Advanced glycation and lipoxidation end products: 
reactive carbonyl compounds-related uraemic toxicity

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Abstract
Most studies on uraemic toxins have focused on 
disorders of enzymatic biochemistry. Recent studies 
to elucidate the pathogenesis of dialysis-related amylo-
oidosis have provided new insights in progressive, 
irreversible protein modifications resulting from non-
enzymatic biochemistry in uraemia. This paper focuses 
on two types of irreversible alterations of proteins: 
advanced glycation and lipoxidation. We investigate 
the causal role of various reactive carbonyl compounds 
(RCOs) accumulating in the serum, speculate on its 
clinical consequences, and discuss the therapeutic 
perspectives.

AGEs/ALEs accumulate in uraemia

The advanced glycation of proteins has been initially 
unravelled by food and nutrition biochemists. The 
Maillard reaction, a non-enzymatic process, is initiated 
when proteins are exposed to glucose or other carbo-
hydrates. It generates first reversible Schiff base 
adducts and subsequently more stable Amadori 
rearrangement products. Through a series of oxidative 
and non-oxidative reactions, it eventually yields the 
irreversible advanced glycation end products (AGEs).

The role of AGEs in human pathology was initially 
highlighted in diabetes with hyperglycemia [1]. The 
correlation between AGE and fructoselysine levels, a 
marker of plasma glucose levels, demonstrates the 
role of glucose in their generation [2]. More import-
antly for their clinical relevance, AGEs are also corre-
lated with the severity of diabetic complications [3,4]. 
The possibility has therefore been considered that 
AGE modification of proteins plays a causal role in the 
development of diabetic complications: AGEs could 
possibly be the mediators of the so-called ‘glucose 
toxicity’.

The discovery that AGEs accumulate in uraemic 
patients to a much greater extent than in diabetics 
came as a great surprise because uraemic patients are 
not necessarily hyperglycaemic. The plasma levels of 
the two well-known AGEs, pentosidine and carboxy-
methyllysine (CML), are one order of magnitude 
higher in haemodialysis patients than those in normal 
or non-uraemic diabetic subjects [5,6]. Other AGE 
adducts also accumulate in uraemia such as glyoxal-
lysine dimmer (GOLD), methylglyoxal-lysine dimmer 
(MOLD), and imidazoline [7].

Among dialysis patients, diabetics and non-diabetics 
have similar plasma pentosidine and CML levels [5,6]. 
In contrast with non-uraemic diabetic patients, neither 
pentosidine nor CML correlate with fructoselysine 
levels in uraemic subjects. It thus became clear that 
factor(s) other than hyperglycaemia are critical for 
AGE formation in uraemia. The fact that >90% of 
plasma pentosidine and CML are bound to albumin 
[5,6] suggests that its accumulation does not result from 
a decreased renal clearance of AGE-modified proteins.

The second approach to irreversible protein modi-
fication in uraemia derives from studies of lipid 
metabolism, especially lipid peroxidation. Proteins are 
modified not only by carbohydrates but also by lipids. 
For instance, proteins modified by malondialdehyde, 
which is derived from the oxidation of polyunsaturated 
fatty acids such as arachidonate, accumulate in plasma 
proteins of haemodialysis patients [6]. Proteins modi-
ified by malondialdehyde, as well as by other lipid 
peroxidation product modified proteins, are called 
advanced lipoxidation end products (ALEs).

Uraemia is thus characterized by irreversible non-
enzymatic protein modifications by carbohydrates 
or lipids. Of note, the levels of AGEs and ALEs 
rise concomitantly in uraemic serum: plasma CML, 
an AGE species, is highly correlated with plasma 
malonyldialdehyde-lysine in patients on chronic
haemodialysis [6], pointing to a common cause in the genesis of AGEs/ALEs.

