating endothelial microparticles)	
	- Loss of vessel wall compliance (ADMA, AGEs)	
	- Vascular calcification (inorganic phosphate, reactive oxygen species, tumor necrosis factor, leptin)	
Connection with the biochemical processes	- Abnormalities of vascular repair (IS, some guanidinocompounds)	3, 10
Organ origin	- Advanced Glycation End-products (AGEs): AGE peptides and "AGE-free adducts" (3-deoxyglucosone, fructoselysine, glyoxal, methylglyoxal, N $\epsilon$ -carboxymethyllysine (CML), N $\epsilon$ -carboxyethyllysine (CEL), pentosidine	
	- Chemical compounds produced by colon microbes: indole (IS, indoxyl glucuronide, 5-hydroxyindole, indole-3-propionic acid), phenyl compounds (p-CS, p-cresol-glucuronide, phenyl sulphate, phenyl glucuronide, alfa-N-phenylacetyl-L-glutamine, phenylpropionylglycine, cinnamoylglycine, 4-ethylphenyl sulfate, hippuric acid)	13, 14, 15, 30
Association with the acute-phase processes	- Compounds with antioxidant, anti-inflammatory and vasodilating properties, which in health can be filtered by the glomeruli (molecular weight <58 kD) (proinflammatory cytokines, $\alpha$ 1-acid glycoprotein, neopterin, calcitonin)	13, 23, 30, 31
	- Several acute phase proteins with large (>58 kD) molecules (CRP, $\alpha$ 2-macroglobulin, fibrinogen, myeloperoxidase) which are directly or indirectly involved in inflammation	

Table 1: Methods of classification of the uremic compounds.

the loss of kidney function, glomerular sclerosis and tubulointerstitial injury. Such factors as infections, toxins, hypoxia, hypertension, genetic or metabolic disorders and autoimmune disease may induce both AKI characterized by sudden development of renal dysfunction and CKD where structural or functional alterations in the kidney develop over a period of at least 3 months. Inflammation plays a key role in mediating CKD progression in response to infectious and noninfectious kidney damage. The effect of impaired intestinal barrier function and potential toxins produced in the intestine (IS, p-CS and phenylacetic acid) has been suggested as a likely clinical mechanism underlying the development of inflammatory response in the kidneys of patients with CKD [13,16-18,21-23].

### Uremic compounds and the development of the cardiorenal syndrome

The term "cardiorenal syndrome" refers to the bidirectional relationship between heart disease and kidney disease. The cardiorenal syndrome is classified into five types in which acute or chronic dysfunction of the heart or kidneys can induce acute or chronic dysfunction of the other organ. Better understanding of the so far unclear pathomechanism of the relationship between renal disease and cardiovascular disease is needed for developing effective treatment strategies and the activity of endogenous uremic toxins is considered by many researchers to be a likely factor [2,3,5,9,13,14,24-26].

### Uremic compounds and function of the intestinal mucosal barrier

Experimental studies confirm that uremia disturbs the function of key proteins of epithelial tight junctions. Pathogen overgrowth (dysbiosis) with additional elimination of creatinine through the intestinal wall is common in the course of CKD. Impaired function of the intestinal mucosal barrier and fluid overload in the early stages of kidney disease are thought to be directly responsible for the translocation of both intact bacteria and their fragments or bacterial bioproducts across the intestinal mucosal barrier into the circulation. The bacteria

and their products affect the activation of the innate immune system which explains the persistence of systemic inflammation in the course of CKD [13,14,17,27-31].

### Uremic toxins and the development of the hepatorenal syndrome

The hepatorenal syndrome is progressive renal failure in the course of chronic liver disease. The liver and kidneys together comprise an organ system responsible for removal of toxic compounds from the body. Renal function loss in patients with cirrhosis has been associated with worse prognosis. Liver failure causes an increase in splanchnic vasodilatation which leads to a fall in systemic vascular resistance and effective hypoperfusion of kidneys and in response renal vasoconstriction. The pathogenesis of the hepatorenal syndrome is unknown and possible biochemical factors include the action of different cytokines and vasoactive mediators, such as nitric oxide, thromboxanes, endotoxins not excreted by the liver or endothelins, on the renal circulation and other vascular beds [32,33].

### Methods to Prevent Damage from Uremic Toxins

According to recent reports two main directions of search for improved treatment methods from uremic toxins have emerged: more effective removal of uremic toxins by dialysis (the protein-bound toxins cannot be sufficiently eliminated using the current dialysis strategies) and use of pharmacological agents to interfere with the production and absorption of colon-derived solutes. The suggested approaches include the use of probiotics (products containing *Bifidobacteria*), prebiotics (resistant starch, oligofructose-enriched inulin) and antibiotics influencing the growth and metabolism of intestinal bacteria. Reducing dietary protein intake and increasing the amount of dietary fiber may be an easy way to decrease the production of colon-derived uremic solutes such as IS and p-CS generated by intestinal bacteria [7,9]. Additionally, intestinal protein absorption is disturbed in renal failure with the resulting increase in the number of intestinal substrates derived from dietary amino acids (phenylalanine, tryptophan) for colon microbes.

Preventing constipation, including the use of laxatives as decreasing the duration of protein transit in the large intestine reduces the metabolism of amino acids to potentially toxic uremic compounds. Intestinal adsorption of uremic compound precursors inhibits their further conversion into active uremic toxins. Decreasing serum IS levels using the intestinal carbon-based sorbent AST-120 (Kremezin) produces reduction of oxidative stress in the kidney, improved renal function and less histological damage *in vivo* [12,15,34].

In summary, the measurement of serum creatinine and urea do not fully reflect the combined toxicity due to the presence of other endogenous organic metabolites. Each of the uremic compounds presents unique biological and kinetic properties and their uremic toxicity cannot be definitively described with the currently available data. Numerous studies have been published recently which prove the toxicity of uremic compounds but are still unable to elucidate the exact mechanism of their relationship with clinical symptoms of organ malfunction.

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