

## Dialysis-Related Amyloidosis: Pathogenesis Focusing on AGE Modification

Toshimitsu Niwa

Department of Clinical Preventive Medicine, Nagoya University Daiko Medical Center, Nagoya, Japan

### ABSTRACT

Dialysis-related amyloidosis (DRA) is a serious complication in long-term dialysis patients, and presents with carpal tunnel syndrome, cystic bone lesions, destructive spondylarthropathy, diffuse arthritis and periartthritis, systemic organ involvement, and dialysis-related spinal canal stenosis (DSCS). Recently a new concept of DSCS has been proposed that includes both destructive spondylarthropathy and myeloradiculopathy induced by extradural thickness.  $\beta_2$ -microglobulin ( $\beta_2$ M) amyloid was demonstrated to be modified with advanced glycation end products (AGEs) such as imidazolone, N<sup>ε</sup>-(carboxymethyl)lysine (CML), and pentosidine. Imidazolone is a reaction product of arginine residue in proteins with 3-deoxyglucosone (3-DG), which is markedly accumulated in uremic serum. Imidazolone is generated under nonoxidative conditions, while CML and pentosidine are formed by oxidative processes. Immunoelectron microscopy demonstrated that AGEs were localized not only in di-

alysis amyloid but also in nonamyloid collagenous structures, supporting the hypothesis that AGE modification of collagen might have pathogenic relevance in the deposition of  $\beta_2$ M on collagen. Serum levels of AGEs are increased in uremic patients. The dimeric form of  $\beta_2$ M in the dialysate and urine of uremic patients is more susceptible to imidazolone modification as observed in dialysis amyloid. However, the major component of dialysis amyloid is a native form of  $\beta_2$ M, while AGE-modified  $\beta_2$ M and truncated  $\beta_2$ M are the minor components. Thus I propose that 3-DG and the other dicarbonyl compounds accumulating in uremic serum promote the modification of  $\beta_2$ M with AGEs mainly after deposition of  $\beta_2$ M as amyloid. For the prevention and treatment of DRA,  $\beta_2$ M should be efficiently eliminated from circulating blood by kidney transplantation, hemodialysis, or hemodiafiltration using high-flux membranes and an adsorbent (Lixelle) column.

Dialysis-related amyloidosis (DRA) is a frequent and major complication in long-term dialysis patients, and presents with carpal tunnel syndrome (CTS), cystic bone lesions, destructive spondylarthropathy, arthritis, periartthritis, and systemic organ involvement (1). We first reported cases with dialysis-related spinal canal stenosis (DSCS) due to DRA (2). Further, it was demonstrated that  $\beta_2$ -microglobulin ( $\beta_2$ M) amyloid deposits are localized extensively in the hearts of long-term hemodialysis (HD) patients (3). This review updates our knowledge on the pathogenesis of DRA, focusing on advanced glycation end product (AGE) modification.

### Dialysis-Related Spinal Canal Stenosis (DSCS)

Recently we proposed a new concept of DSCS, which includes both destructive spondylarthropathy and myeloradiculopathy induced by extradural thickness (2). We first reported three cases operated on for DSCS who had undergone HD for about 20 years. In two cases, cervical

plain X-rays showed only minor spondylotic changes. However, magnetic resonance imaging (MRI), myelography, and computed tomography (CT) showed extradural thickness with compression on the cervical spinal cord and cauda equina. In one case, cervical X-rays showed typical destructive spondylarthropathy and MRI showed compression myelopathy. Surgical treatment on both the cervical and lumbar spine in two patients and on the cervical spine in one patient successfully reduced the symptoms.  $\beta_2$ M-positive amyloid tissue in the extradural thickness was immunohistochemically demonstrated to be modified with AGEs using anti-imidazolone (AG-1) and anti-N<sup>ε</sup>-(carboxymethyl)lysine (CML) (AG-10) antibodies. Inflammatory reaction with histiocytic and giant cell infiltration was also shown around the amyloid tissues. Most of the cells were CD68 positive, and some cells were positive for AGE and  $\beta_2$ M.  $\beta_2$ M accumulation and inflammatory reaction finally promote destruction of connective tissues. MRI, CT, and/or myelography are necessary for diagnosing DSCS.

### DRA in the Heart

$\beta_2$ M amyloid deposits were localized extensively in the hearts of dialysis patients who had undergone HD for more than 10 years (3).  $\beta_2$ M amyloid deposits in the left atrium were localized in the endocardium, the myocar-

*Address correspondence to:* Toshimitsu Niwa, MD, Nagoya University Daiko Medical Center, 1 - 1 - 20, Daiko-minami, Higashi-ku, Nagoya 461-0047, Japan. E-mail: tniwa@med.nagoya-u.ac.jp.

