Advanced Glycation and Lipoxidation End Products: Role of Reactive Carbonyl Compounds Generated during Carbohydrate and Lipid Metabolism

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The uremic syndrome is usually attributed to the retention of a variety of compounds as a result of a deficient renal clearance. These retention solutes are taken to induce biochemical disorders characteristic of uremic complications. However, as recently noted by Vanholder and De Smet (1), only a few of them have an established role as uremic toxins.

Most studies on uremic toxins have focused on disorders of enzymatic biochemistry and/or on toxins that are the result of enzymatic biochemistry. In this review, we concentrate on another aspect of uremic toxicity, related to nonenzymatic biochemistry of proteins. We investigate the causal role of various reactive carbonyl compounds (RCO) accumulating in the serum; postulate the existence of “carbonyl stress” in uremia and analyze its clinical consequences; and, finally, discuss therapeutic perspectives.

Accumulation of Advanced Glycation End Products/Advanced Lipoxidation End Products in Uremia

The advanced glycation of proteins has been initially investigated by food and nutrition biochemists (2). The Maillard reaction, a nonenzymatic process, is initiated when proteins are exposed to glucose or other carbohydrates. It generates first reversible Schiff base adducts and subsequently more stable Amadori rearrangement products. Through a series of oxidative and nonoxidative reactions, it eventually yields the irreversible advanced glycation end products (AGE) linked with amino groups, e.g., lysine residues, of several proteins.

In human pathology, irreversible advanced glycation of proteins is a part of the ageing process. It is markedly amplified in diabetes, as a consequence of hyperglycemia: AGE levels are indeed correlated with those of fructoselysine, taken as a surrogate marker of prevailing plasma glucose concentration (3–5). Of interest, they are also correlated with the severity of diabetic complications, a finding supporting their clinical relevance (6–8).

The subsequent discovery that AGE also accumulate in uremic patients was surprising because most uremic individuals have normal blood glucose levels. Sensitive and specific chemical methods including HPLC (9) and gas chromatography/mass spectrometry (GC/MS) (10) were developed to quantify AGE, such as pentosidine (11) and carboxymethyllysine (CML) (12). The levels of these two AGE adducts in plasma proteins of hemodialysis patients proved markedly higher than in control or diabetic subjects (13,14). Other AGE adducts also accumulate in uremia, such as glyoxal-lysine dimer, methylglyoxal-lysine dimer, and imidazolone (15).

Among dialysis patients, those with diabetes and those without had similar plasma pentosidine and CML levels (13,14). In contrast to nonuremic diabetic patients, neither pentosidine nor CML correlated with fructoselysine levels in uremic subjects. Thus, it became clear that factor(s) other than hyperglycemia are critical for AGE formation in uremia. The fact that more than 90% of plasma pentosidine and CML are albumin adducts (13,14) suggests that its accumulation does not result from a decreased renal clearance of AGE-modified proteins.

The second approach to irreversible protein modification in uremia derives from studies of lipid metabolism, especially lipid peroxidation. Proteins are modified not only by carbohydrates but also by lipids (16). For instance, proteins modified by malondialdehyde, which is derived from the oxidation of polyunsaturated fatty acids such as arachidonate, accumulate in hemodialysis patients (14). Malondialdehyde as well as other lipid peroxidation product modified proteins are called advanced lipoxidation end products (ALE) (17).

Uremia is thus characterized by irreversible nonenzymatic protein modifications by carbohydrates or lipids, i.e., AGE/ALE (Table 1). Of note, the levels of AGE and ALE rise concomitantly in uremic serum: Plasma CML, an AGE species, is highly correlated with plasma malondialdehyde-lysine, an ALE species, in patients on chronic hemodialysis (14). This observation points to a common cause in the genesis of AGE/
ALE. This conclusion is further supported by a recent report that, in the skin of renal failure patients, lipid peroxidation and advanced glycation of matrix collagen increase in close relation to each other (20).

