

## **Oligomers of Wild-Type Transthyretin are Cytotoxic: Understanding the Pathology of Senile Systemic Amyloidosis**

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Senile Systemic Amyloidosis (SSA) is a unique disease predominately affecting men over the age of 60 years. In SSA, the wild-type (WT) human serum protein transthyretin (TTR) becomes destabilized, misfolds and precipitates as amyloid primarily in the heart; amyloid deposits lead to congestive heart failure, arrhythmias, and conduction defects. Previous studies have suggested that heart failure due to amyloidosis may be greatly unrecognized and under-diagnosed. Furthermore, it has been shown that the amount of amyloid in the heart does not directly correlate to disease severity; thus, cytotoxicity may be a contributing factor in SSA pathology. The purpose of this study was to investigate whether non-native WT TTR oligomers (pre-fibrils) are cytotoxic to cells in culture. Recombinantly-generated human WT TTR and two TTR mutants (V30A and V122I) associated with cardiac amyloidosis were each incubated at pH 4.5 to produce soluble amyloid oligomers. TTR oligomers were placed in the culture medium of 3T3 murine fibroblast cells and incubated for 24, 48, or 72 hours. Triplicate samples were run for each time point. The MTT assay was used to assess cell viability and morphological changes were followed by phase contrast light microscopy. The results were as follows: V30A reduced cell viability by at least 60% when incubated with cells for 48 and 72 hours, V122I reduced cell viability by at least 20% when incubated with cells for 72 hours, and WT TTR reduced cell viability by at least 40% when incubated with cells for 24 hour and by at least 20% when incubated with cells for 72 hours ( $p < 0.05$ ). Distinct morphological changes were observed for the cells treated with oligomers; enlargement, vacuolization, and a decrease in cell number were noted. In summary, our results indicate that WT and mutant TTR amyloidogenic oligomers can be cytotoxic as demonstrated by the reduction in cell viability and alteration of cellular architecture in the treated cells. Future studies are aimed at characterizing the specific nature of oligomeric TTR cytotoxicity which may involve apoptosis, reactive oxygen species, or cell cycle arrest.

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