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dium, and the walls of small blood vessels, whereas in the left ventricle they were localized only in the walls of small blood vessels. Most calcification areas near the mitral valve were dotted with  $\beta_2$ M amyloid deposits, while diffuse fine calcification was localized within the  $\beta_2$ M amyloid tissues in some cases. Thus there is a strong affinity between  $\beta_2$ M amyloid deposits and calcification. Imidazolone and CML were localized in massive  $\beta_2$ M amyloid deposits.

### AGE Modification of $\beta_2$ M Amyloid

$\beta_2$ M isolated from the amyloid deposits in patients with DRA was demonstrated to be AGE modified (4). AGE-modified  $\beta_2$ M may be involved in the pathogenesis of DRA by stimulating the chemotaxis of monocytes, the secretion of interleukin (IL)-1 $\beta$ , tumor necrosis factor (TNF)- $\alpha$ , and IL-6 from macrophages, and the subsequent synthesis of collagenase in synovial cells (5).  $\beta_2$ M amyloid was reported to be modified with pentosidine (6). However, it has not yet been proved if AGE-modified  $\beta_2$ M purified from amyloid deposits shows any biological effects toward macrophages, and it is also unknown which chemical structure of AGEs is responsible for the biological effects of the AGE-modified  $\beta_2$ M samples purified from urine or prepared in vitro. Further, a recent study suggests that AGE modification of  $\beta_2$ M may play an inhibitory role for the formation of  $\beta_2$ M amyloid fibril (7).

We demonstrated by using anti-imidazolone (AG-1) (8, 9), and anti-CML (AG-10) (10,11), antibodies that  $\beta_2$ M and its dimer extracted from amyloid deposits was modified with AGEs such as imidazolone (8) and CML (10, 11), and that 3-DG may be involved in the AGE modification of  $\beta_2$ M amyloid (12,13). Further, we recognized by using mass spectrometry that a major component of dialysis amyloid is a native (or deamidated) but not AGE-modified  $\beta_2$ M (10), and that dialysis amyloid contains truncated forms of  $\beta_2$ M as minor components. Thus it is most likely that  $\beta_2$ M is modified with AGEs after its deposition in the tissues as amyloid. Once amyloid is formed, it is difficult to degrade. Then it may react with 3-DG and the other dicarbonyl compounds and become modified with AGEs after a long-term period of deposition as  $\beta_2$ M amyloid. Further, a concomitant generation of oxygen radicals which occurs in HD patients may enhance the aggregation of  $\beta_2$ M amyloid (14, 15).

Imidazolone modification of arginine is formed by reacting guanidino groups of the arginine residues in the proteins with 3-DG (16, 17). We reported that the serum levels of 3-DG are elevated not only in diabetic patients (18), but also in uremic patients (12, 13). Further, we found that the erythrocyte levels of 3-DG are elevated in diabetic uremic patients via the polyol pathway (19) as well as nondiabetic uremic patients (20). 3-DG inactivates intracellular enzymes such as glutathione peroxidase, a key enzyme in the detoxification of hydrogen peroxide. Thus intracellular accumulation of 3-DG may enhance oxidative stress by inactivating the antioxidant enzymes (21).

Incubation of proteins with 3-DG leads to the formation of imidazolone (8, 9), pyrrole (22), and to a much lesser extent, CML (10, 13) and pentosidine (23). Among these AGEs, imidazolone is the most specific marker for the involvement of 3-DG in the modification of tissue proteins in vivo. Thus the detection of imidazolone-modified protein implies that 3-DG is inevitably involved in the modification of proteins with imidazolone under nonoxidative conditions. However, the formation of CML and pentosidine is considered to be more related to oxidative processes.

By using immunoelectron microscopy, Brancaccio et al. (24) confirmed that amyloid deposits strongly reacted with anti- $\beta_2$ M and anti-AGE antibodies [anti-imidazolone (AG-1) and anti-CML (AG-10)]. Macrophage-like synovial (CD68-positive) cells surrounding amyloid deposits were also immunoreactive for  $\beta_2$ M and AGEs, which were detected in lysosomes and in intracellular fibrillar material. Anti-AGE reactivity was also evident in collagenous structures in the absence of  $\beta_2$ M or amyloid deposits. This finding supports the hypothesis that AGE modification of collagen might have pathogenic relevance in the development of DRA. Hou et al. (25) studied in vitro the binding of between  $\beta_2$ M and AGE-modified collagen, and demonstrated an increased binding of  $\beta_2$ M to AGE-modified collagen as compared to unmodified collagen. After binding to AGE-modified collagen,  $\beta_2$ M could be modified further by nonenzymatic glycation during an in vitro incubation with glucose. Thus  $\beta_2$ M can bind to AGE-modified collagen, and this incorporated  $\beta_2$ M can be modified with AGE.

The earliest stage of  $\beta_2$ M deposition occurs in the cartilage, then  $\beta_2$ M subsequently extends to capsule and synovium (26). These two first stages do not require macrophage infiltration. Macrophages are eventually recruited around larger synovial or capsular deposits in the final stage. Marginal bone erosions develop in this late stage.