**Accumulation of Reactive Carbonyl Compounds in Uremia: Carbonyl Stress**

Both AGE and ALE are formed by carbonyl amine chemistry between protein residues and reactive carbonyl compounds (RCO) (17). These RCO are constantly produced by the metabolism of carbohydrates, lipids, and amino acids, all of which are abundantly present throughout the body. RCO, e.g., glyoxal, methylglyoxal, arabinose, glycoaldehyde, 3-deoxyglucosone, and dehydroascorbate, are formed from carbohydrates and ascorbate (21–25). They react nonenzymatically with protein amino groups and eventually yield AGE, e.g., CML, pentosidine, imidazolone, glyoxal-lysine dimer, and methylglyoxal-lysine dimer. Similarly, RCO, e.g., glyoxal, malondialdehyde, hydroxynonenal, and acrolein, are generated by lipid peroxidation of polyunsaturated fatty acids (16,26). In addition, RCO, such as glyoxal, methylglyoxal, acrolein, and glycoaldehyde, are produced during the myeloperoxidase catalyzed metabolism of amino acids (27). These RCO react with proteins and form ALE as well as AGE.

Could the raised levels of AGE and ALE in uremia accrue from an accumulation of carbohydrate- and lipid-derived RCO? Total RCO have been measured in uremic plasma with the 2,4-dinitrophenylhydrazine (DNPH) method. DNPH is known to combine with RCO and to yield hydrazones. The yield of hydrazone is indeed several times higher in uremic than in normal plasma (28). The accumulation of total as well as individual RCO has also been documented by several groups (17,28–31). Table 2 summarizes the various RCO thus far found to be raised in uremic plasma.

Table 1. Accumulation of AGE/ALE in hemodialysis patients

<table>
<thead>
<tr>
<th>AGE/ALE</th>
<th>Precursor(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentosidine</td>
<td>Carbohydrate</td>
<td>13, 18</td>
</tr>
<tr>
<td>Carboxymethyllysine</td>
<td>Carbohydrate, lipid, amino acid</td>
<td>14</td>
</tr>
<tr>
<td>Malondialdehyde</td>
<td>Carbohydrate, lipid, amino acid</td>
<td>14</td>
</tr>
<tr>
<td>Imidazolone</td>
<td>Carbohydrate</td>
<td>19</td>
</tr>
<tr>
<td>Glyoxal-lysine dimer</td>
<td>Carbohydrate, lipid, amino acid</td>
<td>15</td>
</tr>
<tr>
<td>Methylglyoxal-lysine dimer</td>
<td>Carbohydrate, lipid, amino acid</td>
<td>15</td>
</tr>
</tbody>
</table>

*a AGE, advanced glycation end product; ALE, advanced lipoxidation end product.

The production of low molecular weight AGE precursors in uremic plasma has also been demonstrated by *in vitro* incubation experiments (28). Plasma samples were incubated under air for several weeks while the generation of pentosidine in the medium was monitored. Protein-linked pentosidine levels rise much more in uremic than in control plasma. Most precursors of this newly formed pentosidine have a molecular weight below 5000 Da. Indeed, when plasma is ultrafiltrated through a filter with a 5000 Da cutoff, the difference in pentosidine generation between uremic and control plasma ultrafiltrate is sustained. This conclusion is further supported by the observation that the pentosidine yield is higher in pre- than postdialysis plasma samples. Finally, addition of inhibitors of the carbonyl amine reaction, such as aminoguanidine or 2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide (OPB-9195), represses the production of pentosidine, pointing to the RCO nature of the pentosidine precursor(s). Taken together, these results confirm the accumulation of RCO in uremic plasma and suggest that they are indeed precursors of AGE.

In *in vivo* pentosidine levels in plasma are correlated with the level of *in vitro*-generated pentosidine after incubation of the same plasma samples (28). This correlation suggests that the prevailing plasma pentosidine level mirrors the level of its RCO precursor(s). Taken together, these results confirm the accumulation of RCO in uremic plasma and suggest that they are indeed precursors of AGE.

