Pathophysiology of advanced glycation end-products in renal failure

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Abstract. β2-Microglobulin is a major constituent of amyloid fibrils in dialysis-related amyloidosis, a serious complication leading to bone and joint destruction in long-term haemodialysis patients. However, the molecular pathogenesis of this complication remains unknown. Intact β2-microglobulin per se seems an unlikely contributor to the pathogenesis, because no difference in the plasma levels of intact β2-microglobulin has yet been found between haemodialysis patients with and without this complication. Some investigators have therefore focused on the modification of this molecule. Recent studies have revealed a new modification of β2-microglobulin in amyloid fibrils—the advanced glycation end-products (AGEs) formed by a non-enzymatic reaction between sugar aldose and protein. Further studies have suggested that the interaction of AGE-modified β2-microglobulin with monocyte/macrophage and osteoclast/osteoblast gives a plausible albeit partial explanation for the mechanism of bone and joint destruction in dialysis-related amyloidosis. This article discusses the pathophysiology of AGEs in renal failure and the modification of β2-microglobulin with AGEs, especially focusing on their structure and pathological role in dialysis-related amyloidosis.

Key words: AGEs; β2-microglobulin; pathophysiology; renal failure

Introduction

In the Maillard reaction [1] aldehyde groups react with protein amino groups to reversibly form Schiff bases, and upon rearrangement convert into more stable Amadori products over a period of days. Then, over months, some Amadori products are further converted into advanced glycation end-products (AGEs) through a series of chemical rearrangements, dehydration and fragmentation reactions. AGEs constitute a heterogeneous class of structures that are brown in colour, fluoresce and cross-link. AGEs have been thus far implicated in tissue damage associated with the complications of diabetic patients with high glucose concentrations. However, recent studies have indicated a marked increase of AGEs in haemodialysis patients with normal glucose values. This article focuses on the pathophysiology of AGEs in renal failure.

Accumulation of AGEs in renal failure

An increase of the AGEs in the serum or matrix proteins of patients undergoing haemodialysis due to end-stage renal failure has been shown using a radioreceptor assay [2] and enzyme-linked immunosorbent assays using anti-AGE antibodies [3,4], although the AGE structure(s) recognized with their assays remains unknown. Recently, certain AGE structures have been characterized in detail. Pentosidine (Figure 1) is a fluorescent cross-linked molecule involving a lysine and an arginine residue combined in an imidazo-(4,5b)-pyridinium ring [5], and has been postulated to be a glycoxidation marker for AGEs [6]. Recent studies have demonstrated a marked increase of pentosidine in plasma proteins [7], β2-microglobulin (β2m) amyloid fibrils [8] and skin collagens [9] of haemodialysis patients.

The pentosidine in acid hydrolysates of plasma were measured by HPLC (Figure 2). The mean (± SD) pentosidine concentration was significantly greater (P < 0.05) in diabetic patients with normal renal function (2.01 ± 0.83 pmol/mg) than in normal subjects (1.55 ± 0.22 pmol/mg), in good agreement with a previous report [7]. This difference in the pentosidine between diabetic and non-diabetic haemodialysis patients was not evident in patients on haemodialysis whose plasma pentosidine levels (21.75 ± 6.63 pmol/mg for diabetics and 20.33 ± 7.46 pmol/mg for non-diabetics) were elevated >10-fold above normal subjects and diabetic patients with normal renal function (P < 0.0001). Plasma pentosidine was statistically correlated with creatinine in non-diabetic pre-dialysis patients with renal dysfunction, indicating that the plasma pentosidine level is closely related to the level of renal function.
Fig. 1. Structure of pentosidine.

Fig. 2. Plasma pentosidine levels in normal subjects (n = 15), diabetic patients with normal renal function (n = 15), diabetic haemodialysis patients (n = 20) and non-diabetic haemodialysis patients (n = 20).

The pentosidine level in acid hydrolysate of plasma was determined by HPLC [7]. Data are expressed as mean ± SD. *P < 0.05 versus normal subjects, †P < 0.0001 versus normal subjects and §P < 0.0001 versus diabetic patients with normal renal function.

function. Interestingly, plasma pentosidine was exclusively present in the albumin fraction [10].

What is the mechanism of accumulation of albumin-linked pentosidine in renal failure patients? In diabetic patients with normal renal function, increased plasma glucose is thought to accelerate the Maillard reaction and increase generation of albumin-linked pentosidine. By contrast, albumin-linked pentosidine increases by an order of magnitude when renal failure develops, at which time there is no difference between diabetic and non-diabetic haemodialysis patients, meaning that whatever the effect of glucose, it is lost in the renal failure effect. Indeed, it has been shown that there is no statistical correlation between AGEs and glucose [4]. Since albumin-linked pentosidine is not thought to be filtered through the glomerular basement membrane, diminished glomerular filtration is unlikely to cause an increase in albumin-linked pentosidine. Thus, it is likely that substance(s) other than glucose which accumulate in these patients could be a precursor of albumin-linked pentosidine, or that some factor(s) in these patients might catalyse the glycoxidation and accelerate pentosidine formation on albumin even in the absence of increased glucose.