Various RCOs accumulate in uraemia and modify proteins

Both AGEs and ALEs are formed by carbonyl amine chemistry between protein residues and reactive carbonyl compounds (RCOs). RCOs are constantly produced by the metabolism of carbohydrates, lipids and amino acids, all of which are abundantly present throughout the body. RCOs such as glyoxal, methylglyoxal, arabinose, glycoaldehyde, 3-deoxyglucosone and dehydroascorbate are formed from carbohydrates and ascorbate. Similarly, RCOs such as glyoxal, malondialdehyde, hydroxynonenal, and acrolein are generated by lipid peroxidation of polyunsaturated fatty acids. In addition, RCOs such as glyoxal, methylglyoxal, acrolein and glycoaldehyde are produced during the myeloperoxidase-catalysed metabolism of amino acids. These RCOs react with proteins and eventually form AGEs and ALEs.

Could the raised levels of AGEs and ALEs in uraemia acrue from an accumulation of carbohydrate- and lipid-derived RCOs? Total RCOs are indeed several times higher in uraemia than in normal plasma [8]. The accumulation of individual RCOs has also been documented by several groups [9–11]. We have evidence that the prevailing plasma pentosidine levels are correlated with the levels of pentosidine precursor RCOs [8].

The accumulation in uraemic plasma of various RCOs derived from either carbohydrates or lipids, as well as the subsequent carbonyl modification of proteins, suggests that chronic uraemia may be characterized as a state of ‘carbonyl stress’ [12]. Under carbonyl stress, AGEs derived from carbohydrates and also ALEs derived from lipids accumulate in parallel, not only in plasma, but also in tissue proteins.

Causes of uraemic carbonyl stress

Two competing but not mutually exclusive hypotheses should be considered to account for the uraemic carbonyl stress: an increased generation or a decreased removal of RCOs.

Production of RCOs is known to be increased by oxidative stress. Several reports point to the existence of an increased oxidative stress in uraemia, characterized by an augmented production of oxidants and a decreased level of antioxidants [13–15]. The uraemic oxidative stress might be worsened further by some modalities of renal replacement therapy: the haemodialysis treatment activates complement and neutrophils, and releases reactive oxygen species.

Oxidative stress modifies proteins either directly or indirectly. Reactive oxygen species oxidize amino acids directly [16], but we have no evidence that this occurs in uraemia. The indirect pathway is more interesting. Carbohydrates and lipids abundantly present throughout the body are targeted by reactive oxygen species and yield various RCOs that are eventually involved in the formation of AGEs and ALEs. The existence of this latter pathway is supported by the correlation existing in uraemic plasma between levels of pentosidine and oxidative markers such as dehydroascorbate and advanced oxidation protein product (AOPP) [11,13].

The oxidative stress hypothesis, however, is not satisfactory. Indeed, RCOs such as 3-deoxyglucosone and methylglyoxal are derived from non-oxidative chemistry. 3-deoxyglucosone is formed non-oxidatively by rearrangement and decomposition of Amadori compounds or by anaerobic metabolic reactions leading to the formation of fructose-3-phosphate, which decomposes spontaneously to 3-deoxyglucosone. The more reactive RCO, methylglyoxal, is also formed during anaerobic metabolism of acetone and amino acids. Both RCOs react with proteins and form AGEs. Raised levels of 3-deoxyglucosone and methylglyoxal, and of their protein adducts in uraemia [15,17], demonstrate that non-oxidative chemistry is also involved in the generation of carbonyl stress.

The rise of RCOs in uraemia might also be accounted for by a decreased removal. RCOs are constantly detoxified by several enzymatic pathways, such as the aldose reductase, the aldehyde dehydrogenase and the glyoxalase pathways [18]. Redox coenzymes, reduced glutathione (GSH) and nicotinamide adenine denucleotide phosphate (NAD(P)H) contribute to their activity. RCOs such as methylglyoxal and glyoxal react reversibly with the thiol group of glutathione and are subsequently detoxified by glyoxalases I and II into lactate and glutathione. NAD(P)H also replenishes glutathione by increasing the activity of glutathione reductase. Decreased levels of glutathione and NAD(P)H can therefore result in augmented levels of a wide range of RCOs.

In this context, it is of interest to know that the glutathione concentration in red blood cells and the serum activity of glutathione-dependent enzymes are significantly reduced in uraemic patients [19–21]. The hypothesis that a decreased thiol storage capacity contributes to the accumulation of RCOs in uraemia is supported by recent evidence: glutathione peroxidase activities are indeed inversely correlated with the plasma pentosidine levels in haemodialysis patients [21].