**Accumulation of RCO in hemodialysis patients**

Table 2. Accumulation of RCO in hemodialysis patients

<table>
<thead>
<tr>
<th>RCO</th>
<th>Precursor(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Deoxyglucosone</td>
<td>Carbohydrate</td>
<td>29</td>
</tr>
<tr>
<td>Dehydroascorbate</td>
<td>Ascorbic acid</td>
<td>31</td>
</tr>
<tr>
<td>Glyoxal</td>
<td>Carbohydrate, lipid, amino acid</td>
<td>30</td>
</tr>
<tr>
<td>Methylglyoxal</td>
<td>Carbohydrate, lipid, amino acid</td>
<td>30</td>
</tr>
<tr>
<td>Malondialdehyde</td>
<td>Lipid</td>
<td>14</td>
</tr>
<tr>
<td>Arabinose</td>
<td>Carbohydrate</td>
<td>Miyata <em>et al.</em>, unpublished observation</td>
</tr>
</tbody>
</table>

*a RCO, reactive carbonyl compound.*
carbonyl stress, not only AGE derived from carbohydrates, but also ALE derived from lipids accumulate in parallel in plasma as well as in tissue proteins.

**Causes of Uremic Carbonyl Stress**

Two competing but not mutually exclusive hypotheses should be considered to account for the uremic carbonyl stress: an increased generation or a decreased removal (detoxification or clearance) of RCO.

**Oxidative Stress**

Production of RCO is known to be increased by oxidative stress. Several reports point to an increased oxidative stress in uremia, characterized by an augmented production of oxidants and a decreased level of antioxidants. The evidence includes increased serum ratios of oxidized to reduced ascorbate (31), glutathione (32), and albumin (33); increased serum levels of advanced oxidation protein products (34) and of protein car- 

The oxidative stress might be further worsened by some modalities of renal replacement therapy: The hemodialysis treatment activates complement and leukocytes, which release reactive oxygen species (39,40).

The oxidative stress modifies proteins either directly through the oxidation of amino acids by reactive oxygen species (41) or indirectly by an increased generation of RCO (17). Carbohy-

**Impaired RCO Detoxification**

The rise in RCO in uremia might also be accounted for by a decreased removal. RCO are detoxified by several enzymatic pathways, such as aldose reductase, aldehyde dehydrogenases, and the glyoxalase pathway (22). Redox coenzymes, reduced glutathione (GSH) and nicotinamide adenine denucleotide (phosphate) (NAD(P)H), contribute to their activity. RCO such as methylglyoxal and glyoxal react reversibly with the thiol

![Figure 1](https://example.com/fig1.png)

**Figure 1.** Routes to generation and detoxification of reactive carbonyl compounds (RCO). The level of oxidative stress is depicted as the balance between the overall rate of formation of reactive oxygen species (ROS) \((k_1 \times [O_2])\) and the rate of their inactivation by antioxidant defenses \((k_2 \times [O_2]^\cdot)\). ROS react with carbohydrates, polyunsaturated fatty acids, or amino acids \((k_3 \times [O_2]^\cdot \times [Substrate])\) to yield RCO. RCO are also formed by nonenzymatic reactions \((k_4; e.g., 3
deoxyglucosone) and by anaerobic metabolism \((k_5; e.g., methylglyoxal). RCO are detoxified \((k_6 \times [RCO])\) by a variety of metabolic pathways (e.g., the glyoxalase pathway) or probably cleared from the body \((k_7). The RCO subsequently react with protein \((k_8 \times [RCO] \times [Proteins])\) to form advanced glycation end products (AGE)/advanced lipoxidation end products (ALE). The increase in RCO in uremia results from an increase in their production \((k_3 + k_4 + k_5)\) and/or a decrease in their removal \((k_6 + k_7). The AGE/ALE formation depends on the balance between their rates of formation \((k_8)\) and degradation \((k_9)\).
group of glutathione and are subsequently detoxified by glycoxalases I and II into lactate and glutathione. NAD(P)H 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 RCO ($k_n$).

