UREMIC TOXINS

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own-regulated in

\textit{Miner}

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16.1 CHEMICAL STRUCTURE AND MOLECULAR WEIGHT

\( \beta_2 \)-microglobulin is a polypeptide of 99 residues that has a molecular weight of 11.8 kDa. It forms the beta chain of the human leukocyte antigen (HLA) class I molecule and has a well-known \( \beta \)-sandwich structure that involves a seven-strand \( \beta \)-pleated structure stabilized with a single disulphide bond (Cys25–Cys80) (Fig. 16.1a). \( \beta_2 \)-microglobulin changes conformation under various \textit{in vivo} or \textit{in vitro} conditions. Far-UV spectra show that the fractions of \( \beta \)-sheet and \( \beta \)-turn decrease and the fractions of \( \alpha \)-helix and random structure increase with several kinds of treatment, such as acidic pH, 2,2,2-trifluoroethanol, sodium dodecyl sulfate (SDS), lyso-phospholipids, nonesterified fatty acids, heating, and agitation. A recent finding shows that the \( \beta_2 \)-microglobulin mutant, K58P-W60G, where a Pro residue has been introduced in the type I \( \beta \)-turn, improves chemical and temperature stability and makes folding faster relative to native \( \beta_2 \)-microglobulin. These conformational changes and stability are pivotal for \( \beta_2 \)-microglobulin-related amyloid fibril formation/extension (Fig. 16.1b), which induces dialysis-related amyloidosis (DRA).

16.2 METABOLISM AND BIOLOGY

\( \beta_2 \)-microglobulin is a component of MHC class I molecules, which are present on all nucleated cells. Multiple myeloma, lymphatic leukemia, and malignant lymphoma,
as well as diseases with activation of the cellular immune system induce increased production of β₂-microglobulin in serum.

Most β₂-microglobulin is normally eliminated by the kidney via glomerular filtration and subsequent tubular catabolism. Megalin is a multiligand endocytic receptor involved in the endocytosis into lysosomes and metabolism of a wide variety of glomerular-filtered proteins, including β₂-microglobulin in the proximal tubule. These proteins are metabolized in proximal tubular epithelial cells and the metabolites are recovered from general circulation via peritubular capillaries. Thus, proximal tubular injury increases β₂-microglobulin excretion in urine, while severe kidney damage induces the retention of β₂-microglobulin in serum due to impaired excretion from the kidney.

16.3 QUANTIFICATION METHODS

Serum level of β₂-microglobulin is usually measured using immunoassays, such as an indirect solid phase enzyme-linked immunosorbent assay (ELISA). β₂-microglobulin is the precursor protein for β₂-microglobulin-related amyloid fibrils and needs conformational change for in vitro fibril formation, as described above. Recent clinical studies have attempted to identify the conformational intermediate form of β₂-microglobulin. Capillary electrophoresis reveals that patients with chronic kidney disease (CKD) undergoing hemodialysis (HD), but not healthy control subjects, have the conformational intermediate form of β₂-microglobulin in serum. The level of predialysis serum β₂-microglobulin intermediate was 2.7 ± 1.4 mg/L and native β₂-microglobulin was 29.4 ± 6.8 mg/L in 31 HD patients. HD using a polymethylmethacrylate membrane decreased the conformational intermediate form of microglobulin treatment. It is a cellular space with tissues (cartilage, tumors) which was found in subjects. Ho containing β₂-microglobulin the precursor continuous high of perfusion rate.

16.4 PLASM SUBJECTS

Advanced CKD impaired metabolic levels of β₂-microglobulin to those in normal that the impair HD patients; microglobulin is the precursor of microglobulin membrane, an

16.5 TOXIC

16.5.1 Adeq

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16.5.2 Mort

In the random β₂-microglobulin
polymethylmethacrylate and online hemodiafiltration (HDF) with a polysulfone (PS) membrane decreased the level of the native form, while any change in the intermediate form was variable.\textsuperscript{10} These results indicate that intermediate $\beta_2$-microglobulin is increased in HD patients and is difficult to remove with dialysis treatment. It may suggest that the intermediate form is immobilized in the extracellular space where $\beta_2$-microglobulin-related amyloid has a marked affinity for joint tissues (cartilage, capsule, and synovium). In addition, immunoaffinity-liquid chromatography-mass spectrometry analysis and immunoassay revealed the generation of lysine-58-cleaved and truncated $\beta_2$-microglobulin ($\Delta$K58-$\beta_2$-microglobulin), which was found in serum from 20–40\% HD patients but not in serum from control subjects.\textsuperscript{11} However, this truncated form has not been demonstrated in the tissue containing $\beta_2$-microglobulin-related amyloid.\textsuperscript{12} It is not certain whether the conformational intermediate or the truncated form of $\beta_2$-microglobulin has a critical role of onset/progress of DRA, and future studies will be needed to understand the relationship.

