‘Carbonyl stress’ and dialysis-related amyloidosis

T. Miyata, Y. Ueda, A. Saito and K. Kurokawa

Institute of Medical Sciences and Department of Medicine, Tokai University School of Medicine, Isehara, Kanagawa 259–1193, Japan

Abstract

Advanced glycation end products (AGEs) are formed by nonenzymatic glycation and oxidation (glycoxidation) reactions. As AGE formation is related to hyperglycaemia, they have been implicated in the pathogenesis of diabetic complications. They also increase in normoglycaemic uraemic patients: AGEs, such as pentosidine and carboxymethyllysine (CML), are elevated in both the plasma proteins and skin collagen of uraemic patients, being several times greater than in normal subjects and nonuraemic diabetic patients. However, AGE concentrations do not differ between diabetics and non-diabetics in uraemia. AGE accumulation in uraemia, therefore, cannot be attributed to hyperglycaemia, or simply to the decreased removal by glomerular filtration of AGE-modified proteins as over 90% of plasma pentosidine and CML are linked to albumin. Recently, evidence has suggested that, in uraemia, the increased carbonyl compounds, derived from both carbohydrates and lipids, modify proteins both by the glycoxidation reaction (leading to augmented AGE production) and also by the lipoxidation reaction (leading to the augmentation of the advanced lipoxidation end product, ALE, production). Thus, uraemia might be a state of carbonyl overload with potentially damaging proteins ('carbonyl stress'). Carbonyl stress in uraemia appears to be relevant to long-term complications associated with chronic renal failure and dialysis, such as dialysis-related amyloidosis. Immunohistochemical studies, with specific antibodies to AGEs and ALEs, identified carbonyl stress in long-lived \( \beta_2 \)-microglobulin amyloid deposits. Furthermore, proteins modified with carbonyl stress exhibit several biological activities through interactions with several types of cell, e.g. monocytes/macrophages, synovial cells and osteoclasts/osteoblasts, which might partially account for dialysis arthropathies.

Increase of the advanced glycation endproduct modification in uraemia

Dialysis-related amyloidosis is a serious complication associated with chronic renal failure (CRF) and long-term dialysis [1]. Amyloid fibrils consisting of \( \beta_2 \)-microglobulin deposit preferentially in osteoarticular tissues and then, over the years, lead to bone and joint destruction. Two stages may be considered in the development of dialysis-related amyloidosis. The first is asymptomatic and is diagnosed mainly by pathology several years before the onset of clinical or radiological signs of this complication [2]. However, at this early stage, neither macrophage nor bone destruction is detectable in the vicinity of amyloid deposits. The second stage is symptomatic and is accompanied by an inflammatory reaction. Recent histological studies have shown the accumulation of monocytes/macrophages around amyloid deposits [3,4]. The question thus arose as to what transforms silent early deposits into clinically manifest evidence of bone and joint destruction over the years.

A clue was provided by the demonstration of the existence of an acidic isoform of \( \beta_2 \)-microglobulin, which is a major form in amyloid [5,6]. This finding suggests a potential link between acidic \( \beta_2 \)-microglobulin and the pathogenesis. Thus, the chemical modification responsible for acidic \( \beta_2 \)-microglobulin was investigated and a new chemical modification, the advanced glycation end products (AGEs), were recently identified [7,8]. AGEs are the end result of the Maillard or browning reaction to non-enzymatically form Schiff’s base between the protein amino group and carbonyl group [9]. AGEs display characteristic physicochemical properties, such as fluorescence, brown colour and a polymerization tendency.