It is of interest to know in this context that the glutathione concentration in red blood cells and the serum activity of glutathione-dependent enzymes are significantly reduced in uremic patients (32,36,37). The hypothesis that a decreased thiol storage capacity contributes to the accumulation of RCO in uremia is supported by recent evidence. Glutathione peroxidase activity is indeed inversely correlated with the pentosidine levels in the plasma of hemodialysis patients (37). Of course, other as yet unexplored enzymatic mechanisms might contribute to a decreased removal of RCO and thus to the uremic carbonyl stress.

It should be pointed out that the decreased thiol concentration in uremia might reflect its consumption during detoxification of reactive oxygen species generated under uremia-associated oxidative stress. Still, as yet unproven in uremia, a nonoxidative pathway to decrease thiol concentration has been recognized in diabetes. The polyol pathway is activated by hyperglycemia and consumes NAD(P)H for the reduction of glucose to sorbitol, a process catalyzed by aldose reductase (46). The decrease of NAD(P)H availability for GSH reductase lowers GSH levels. This process depends on glucose concentration but is independent of oxidative stress. It remains to be seen whether the decrease of thiol concentration in uremia derives only from oxidative stress or from other nonoxidative pathways.

**Decreased Glomerular Filtration of RCO**

RCO derived from both oxidative and nonoxidative chemistry of both carbohydrates and lipids have a rather low molecular weight. A decreased renal clearance may contribute to the uremic carbonyl stress ($k_n$). In fact, the pentosidine level in both uremic and diabetic patients is strongly influenced by residual renal function (8,13,47). If pentosidine levels are taken as markers of RCO precursors (28), these data point to a significant role of the failing kidney in raising plasma RCO levels.

**Implications of Carbonyl Stress**

The consequences of the uremic carbonyl stress and its attendant AGE and ALE modification of proteins are discussed below.

**Cellular Effects of RCO**

Several lines of evidence suggest that RCO interfere with various cellular functions independently of their effect on AGE and ALE modification of proteins. RCO are biologically active, initiate a variety of cellular responses, and induce structural and functional alterations of proteins (17,48).

For example, renal failure is associated with resistance to the action of calcitriol (1,25-dihydroxyvitamin D) (49), which is partly attributed to the inhibition by unknown uremic toxins of the interaction between the vitamin D receptor and vitamin D response elements (50). Subsequently, Patel and coworkers (51) demonstrated that RCO capable of Schiff base formation with lysine residues of the vitamin D receptor inhibit its interaction with the vitamin D response element.

In another model, exposure in vitro of cultured mesothelial and endothelial cells to methylglyoxal increases mRNA and protein synthesis of vascular endothelial growth factor (VEGF) (52). VEGF also increases in vivo in the peritoneal tissue of rats given repeated intraperitoneal loads of methylglyoxal (52).

Carbonyl stress also influences the intracellular signaling by multiple pathways. First, AGE, upon interaction with the receptor for AGE, trigger the signaling involving ras pathway (53). Second, exposure of fibroblasts to glyoxal activates protein kinases such as c-Src and increases intracellular tyrosine phosphorylation of several cellular proteins (54). 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 (54). Third, hydroxynonenal causes a capping of epidermal growth factor (EGF) receptor on the cell surface, mimics the effect of EGF on the downstream signaling pathways that involve mitogen-activated protein kinases, and contributes to oxidative stress-induced apoptotic cell death (55). Fourth, hydroxynonenal also triggers oxidative stress-induced apoptotic cell death by activating caspase-3 through a Fas-independent but GSH-dependent redox pathway (56). Finally, methylglyoxal induces apoptosis in Jurkat leukemia T cells by activating c-Jun N-terminal kinase (57).