16.4 PLASMA/SERUM LEVELS IN UREMIC PATIENTS AND HEALTHY SUBJECTS

Advanced CKD induces to elevate the serum level of $\beta_2$-microglobulin due to the impaired metabolism and excretion in the kidney. The average serum concentration levels of $\beta_2$-microglobulin in patients undergoing HD is significantly higher compared to those in normal subjects (25–45 mg/L vs. 1–2 mg/L).\textsuperscript{10,13–16} It is clearly understood that the impairment of metabolism in the kidney is the main cause of fluid retention in HD patients; however, it is not clearly understood whether the production of $\beta_2$-microglobulin is increased with CKD and/or dialysis treatment. $\beta_2$-microglobulin is the precursor protein of $\beta_2$-microglobulin-related amyloid fibrils in DRA, and continuous higher serum levels of $\beta_2$-microglobulin probably indicate the presence of carpal tunnel syndrome, one of the major symptoms induced by DRA.\textsuperscript{13}

16.5 TOXICITY AND CLINICAL RELEVANCE

16.5.1 Adequacy of Dialysis Treatment

$\beta_2$-microglobulin, a middle-size protein, is a marker for adequacy of dialysis. A high serum $\beta_2$-microglobulin level and a low $\beta_2$-microglobulin $Kt/V$ indicate that adequate dialysis for middle-size proteins has not been achieved. Generally, serum $\beta_2$-microglobulin is increased by reduced residual renal function, use of low-flux dialyzer membrane, and long-term dialysis treatment even with high-flux membrane.\textsuperscript{15}

16.5.2 Mortality

In the randomized Hemodialysis (HEMO) Study, the relationship between serum $\beta_2$-microglobulin levels or dialyzer $\beta_2$-microglobulin clearance and mortality over
a period of 2.84 years was analyzed. In time-dependent Cox regression models, predialysis serum β₂-microglobulin levels were associated with all-cause mortality after adjustment for residual kidney urea clearance and the number of prestudy years on dialysis. In addition, Okuno et al. reported that higher serum β₂-microglobulin levels showed higher all-cause mortality and noncardiovascular mortality during 3.33 years. In this study, there was no correlation between serum β₂-microglobulin and cardiovascular mortality. However, another cross-sectional study revealed that serum β₂-microglobulin is related with heart valve calcification, which is associated with carotid intima-media thickness in HD patients. Furthermore, serum β₂-microglobulin levels were positively correlated with several cardiovascular risk factors, such as highly-sensitive C-reactive protein, troponin-T, myeloperoxidase, N-terminal pro-B-type natriuretic peptide and inversely correlated with prealbumin and ankle-brachial index. These reports indicate that increased clearance for middle-size molecules, such as β₂-microglobulin, may induce less all-cause mortality or less cardiovascular event/mortality.

16.5.3 Dialysis-Related Amyloidosis

DRA is a form of general amyloidosis characterized by the deposition of β₂-microglobulin-related amyloid fibrils. Long-term dialysis patients often have DRA-related clinical manifestations, such as carpal tunnel syndrome and destructive arthropathy associated with cystic bone lesions (Fig. 16.2). The tissue retention of β₂-microglobulin with dialysis treatment due to the persistence of high serum concentrations for long durations appears to be a prerequisite for the onset of DRA. In addition to the retention of β₂-microglobulin, partial unfolding of β₂-microglobulin is believed to be needed for its assembly into β₂-microglobulin-related amyloid fibrils, in combination with interaction of disease-related molecules, such as apolipoprotein E, glycosaminoglycans, proteoglycans, and phospholipids. Thus, accumulated β₂-microglobulin in dialysis patients forms amyloid fibrils with conformational change and stabilization of amyloid fibrils with several disease-related molecules. In addition to deposition of β₂-microglobulin-related amyloid fibrils, inflammation after infiltration of inflammatory cells induces various DRA-related manifestations. In the case of DRA-osteopathy, chronic inflammation is found in the lesion. Inflammatory cells often infiltrate into the synovial and/or disc tissue that contain β₂-microglobulin-related amyloid deposits, and these inflammatory cells release inflammatory cytokines. Thus, amyloid deposition induces local osteolysis through synovial inflammation and subsequent osteoclastogenesis and/or osteoclast activation through three possible pathways: (i) indirect action of inflammatory cytokines through the expression in osteoblasts of receptor activator of nuclear factor-κB ligand/osteoprotegerin ligand (RANKL/OPGL), (ii) direct action of inflammatory cytokines, and (iii) RANKL/OPGL expression in inflammatory cells (Fig. 16.3).
session models, cause mortality of prestudy ter serum β2-cardiovascular ation between another cross- heart valve xness in HD rely correlated ve C-reactive uretic peptide ndex. These s, such as β2-vascular event/

