Thesis title: STUDY OF DIALYSIS-RELATED β2-MICROGLOBULIN AMYLOID FIBRILLATION AND ITS INHIBITION

Student's name: DEVANSHU MEHTA

Entry Number: 2016BLZ8127

ABSTRACT:

In the present work, the *in vitro* process of β2 microglobulin (β2m) amyloid fibrillation and the molecular insights into its inhibition orchestrated by a rationally designed peptidomimetic have been investigated. β2m protein is involved in dialysis-related amyloidosis (DRA), a protein misfolding disorder that affects renal failure patients when subjected to prolonged hemodialysis. DRA involves the accumulation of long fibrillar aggregates (called amyloids) of β2m specifically into the musculoskeletal system of the patients. The deposition of β2m amyloids results in the inflammation of joints and bones, followed by their degradation causing chronic pain. Treatment methods currently available to manage DRA include surgery to remove amyloid deposits, medications for symptomatic relief, improved dialysis columns for β2m removal from the patient's serum, and kidney replacement therapy. However, these methods are plagued with the high cost and low success and only provide palliative care to the patients. With no drug-based treatment method currently available for managing DRA, our study explores the potential of a rationally designed peptidomimetic against *in vitro* process of β2m amyloid fibrillation.

Peptidomimetics are a class of molecules that constitute a molecular scaffold intrinsically employed in their structure to provide them a specific overall structure. In contrast to small molecular drugs and flexible peptides, which require a well-defined protein pocket for binding and often fail to provide the desired effect, peptidomimetics can provide active and selective inhibition of processes like amyloid fibrillation, which are fundamentally driven by protein-protein interactions (PPIs). The $B(LVI)_2$ peptidomimetic investigated in this study constitutes a centrally located, hydrophobic bispidine moiety to which hydrophobic amino acids leucine (L), valine (V), and isoleucine (I) have been attached on both sides. The bispidine scaffold provides $B(LVI)_2$ an overall structure that mimics the toxic PPI interfaces typically observed during amyloid fibrillation. With its intrinsic hydrophobic nature and a β-

strand conformation, $B(LVI)_2$ was hypothesized to interfere with hydrophobic, amyloid-competent, β-sheet driven intermolecular associations among β2m amyloid precursor species to inhibit the formation of long and ordered amyloid-like fibrils.

This thesis investigates and introduces a peptidomimetic-based approach to help develop future therapeutics against DRA.

The first part of this study explores the effect of $B(LVI)_2$ against *in vitro* developed process of β2m amyloid fibrillation. Thioflavin-T, SDS-PAGE, and transmission electron microscopy analysis show that $B(LVI)_2$ significantly reduces the yield and extends the time required to generate long and straight amyloid-like β2m fibrils in a dose-dependent manner. β2m is observed to develop into morphologically distinct short and straight amyloid-like fibril species in the presence of $B(LVI)_2$, indicative of an altered route from typical β2m amyloid fibrillation. The second part of this study delves into the molecular mechanism of $B(LVI)_2$-effected inhibition of β2m amyloid fibrillation. In the absence of $B(LVI)_2$, circular dichroism data shows that β2m species undergo typical β-sheet-rich amyloid-competent conformational rearrangements. However, in the presence of $B(LVI)_2$, β2m species exist in a non-amyloidogenic, non-β-sheet containing conformation indicative of β2m incompetence to undergo required conformational rearrangements for generation of control-like long and ordered amyloid-like fibrils. $B(LVI)_2$ is further observed using dynamic light scattering to interfere with the amyloid-competent intermolecular associations among β2m species. Our study demonstrates that the long and straight β2m amyloid-like fibrils are cytotoxic to human synovium cell lines, while the morphologically distinct $B(LVI)_2$-induced non-β-sheet containing soluble, non-fibrillar β2m species and β-sheet rich, insoluble, fibrillar β2m species are found to be non-cytotoxic. $B(LVI)_2$ peptidomimetic is non-cytotoxic at lower concentrations (250 µM) which, as per DRA relevant serum β2m levels, may prove promising for future study under *in vivo* setup.

Overall, this thesis reports the anti-fibrillation property of a novel rationally designed peptidomimetic '$B(LVI)_2$' against dialysis-related β2m amyloid fibrillation, which may help bring a paradigm shift in therapeutic approaches against DRA.