**AGE and ALE Biologic Effects**

Carbonyl stress is also implicated in the development of several uremic complications. Dialysis-related amyloidosis is a serious bone and joint destruction associated with chronic renal failure (58). The cause of $\beta_2$-microglobulin amyloid fibril deposition is not fully elucidated. The role of elevated plasma $\beta_2$-microglobulin levels is disputed because no difference has been found between dialysis patients with and those without clinical evidence of complications (59). Efforts have therefore been directed toward identification of chemical modifications of $\beta_2$-microglobulin. Although these studies are incomplete, immunohistochemical and chemical analyses indicate that $\beta_2$-microglobulin amyloid deposits are modified by carbonyl stress. Long-lived $\beta_2$-microglobulin amyloid plaques react with anti-AGE (CML, pentosidine, and imidazolone) and anti-ALE (malondialdehyde-lysine and hydroxynonenal-protein adduct), as well as anti-receptor for AGE antibodies (60–65). $\beta_2$-Microglobulin amyloid fibrils isolated from plasma and urine of uremic patients also reacts with these antibodies (9,60).

Atherosclerosis is a major complication of chronic renal failure. The levels of AGE in randomly collected arterial tissues are higher in dialysis patients than in healthy subjects (66). AGE and ALE are detectable by immunohistochemistry in the fatty streak and in the thickened neointima (43). The staining patterns of AGE and ALE correspond with that for protein carbonyls, a biomarker of oxidative protein damage (42). The formation of AGE/ALE in vascular lesions is not
specific to uremia, but might be a phenomenon common to most, if not all, types of vascular damage, regardless of whether the vascular injury is caused by metabolic or mechanical factors: AGE/ALE are identified in vascular lesions not only in uremic patients but also in aged and diabetic subjects (42), and in nonuremic rats whose carotid artery has been injured by a balloon (43). Colocalization of AGE/ALE and protein carbonyls in the vascular tissue indicates a wide range of chemical modifications in vascular matrix proteins. The quality of the peritoneal membrane deteriorates progressively with peritoneal dialysis (PD) duration (67). Membrane alterations, characterized by interstitial fibrosis, disappearance of mesothelial cells, vascular wall thickening, vasodilatation, and increased angiogenesis develop progressively, together with changes in permeability characteristics. The glucose content of PD fluid has been incriminated. Glucose is degraded during heat sterilization into a variety of RCO, such as methylglyoxal, glyoxal, and 3-deoxyglucosone (68–70). These RCO might play a role in the development of AGE and ALE accumulating in the mesothelial layers and in the vascular walls of the peritoneum (70,71). Interestingly, AGE such as CML and pentosidine colocalize in the peritoneum with VEGF (52,72). These findings support the implication of peritoneal modification by RCO in the liberation of VEGF, a potent factor enhancing vasodilatation, vascular permeability, angiogenesis, and nitric oxide synthase production (73), all of which may contribute to peritoneal membrane deterioration. In these three examples, it remains to be seen whether the presence of AGE and ALE merely result 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. AGE- and ALE-modified proteins prepared in vitro initiate a range of inflammatory responses, including stimulation of monocyte chemotaxis (74,75), secretion of inflammatory cytokines from macrophages (74–76), stimulation of collagenase secretion from synovial cells (74), stimulation of osteoclast-induced bone resorption (77), proliferation of vascular smooth muscle cells (78), stimulation of aggregation of platelets (79), stimulation of VEGF (80) and platelet-derived growth factor (81) production from endothelial cells, induction of insulin-like growth factor I from monocytes (82), inhibition of antibacterial activity of lysozyme and lactoferrin (83), and quenching nitric oxide activity (84).

**Therapeutic Approaches**

Carbonyl stress is clearly implicated in the development of several uremic complications. Its manipulation should provide new therapeutic insights. Among them are redox modulation, the use of inhibitors of carbonyl amine chemistry, and the improvement of dialyzer membrane biocompatibility.

**Redox Therapy**

The decrease in thiol concentration impairs the detoxification of RCO and potentiates the formation of AGE and ALE. Repletion of thiol might prove possible and useful by supple-mentation of glutathione, N-acetylcysteine, or cysteine. Addition of these thiol compounds in both uremic and normal plasma as well as in glucose-based PD fluid lowers the generation of AGE after incubation in vitro (our unpublished observation). Several other compounds may also prove helpful. Vitamin E and ubiquinol relieve the demands on the activity of glutathione. Lipoic acid is reduced by mitochondrial dehydrogenases to dihydrolipoate, which subsequently reacts with reactive oxygen species and replenishes glutathione (85). Inhibitors of aldose reductase are other candidates to replenish glutathione, as they prevent the polyol pathway activation and replenish NAD(P)H and glutathione available for both aldose reductase and glutathione reductase (86). Administration of lipoic acid (87) or of the aldose reductase inhibitor Stattil (88) to rats with streptozotocin-induced diabetes increases thiol concentration and/or decreases methylglyoxal in tissues and blood.