AGE research focused initially on diabetic patients with high glucose concentrations. Indeed, a marked increase in AGE has been demonstrated in the plasma and skin collagen of diabetic patients [9,10]. This increase was ascribed to hyperglycaemia. Indeed, a correlation was found between the concentration of AGEs and fructoselysine, a marker of prevailing glucose concentration [10]. Of great interest was the further demonstration of a relationship between serum and tissue AGE and the severity of diabetic complications [11,12]. It was thereby suggested that AGE modification of proteins played a causal role in the development of diabetic complications.
Subsequently, it was discovered that AGEs accumulate markedly in the skin and plasma proteins of uraemic patients [13–15]. Pentosidine [16] and carboxymethyllysine (CML) [17] are well-known AGE structures. Serum concentrations of pentosidine determined by HPLC assay [14] and CML determined by GC-MS [18], in haemodialysis patients were several times greater than those of normal subjects and non-uraemic diabetic patients. However, there was no statistically significant difference in the plasma pentosidine and CML between diabetic and non-diabetic haemodialysis patients [14]. These findings suggest that the plasma AGE in uraemia was unrelated to elevated glucose. This assumption was supported by the fact that, in contrast to diabetics, there is no correlation in uraemia between serum pentosidine and fructoselysine, and between serum CML and fructoselysine [14 and our unpublished observation].

**Mechanism of AGE accumulation in uraemia: ‘carbonyl stress’**

As pentosidine and CML are linked mainly to albumin in the serum [14 and our unpublished observation], their accumulation cannot be attributed to decreased removal of pentosidine- and CML-linked albumin by glomerular filtration. Furthermore, as discussed above, their accumulation cannot be attributed to an increased glucose concentration. Obviously, uraemic sera contain either unknown precursors and/or catalysts of the Maillard reaction. Recently, evidence has suggested that an increased oxidative stress in uraemia stimulates the auto-oxidation of a variety of precursor substances, which are unable, under normal conditions, to augment AGE production [19].

First, the formation of pentosidine and CML is known to be closely linked to oxidation [20]. Both pentosidine and CML require oxygen for their formation. Pentosidine was identified as a protein cross-linker between lysine and arginine residues [16]. CML was originally identified as a product formed by the oxidative cleavage of a glucose-derived Amadori compound [17]. However, it was recently demonstrated that the auto-oxidation glucose and ascorbate products formed under oxidative stress, such as glyoxal, arabinose, 3-deoxyglucosone and dehydroascorbate are efficient precursors of CML and pentosidine [21–24]. The characteristic structure among these intermediate compounds is the presence of a carbonyl group which forms Schiff’s base with protein. AGEs, such as pentosidine and CML, are thus products of the combined process of glycation and oxidation (‘glycoxidation’) and are referred to as glycoxidation products.

Secondly, chronic uraemia appears to be a state of increased oxidative stress as suggested by an increased ratio of oxidized to reduced glutathione [25], an increased ratio of oxidized to reduced serum albumin [26], decreased activity of glutathione-dependent enzymes [27], increased serum ‘advanced oxidation protein products’ (AOPP) [28] and an increased ratio of oxidized to total ascorbate [24].

Thus, we hypothesize that an augmented oxidative stress accelerates AGE formation in uraemia [19]. This notion is supported by recent findings by our and other groups that there are significant correlations between serum pentosidine and oxidative markers such as AOPP [28] and oxidized ascorbate [24], in contrast to the absence of correlation between serum pentosidine and fructoselysine.

Lipid peroxidation also occurs in response to oxidative stress and forms a variety of carbonyl compounds, such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE) [29]. These aldehydes are highly reactive with proteins, leading to formation of MDA–protein and HNE–protein adducts, called lipoxidation products or the advanced lipoxidation end products (ALEs). Therefore, under oxidative stress, carbohydrate, polyunsaturated fatty acids (PUFAs) and ascorbate may be simultaneously and independently auto-oxidized and converted into carbonyl compounds, which react with proteins, forms Schiff’s base and eventually ALEGs and ALEs. AGEs and ALEs are thus formed by carbonyl amine chemistry between a protein amino group and a carbohydrate- or lipid-derived carbonyl group. We propose to call this process the ‘carbonyl stress’ pathway [30].