Garcia et al. (27) reported that infiltrating macrophages around  $\beta_2$ M amyloid deposits are involved in phagocytosis and not synthesis of amyloid fibrils. However, an impaired lysosomal processing specific for  $\beta_2$ M was suggested, as the other compounds of the amyloid fibrils, such as P component, are significantly cleared.

Recently transforming growth factor (TGF)- $\beta$  was reported to be involved in the pathogenesis of DRA (28). TGF- $\beta$  is a multifunctional cytokine that has chemotactic activity for monocytes at very low concentrations and inhibits proinflammatory cytokine production of macrophages. TGF- $\beta$  and its receptors were localized in the infiltrated macrophages, synovial lining cells, as well as vascular walls around amyloid deposition. AGE- $\beta_2$ M induced TGF- $\beta$ 1, TNF- $\alpha$ , and IL-1Ra production by macrophage. TGF- $\beta$ 1 decreased AGE  $\beta_2$ M-induced TNF- $\alpha$  production and increased IL-1Ra production. Thus there is a pathophysiologic link between TGF- $\beta$  and DRA.

### Serum AGEs

Serum levels of pentosidine are affected by the time on dialysis, dialyzer membrane pore size, and predialysis

BUN (29). Serum levels of CML are affected by the time on dialysis and predialysis BUN. The primary mechanism of formation of pentosidine is through the Amadori pathway from an unidentified reactive low molecular weight precursor. The primary mechanism of formation of CML is through metal-chelated auto-oxidation of reducing sugars. The formation of both AGEs is markedly enhanced by metal ion-mediated oxidative stress in uremia.

Münch et al. (30) demonstrated by using competitive ELISA that the serum levels of imidazolone are increased in HD patients. Franke et al. (31) demonstrated by using Western blotting that the dimeric form of  $\beta_2$ M in the dialysate and urine of uremic patients is modified with imidazolone, whereas the monomeric and dimeric forms of  $\beta_2$ M are modified with CML. We also demonstrated that the dimeric form of  $\beta_2$ M extracted from dialysis amyloid is modified with imidazolone (8). Recently oligomers of  $\beta_2$ M from patients with DRA were reported to induce complement activation via the classic pathway (32). Soluble  $\beta_2$ M purified from peritoneal dialysates was found to be a potent complement activator, while  $\beta_2$ M purified from urine exhibited lower activity, a difference which may be explained by differences observed in oligomers and isoforms. However, it is not determined if these oligomers of  $\beta_2$ M are modified with AGEs or not.

### Risk Factors and Treatment of DRA

The histologic prevalence of DRA is much greater than suspected on clinical grounds: one-third of patients are affected after less than 4 years on HD and more than 90% are affected after more than 7 years on HD. The prevalence of  $\beta_2$ M amyloidosis in continuous ambulatory peritoneal dialysis (CAPD) patients was comparable to that reported in HD populations (33). Many of the amyloid deposits demonstrated by serum amyloid P component (SAP) scintigraphy were not associated with symptoms. Risk factors include the time on dialysis, the type of HD membrane, and the age of the patient at the onset of dialysis (34). The protective effect of high-flux membranes such as AN69 probably results mainly from the greater clearance of  $\beta_2$ M. Other potential but more controversial explanations include a protective influence on residual renal function, a lower stimulation of  $\beta_2$ M synthesis or release, or a beneficial influence on AGE products. The higher risk of DRA in older patients has recently been suggested to result from an age-related AGE modification of osteoarticular collagen.

The best prevention and treatment of DRA is successful renal transplantation. In patients unsuitable for transplantation, high-flux membranes with high clearance for  $\beta_2$ M and good biocompatibility should be used from the start of dialysis. Convective treatments are associated with a trend toward better survival and significantly delay the need for CTS surgery (35). An older age and the presence of diabetes and heart disease are other important risk factors for CTS surgery. An adsorbent (Lixelle) column is used in combination with a dialyzer in series to eliminate  $\beta_2$ M selectively from circulating blood of

DRA patients (36). Palliative treatment includes analgesics, low-dose prednisone in severe cases, and surgical treatment of complications.

The Lixelle column has such a high capacity for adsorbing  $\beta_2$ M that the most intensive removal of  $\beta_2$ M has been possible (36). In clinical trials of the column, the obvious improvement of subjective symptoms, such as decreases in the frequency of nocturnal awakening, the joint pain severity index, and the joint mobility index, were observed. Hypotension has been the most frequent adverse event observed during treatment since the column was put on the market. The Lixelle column adsorbed  $\beta_2$ M and various inflammatory cytokines such as IL-1 $\beta$ , IL-1Ra, IL-6, IL-8, TNF- $\alpha$ , and soluble TNF receptor (37). Therefore the removal of both  $\beta_2$ M and inflammatory cytokines may play an important role in the treatment of DRA.

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