RESEARCH FINAL REPORT

on

Cytotoxicity of transthyretin amyloid to peripheral tissue and use of the peripheral tissue for cell based screening of the amyloid inhibitors

by

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ABSTRACT

Amylodosis is a group of diseases in which proteins or protein fragments change from their native soluble forms into an insoluble amyloid fibrils that have been found accumulated in a variety of organs and tissues of the body including central nervous system and peripheral tissues. Different cell susceptibility to toxic amyloids depends on the efficiency of each cell type to accumulate amyloid precursors at its plasma membrane. Recently, a marked increase in membrane lipoperoxidation was reported in primary fibroblasts from patients with familial Alzheimer's disease (AD). This led to reduction of ability to counteract amyloid attack and, thus, more susceptibility of cell to toxic amyloid. The question arose whether peripheral tissues from Down Syndrome (DS) also more susceptible to the toxic molecules than that from healthy. To reveal, comparative determination of amyloid toxicities to peripheral tissues from patient with DS and that from healthy are required. In this research, two groups of amyloid proteins i.e. amyloid β peptide (Aβ) and transthyretin (TTR) variants including V30M and L55P were determined for their toxicities to peripheral tissues include fibroblast, lymphoblast and neuroblast from healthy and DS. Recombinant V30M and L55P were produced using the heterologous gene expression system of *Pichia pastoris*. Their physicochemical properties were examined and it confirmed proper structure of the recombinant proteins produced by *Pichia*. Recombinant TTR variants were induced to form amyloid and toxicity of the amyloid was *in vitro* confirmed. The susceptibility of peripheral tissues to toxic amyloids was determined by LDH release quantification. The results showed different susceptibilities of different cell types, and, from different sources. Among cells from healthy, lymphoblast and Lan-5 were more susceptible to Aβ than fibroblast. In contrast, DS lymphoblast showed less susceptibility to Aβ than fibroblast. Moreover, while healthy fibroblast had high susceptibility to Aβ, DS fibroblast was most susceptible to L55P. These data not only reveal the susceptibility characteristics of each cell types from different sources, but also pointed to importance of the appropriate cell to be used in cell based assay.