Recently, we found that a small fraction of pentosidine in the circulation of haemodialysis patients exists in free form, which was undetectable in the plasma of either normal subjects or diabetic patients with normal renal function [10]. Since the molecular weight of pentosidine (379 daltons) is low enough to allow complete filtration through the glomerular basement membrane, one of the causes of accumulation of plasma free pentosidine in haemodialysis patients irrespective of the presence or absence of diabetes may be the loss of glomerular filtration. Kinetic study after intravenous or oral administration of pentosidine to intact or nephrectomized rats has revealed that plasma free pentosidine is closely linked to renal dysfunction, and that dietary pentosidine can be absorbed by the gastrointestinal tract into the circulation, thus being one possible origin of circulating free pentosidine [10]. The dietary intake of pentosidine might thus prove to be of importance in the management of patients with chronic renal failure. In addition, since several matrix proteins and albumin contain a significant amount of pentosidine [7,9], plasma free pentosidine might be released during the degradation processes of these proteins.

Further studies to elucidate the precursor of albumin-linked pentosidine, and the origin and pathological significance of plasma free pentosidine accumulating in renal failure patients are needed to improve the understanding of the pathophysiology of AGEs in renal failure.

Pathological role of AGEs in renal failure

Several lines of evidence have suggested that AGE proteins may play a role in normal tissue remodeling, i.e. the removal and replacement of senescent extracellular matrix components [1]. However, under pathological conditions such as diabetes and renal failure, the accumulation of AGE proteins might result in tissue damage through a variety of mechanisms: through an alteration of the structure and function of proteins [1]; by the stimulation of cellular responses via receptors specific for AGE proteins [11,12]; or by the generation of reactive oxygen intermediates [6].

What is the pathological significance of AGEs in renal failure patients who accumulate large amounts of AGEs in the plasma proteins and matrix tissues? Recent studies have shown the possible involvement of AGEs in the complications of long-term haemodialysis patients such as atherosclerosis [2], hyperlipidaemia [13] and dialysis-related amyloidosis (DRA) [8,14–18]. The role of AGEs in the pathogenesis of DRA will be focused on in detail.

Role of AGEs in dialysis-related amyloidosis

DRA is a serious complication recognized with high frequency among long-term haemodialysis patients
joint destruction is not understood well. This electrophoretically acidic isoform of p2m was also migrated to a more acidic position than normal p2m. AGEs in renal failure.[19] Amyloid fibrils predominantly constituted of βm deposit in joint structures, especially in periarticular bones, and lead to haemodialysis arthropathy. The incidence of DRA increases with the age of the patient and the duration of haemodialysis. However, the molecular mechanism of bone and joint destruction in DRA remains unknown. Although recent histological studies have shown the accumulation of monocytes/macrophages around amyloid deposits, the causative factor for their infiltration and the pathological involvement of these inflammatory cells in bone and joint destruction is not understood well.

When a mixture of amyloid fibril proteins isolated from patients with DRA and purified normal serum βm were analysed by two-dimensional polyacrylamide gel electrophoresis, the βm from the amyloid deposit migrated to a more acidic position than normal βm. This electrophoretically acidic isoform of βm was also found in a small fraction (5–10%) of βm of the serum and urine of long-term haemodialysis patients. Of particular interest, the acidic βm purified from the patients' urine showed the characteristic properties of AGE proteins, i.e. brown colour, fluorescence and tendency to polymerize [14]. Furthermore, purified acidic βm as well as amyloid fibril βm exhibited positive immunoreactivity to anti-AGE antibody [14]. A recent immunohistochemical study using anti-AGE antibody revealed positive immunostaining for AGEs in long-lived amyloid deposits with macrophage infiltration [8]. These findings indicate that AGEs are present in amyloid fibril βm of DRA.

Chemical analyses were performed on the purified acidic βm to determine the Maillard site, the structure of Maillard products and the extent of Maillard modification. The α-amino group of the aminoterminal isoleucine was determined to be the major Maillard site in human βm [16]. Computer graphics analysis of the three-dimensional structure of human βm suggested that the imidazole group of histidine-31 was uniquely positioned very close to the α-amino group of isoleucine-1, and could act as an acid–base catalyst for the Amadori rearrangement, thus accelerating the Maillard reaction at isoleucine-1 [16]. The major structure of Amadori products in acidic βm was identified as Ne-(1-deoxyfructosyl)-isoleucine, while Ne-(1-deoxyfructosyl)-lysin was identified as the minor structure [18]. Utilizing the radioactive incorporation of sodium borotritide, which reduces the Amadori products, we demonstrated Maillard products in ~10% of the circulating acidic βm [16].