**Carbonyl Stress Inhibitor**

AGE/ALE are formed by carbonyl amine chemistry between RCO and proteins. Trapping of RCO with substances such as aminoguanidine (89) and OPB-9195 (90) should inhibit the formation of AGE and ALE. Indeed, we have demonstrated that both compounds inhibit the in vitro formation of AGE from a variety of individual precursors such as ribose, glucose, and ascorbate, as well as that of AGE, malondialdehyde-lysine, and hydroxynonenal-protein adduct from arachidonate (91). Pentosidine generation in uremic plasma (28) and in glucosereich PD fluid (91) incubated for 4 wk is also inhibited by aminoguanidine and OPB-9195.

On a molar basis, OPB-9195 is more effective than aminoguanidine because the latter’s hydrazine nitrogen atom has a decreased nucleophilicity due to the proximity of the guanidinium cation. OPB-9195 as well as aminoguanidine might act by an antioxidative mechanism inhibiting the production of RCO from the various substrates. Alternatively, it may trap the available RCO and thus prevent AGE formation. The observation that OPB-9195 markedly decreases in vitro the level of RCO present in glucose-based PD fluid (91) strongly supports the latter hypothesis. Trapping may result from the reaction of the hydrazine nitrogen atom of aminoguanidine and OPB-9195 with carbonyl groups, leading to the eventual formation of hydrazones.

Interestingly, OPB-9195 corrects several biologic effects of RCO. In murine thymocytes and fibroblasts, it inhibits the phosphorylation of tyrosine residues of a number of intracellular proteins induced by glyoxal (54). Given to Otsuka-Long-Evans-Tokushima-Fatty (OLETF) rats, a model of non-insulin-dependent diabetes mellitus, OPB-9195 reduces urinary albumin excretion and improves the morphology of glomeruli (90). 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 (43).

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 RCO from blood during dialysis therapy.

Membrane Biocompatibility

An unexpected observation has yielded an additional therapeutic insight. A cross-sectional and longitudinal study of dialyzed patients has shown that both protein-linked and free pentosidine levels are lower in the plasma of patients given polysulfone dialysis than in those treated with several other membranes (47,92), indicating the equally biocompatible toward the complement and leukocyte system high-flux AN69 membrane and the less biocompatible low-flux cuprophane membrane. Pentosidine levels are lower in Belgian, Japanese, and German patients given dialysis with polysulfone membranes produced by two different companies.

There is at present no obvious explanation for these results. The effect is unrelated to the porosity or to the clearing ability of the membrane for pentosidine: Hemodialysis itself does not modify protein-linked pentosidine levels (more than 90% of pentosidine is linked to nondiffusible albumin), and the clearance of free pentosidine (379 Da) during a single dialysis session is similar for all membranes (18). The lower level of pentosidine probably reflects a lower generation of RCO, precursors of pentosidine through an as yet undefined mechanism. Much research remains to be done to demonstrate the clinical relevance of these observations.

Until recently, membrane biocompatibility has been discussed in relation to acute, enzymatic biochemistry, such as leukocyte and complement activation, and production of cytokines. However, long-term, nonenzymatic biochemistry may also be equally important in terms of membrane biocompatibility.

Conclusion

Research on AGE and ALE has led to new insights in nonenzymatic biochemistry in renal failure. It has revealed the accumulation of RCO derived from carbohydrates and lipids, the so-called carbonyl stress. Carbonyl stress alters the structure and function of cellular and matrix proteins and might underlie the development of long-term complications. It may prove amenable to therapeutic interventions. Carbonyl stress should therefore be considered a major contributor to uremic toxicity.

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References