FIGURE 16.2 Destructive arthropathy in hip joint due to DRA. A 68 year-old man who had undergone hemodialysis for 34 years had rt. hip joint pain and gait disturbance. Bone X-ray showed destructive arthropathy of rt. femur neck (a), and the man underwent joint replacement (b). Congo-red (c) stained the tissue with orange-green birefringence under polarized light (d).

16.6 THERAPEUTIC METHODS TO REMOVE THE TOXINS

16.6.1 Dialysis Modality

Therapeutic options to improve serum β2-microglobulin levels are to use several dialysis modalities, such as HD, HDF, and peritoneal dialysis. A significant inverse relationship is observed between residual renal function and serum β2-microglobulin level. This suggests that peritoneal dialysis may keep lower serum levels of β2-microglobulin because of better maintenance of intrinsic renal function than HD, while the prevalence of histological DRA in peritoneal dialysis patients is not significantly different from that observed in a group of HD patients matched for age and dialysis duration. A radical approach to reducing serum β2-microglobulin is renal transplantation, which decreases it from 39 ± 6 to 3.8 ± 1 mg/L as well as improving DRA symptoms and inhibiting the progress of DRA.

16.6.2 HD with High-Flux Membrane

The use of high-flux dialyzer membrane leads to a reduction in the serum level of β2-microglobulin as compared to using low-flux dialyzer membrane. In the HEMO
more P2-microglobulin on the cell surface in the patients undergoing HD using high-flux dialyzers and biopsy samples. These cells have processes that change, but this does not appear to be a major factor. If the membrane produces a lower level of serum P2-microglobulin in a fixed area, it is not clear why. The reason why high-flux membrane produces a lower level of serum P2-microglobulin is not clear. In addition, the rate of increase during 36 months was low in the high-flux membrane group. In another study, the rate of increase in the high-flux membrane level was lower in the high-flux membrane group than in the low-flux membrane group (3.3 ± 1.7 mg/mL vs.

Figure 16.3 Possible pathways of bone resorption in DRA-associated: (a) Immunochemistry.

Figure 16.4 Bone remodelling.
flux membrane compared to patients using low-flux membrane, while there is no significant difference in β2-microglobulin mRNA expression in the cells among the groups.\textsuperscript{29} Thus, high-flux membrane decreases serum β2-microglobulin levels due to better clearance and less release from the surface of cells. These results indicate fewer onsets of DRA \textsuperscript{28} as well as better survival\textsuperscript{30} with HD using high-flux membrane compared to HD using low-flux membrane.

16.6.3 HD with β2-Microglobulin Adsorption Column

A β2-microglobulin adsorption column has been developed as a way to directly eliminate serum β2-microglobulin, although limitations are encountered in removing β2-microglobulin by the improvement of high-flux membrane alone. This adsorption column system is designed for direct hemoperfusion (Fig. 16.4). Adsorption of β2-microglobulin by this column is a result both of hydrophobic and molecular size-dependent interactions between the ligand in the column and β2-microglobulin molecule. At each standard HD treatment, the adsorption column is connected in series before arterial blood enters the dialyzer. According to a prospective multicenter study, a β2-microglobulin adsorption column that was placed in series with a polysulfone dialyzer increased serum β2-microglobulin reduction in patients undergoing HD as compared to the control group (74.1 ± 6.1% vs. 60.1 ± 6.3%).\textsuperscript{31} This study also showed improvement of DRA-related symptoms, such as joint pain, and it

![Diagram of blood flow through dialyzer and adsorption column](image)

**FIGURE 16.4** Schematic (a) and photographic (b) representation of blood flow through the dialyzer and β2-microglobulin adsorption column.
may suggest that the column absorbs not only β₂-microglobulin, but also other molecules related to inflammation.

16.6.4 Hemodiafiltration

HDF has better clearance of middle-size molecules than HD and is known to reduce the risk of progression of DRA. A recent multicenter prospective randomized study examined the treatments, A: HD with low flux for 6 months after high-efficiency online HDF for 6 months or B: high-efficiency online HDF for 6 months after HD with low flux for 6 months. Online HDF showed greater efficiency than HD with low-flux membrane in reducing the basal level of β₂-microglobulin (22.2 ± 7.8 mg/L vs. 33.5 ± 11.8 mg/L) and of small sized molecules.

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