Thus far, two AGE structures have been identified in both the acidic βm and amyloid fibril βm. One structure is pentosidine [8] and the other is Ne-(carboxymethyl)lysine, which was recently determined to be the epitope structure for the monoclonal anti-AGE antibody [20]. Since a recent study demonstrated that formation of pentosidine and Ne-(carboxymethyl)lysine is closely related to oxidative stress [6], not only glycation but oxidative stress associated with pentosidine formation might function in the development of bone and joint destruction of DRA.

The βm modified with AGEs (AGE βm) was shown to enhance chemotaxis and chemokinesis of monocytes, but normal βm did not enhance any migratory activity of monocytes [15,18]. AGE βm, but not normal βm, also stimulated monocyte-derived macrophages to secrete interleukin 1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin 6 (IL-6) [15,17,18], all of which are potent bone-resorbing cytokines which are deeply involved in bone and joint disorders such as rheumatoid arthritis and osteoporosis. These findings are in good agreement with a previous histological observation that amyloid deposits of long-term haemodialysis patients with severe DRA were surrounded by a number of monocytes/macrophages immunochemically stained positive for IL-1β and TNF-α [21].

We then evaluated the effect of AGE βm on osteoclast-induced bone resorption using an unfractionated bone cell culture system containing mature osteoclasts from the femur and tibia of newborn mice. When the cells were cultured on dentin slices, AGE βm increased the number of resorption pits formed by osteoclasts but normal βm did not [22], suggesting that AGE βm accelerates bone resorption of osteoclasts. This contention was also supported by the observation that AGE βm enhanced net calcium efflux from cultured neonatal mouse calvariae to a much greater extent than normal βm [S. Sprague and T. Miyata, unpublished observation]. In addition to bone resorption, the amount of cytokines secreted from macrophages by AGE βm was sufficient to stimulate collagenase synthesis in cultured human synovial cells [15].

Recently, we tested whether the biological effect of AGE βm is mediated by the receptor for AGEs (RAGE), a newly identified member of the immunoglobulin superfamily which selectively interacts with AGE proteins and is expressed on monocytes/macrophages [13]. Radiolabelled AGE βm, but not normal βm, bound to monocytes in a dose-dependent and saturable manner. The binding of AGE βm was inhibited by excess soluble RAGE, an extracellular domain of RAGE. Monocyte chemotaxis stimulated by AGE βm was also blocked by anti-RAGE antibody. Furthermore, induction of TNF-α from macrophages by AGE βm was also blocked by anti-RAGE antibody. All these findings indicate that interaction of AGE βm with monocytes/macrophages is mediated by RAGE [23].

Taking these findings together, we hypothesized the mechanism of bone resorption and joint destruction in DRA as follows. AGE βm is present in amyloid deposits, inducing monocyte chemotaxis and recruiting them into amyloid deposits from the circulation, where monocytes accumulate through engagement with RAGE. Then AGE βm stimulates monocyte-derived macrophages in situ to secrete potent bone-resorbing cytokines due to interaction with RAGE. These cytokines activate osteoclasts, leading to bone resorption,
probably in concert with osteoblasts. Furthermore, these cytokines stimulate synovial cells to produce collagenase, leading to matrix degradation.

The mechanism of AGE formation in amyloid fibril βm remains unknown. Since the AGE modification occurs preferably in long-lived proteins and amyloid fibril proteins are known to have a long life-time due to extreme insolubility and resistance to proteases, one explanation may be that the AGE modification might take place directly in situ in long-lived amyloid fibril βm. An alternative explanation may be that AGE-modified βm formed in the circulation might be taken up into amyloids due to intermolecular cross-linking with amyloid fibrils or through engagement with RAGE on macrophages surrounding amyloid fibrils in an advanced step of DRA.

Although we have not yet compared the βm-linked pentosidine between diabetic and non-diabetic haemodialysis patients, similar levels of albumin-linked pentosidine between the two groups, as described above, strongly suggest that βm-linked pentosidine may also be similar between diabetics and non-diabetics since the pentosidine precursor is thought to be identical in albumin and βm. This agrees with the finding that diabetic haemodialysis patients do not seem more prone to develop DRA [C. van Ypersele, unpublished observation].

Acknowledgements. We thank Dr C. van Ypersele for invaluable comments and helpful discussions. This study was supported in part by a grant from the Ministry of Education, Science, Sports, and Culture of Japan, and a grant from the Baxter Extramural Grant Program.

References


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