Implications of carbonyl stress

The consequences of the uraemic carbonyl stress and its attendant AGE and ALE modification of proteins remain to be discussed. Carbonyl stress is, however, detected chemically and immunohistochemically in several uraemic complications, such as dialysis-related amyloidosis [22,23], atherosclerosis [24] and peritoneal
membrane deterioration, in patients undergoing long-term peritoneal dialysis (PD) [25–27].

It remains unknown whether the presence of AGEs and ALEs merely results from the long-term accumulation of protein modifications and is therefore an inert surrogate marker for carbonyl stress, or, alternatively, whether it plays an active role in the pathogenesis of these complications. Recent studies support the latter hypothesis. Proteins modified with AGEs and ALEs initiate a range of cellular responses [28–30], which partly account for the pathogenesis of joint and vascular complications in uraemia. Furthermore, RCOs themselves interfere with various cellular functions, independently of their effect on AGE and ALE modification of proteins: RCOs are biologically active, initiate a variety of cellular responses, and induce not only structural but also functional alterations of proteins. They also influence intracellular signalling by multiple pathways. Exposure of fibroblasts to glyoxal activates protein kinases such as c-Src and increases intracellular tyrosine phosphorylation of several cellular proteins [31]. This effect is mediated by the formation of Schiff base on cell surface protein since it is prevented by an inhibitor of the carbonyl amine reaction, OPB-9195. Hydroxynonenal causes a capping of epidermal growth factor receptor (EGFR) on the cell surface, mimics the effect of EGF on the downstream signalling pathways that involve mitogen activated protein kinases (MAPKs), and contributes to oxidative stress-linked induced apoptotic cell death [32]. Furthermore, hydroxynonenal also triggers oxidative stress-linked induced apoptotic cell death by activating caspase-3 through a Fas-independent, but glutathione-dependent, redox pathway [33].

**Insights into new therapeutic approaches**

If carbonyl stress is not a passive bystander but rather an active contributor to the pathogenesis, its manipulation may reveal new therapeutic approaches. Among them are redox modulation and the use of inhibitors of carbonyl amine chemistry.

As described above, the decrease in glutathione concentration impairs the detoxification of RCOs and potentiates the formation of AGEs and ALEs. Repletion of glutathione might prove possible and useful by supplementation of glutathione, N-acetylcysteine or cysteine. Addition of these thiols compounds in both uraemic and normal plasma as well as in glucose-based PD fluid indeed lowers the generation of AGEs after incubation in vitro. Several other compounds may also prove helpful. Vitamin E and ubiquinol relieve the demands on the activity of glutathione. Inhibitors of aldose reductase are other candidates to replenish glutathione as they prevent the polyol pathway activation and replenish NADPH and glutathione available for both aldose reductase and glutathione reductase.

A second approach is the use of the inhibitors of carbonyl amine chemistry. AGEs/ALEs are formed by carbonyl amine chemistry between RCOs and proteins. Trapping of RCOs with substances such as aminoguanidine and 2-isopropyldenedihydrazono-4-oxo-thiazolidin-5-ylacetanilide (OPB-9195) should inhibit the formation of AGEs and ALEs. Indeed, we have demonstrated that both compounds inhibit the in vitro formation of AGEs from a variety of individual precursors such as ribose, glucose and ascorbate, as well as that of ALEs, malondialdehyde-lysine and hydroxynonenal-protein adduct from arachidonate [34]. Pentosidine generation in uraemic plasma and in glucose-rich PD fluid incubated for 4 weeks is also inhibited by aminoguanidine and OPB-9195 [8,34]. Interestingly, OPB-9195 corrects several biological effects of RCOs. In murine thymocyte and fibroblasts, it inhibits the phosphorylation of tyrosine residues of a number of intracellular proteins induced by glyoxal [31]. Furthermore, oral administration of OPB-9195 to rats after balloon injury of their carotid arteries effectively reduces neointima proliferation in arterial walls, an early and major step in the development of atherosclerotic lesions [24].

The development of less toxic and more specific carbonyl stress inhibitors should prove an important new therapeutic avenue. Such compounds immobilized in cartridges might enhance extraction of RCOs from blood during dialysis therapy.

**References**