Several lines of evidence support the assumption that uraemia might be a state of increased carbonyl stress. First, GC-MS and HPLC were applied to measure protein adducts formed by glycoxidation and lipoxidation reactions: pentosidine, CML and MDA-lysine. All three adducts were elevated in uraemic plasma in the absence of a correlation with hyperglycaemia and hyperlipidaemia (our unpublished observation). Interestingly, plasma concentrations of CML correlated with those of MDA-lysine. These findings indicate the simultaneous increase of AGEs and ALEs in uraemic plasma.

Secondly, when osteoarticular amyloid tissues from haemodialysis patients were examined immunohistochemically, with specific antibodies to detect AGEs and ALEs, both AGEs and ALEs were identified in macrophage-rich β₂-microglobulin amyloid deposits [8,24, and our unpublished observation]. The antibodies used to detect carbonyl stress end products recognize distinct structures and apparently do not cross-react with the other structures, based on inhibition by modified bovine serum albumin (BSA) competitors [31]. Therefore, the co-localization of AGEs and ALEs in amyloid tissues provides independent evidence for increased glycoxidation and lipoxidation reactions in amyloid deposits. It is notable that the staining patterns of these biomarkers topographically coincided with those of protein carbonyls (our unpublished observation), a biomarker of oxidative protein damage [32].

Thirdly, when ultrafiltered plasma was exposed to 2,4-dinitrophenylhydrazine, the yield of hydrazones, formed by an interaction with carbonyl groups and detected using a spectrophotometric assay, was several times greater in uraemic than in control patients (our
unpublished observation). Although, at present, it remains unknown whether these carbonyl compounds, which accumulated in uraemic plasma, contribute to the augmentation of AGE and ALE, production these in vitro studies demonstrate the accumulation of carbonyl compounds in uraemic plasma.

Pathological role of ‘carbonyl stress’ in uraemia

The relevance of ‘carbonyl stress’ in uraemia was suggested in dialysis-related amyloidosis. Several lines of evidence demonstrated the presence of AGEs in acidic β2-microglobulin and amyloid fibrils: physicochemical properties of AGEs [7], immunoreactivity to anti-AGE antibodies [7,8], detection of several AGE structures [8,24] and binding to the receptor for AGEs [33].

At present, it remains unknown whether the AGE-modification derived from carbonyl stress plays an active role in the pathogenesis of β2-microglobulin amyloidosis, or is merely a long-term transformation of long-lived amyloid fibrils. However, recent studies have demonstrated that AGEs are endowed with biological activities that can partly account for dialysis arthropathies: monocyte chemotaxis [34], macrophage secretion of inflammatory cytokines [34–36], synovial cell production of collagenase [34] and osteoclast bone resorption [37,38]. Uraemic arthropathies might thus be the combined result of AGE accumulation in long-lived amyloids linked to a heightened cellular response. The progressive accumulation of AGEs in amyloid deposits might be the factor transforming the silent early deposits into clinically manifest osteoarticularitis at the advanced stage. More studies will, of course, be necessary to address this issue.

Conclusions

The mechanism of increased carbonyl compounds in uraemia remains incompletely understood. However, there has been much progress in understanding of the mechanism of increased AGE in uraemic patients. Several lines of evidence suggest that, in uraemia, increased carbonyl compounds, derived from both carbohydrates and lipids, modify proteins not only by glycoxidation, but also by lipoxidation reactions. The increased AGEs and ALEs in blood and tissues in uraemic patients may thus betray a broad derangement in nonenzymatic biochemistry involving alterations in both carbohydrates and lipids. Yet, it remains important to further investigate whether carbonyl stress actively plays a role in the pathogenesis of dialysis-related amyloidosis or is merely a result of the long-term accumulation in amyloid fibrils. Further study will undoubtedly be necessary to understand the pathological role of carbonyl stress in uraemia and develop an effective treatment strategy.

Acknowledgements. This study was supported by a grant from Research for the Future Program of the Japan Society for the Promotion of Science (96L00303).

References