Implication of carbonyl stress in long-term uraemic complications

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Abstract Advanced glycation end products (AGEs) are formed during non-enzymatic glycation and oxidation (glycoxidation) reactions. AGEs, such as pentosidine and carboxymethyllysine are increased in plasma proteins and skin collagen of uraemic patients several times more than in normal subjects and non-uraemic diabetic patients. However, AGEs do not differ between diabetics and non-diabetics in uraemic patients. The AGE accumulation in uraemia, therefore, cannot be attributed to hyperglycaemia, nor simply to a decreased removal by glomerular filtration of AGE-modified proteins. Recent evidence has suggested that, in uraemia, the increased carbonyl compounds, derived from both carbohydrates and lipids, modify proteins not only by glycoxidation but also by lipoxidation reactions, leading to the increased production of AGEs and advanced lipoxidation end products (ALEs). Thus, uraemia might be a state of increased carbonyl compounds with potentially damaging proteins ('carbonyl stress'). Carbonyl stress in uraemia appears relevant to long-term complications, such as dialysis-related amyloidosis. The increased AGEs and ALEs in uraemic plasma and tissue proteins may indicate alterations in the non-enzymatic chemistry involving both carbohydrates and lipids in uraemia.

Increase of advanced glycation end products (AGEs) in uraemia

AGEs are formed by non-enzymatic glycation and oxidation (glycoxidation) reactions. AGE research initially focused on diabetic patients with hyperglycaemia. Indeed, a marked increase in AGEs has been demonstrated in the plasma and skin collagen of diabetic patients [1]. This increase in AGEs was ascribed to hyperglycaemia, because a correlation was found between AGEs and fructoselysine, a biomarker of prevailing plasma glucose concentration [1].

Subsequently, it was discovered that AGEs accumulate markedly in the plasma and collagenous tissues [1,2] in normoglycaemic uraemic patients. Pentosidine [3] and N’-(carboxymethyl)lysine (CML) [4] are well-characterized AGE structures. Serum pentosidine determined by high performance liquid chromatographic assay and CML determined by gas chromatography/mass spectrometry, were elevated in haemodialysis patients several times above those of normal subjects and non-uraemic diabetic patients [2, Miyata et al., submitted]. Of particular note is the finding that there is no significant difference statistically in the plasma pentosidine [2] and CML [Miyata et al., submitted] between diabetic and non-diabetic haemodialysis patients. This finding suggests that the plasma AGEs in uraemia appear unrelated to elevated glucose. This assumption was further supported by the fact that, in contrast to diabetics, there was no correlation in uraemia between serum pentosidine and fructoselysine [2] or between serum CML and fructoselysine [Miyata et al., submitted].

Mechanism of increased AGEs in uraemia

Recent studies have demonstrated that over 90% of plasma pentosidine and CML were linked to albumin [2, Miyata et al., submitted]. These high molecular weight modified albumins are also unlikely to be cleared from the circulation by glomerular filtration. Therefore, the AGE accumulation in uraemia cannot be attributed merely to a decreased removal of pentosidine- and CML-linked albumin by glomerular filtration.

Furthermore, as already described, the AGE accumulation in normoglycaemic uraemia cannot be attributed to hyperglycaemia. Obviously, uraemic sera contain either unknown precursor(s) and/or catalyst(s) for the Maillard reaction. In vitro studies demonstrated that pentosidine originates from glucose and ribose [3] and that CML is formed of proteins exposed to glucose [4]. Subsequently, ascorbic acid proved to be a source of both CML and pentosidine [5]. However, it has recently become apparent that CML can be derived not only from carbohydrates but also from lipid
sources such as polyunsaturated fatty acids (PUFAs) [6].

The assumption that glucose is not the sole precursor of AGEs in uraemic patients is supported by recent findings by ourselves and by other groups that there are significant correlations in haemodialysis patients between serum pentosidine and dehydroascorbate [5] and between serum CML and malondialdehyde [Miyata et al., submitted], in contrast to the absence of correlation between the serum AGE (pentosidine and CML) and glucose.

Recent studies have emphasized that both CML and pentosidine are products of the combined processes of glycation and oxidation (glycoxidation) and are referred to as glycoxidation products (GOPs). Therefore, the increase of pentosidine and CML in skin collagen and plasma proteins in uraemia, in the absence of hyperglycaemia, suggests that oxidative stress, rather than glycative stress, is increased in uraemia. Several lines of evidence have suggested that chronic uraemia appears to be a state of increased oxidative stress [5,7].

Augmented oxidative stress might therefore accelerate AGE formation in uraemia [8]. This assumption is supported by recent findings by ourselves and by other groups that there are significant correlations between the serum pentosidine and oxidative markers such as advanced oxidation protein products [7] and oxidized ascorbate [5] and between the serum CML and a lipid peroxidation marker [Miyata et al., submitted], in contrast to the lack of correlation between the serum levels of pentosidine and fructoselysine.

**Carbonyl modification of proteins: carbonyl stress**

Oxidative stress has been implicated in several diseases. Carbohydrates and lipids are the major targets of reactive oxygen species. As already described, oxidative stress conspires with non-enzymatic glycation to form AGEs/GOPs on proteins. Several intermediates resulting from autoxidation of carbohydrates, ascorbate, and the following PUFAs have been identified as precursors of GOPs, glyoxal, methylglyoxal, arabinose, glycolaldehyde, and dehydroascorbate [9]. A common structural feature of these intermediates is the presence of carbonyl group(s), which are capable of reacting with protein amino groups, leading to the formation of GOPs on proteins.

Lipid peroxidation also occurs in response to oxidative stress and forms a variety of carbonyl compounds, such as malondialdehyde (MDA) and 4-hydroxynonenal [10], both are highly reactive with proteins, leading to the formation of covalent adducts, called lipoxidation products (LOPs) or advanced lipidation end products (ALEs).

In addition to oxidative mechanisms, some reactive carbonyl compounds may also be produced by non-oxidative pathways e.g. 3-deoxyglucosone and methylglyoxal. Both 3-deoxyglucosone and methylglyoxal are known to react with protein amino groups and to form GOPs on proteins.

Therefore, reactive carbonyl compounds derived from carbohydrates and lipids by both oxidative and non-oxidative mechanisms, are increased simultaneously in uraemic plasma, leading to the formation of GOPs/AGEs and GOPs/ALEs. This condition can be described as ‘carbonyl stress’ [9]. Indeed, all these biomarkers were elevated in uraemic plasma [Miyata et al., submitted] and there was a correlation between plasma CML and MDA-lysine.

**Implication of ‘carbonyl stress’ in uraemia**

‘Carbonyl stress’ has been implicated in dialysis-related amyloidosis [11]. Several lines of evidence have demonstrated the presence of AGEs in β₂-microglobulin amyloid fibrils: physicochemical properties of AGEs [12], immunoreactivity to anti-AGE antibodies [12,13], detection of several AGE structures [5,13], and binding to the receptor for AGEs [14]. At present, it remains unknown whether the modification with carbonyl stress plays an active role or is merely long-term transformation of long-lived amyloid fibrils. However, recent studies have demonstrated that AGE-modified proteins are endowed with biological activities which can partly account for the bone and joint destruction in dialysis-related amyloidosis, e.g. monocytic chemotaxis, macrophage secretion of inflammatory cytokines, synovial cell production of collagenase, and osteoclast bone resorption [15,16]. Uraemic arthropathies might thus be the combined result of the accumulation of carbonyl stress in long-lived amyloid fibrils linked to a heightened cellular response. Further studies are necessary to address this issue.

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**References**


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