Introduction

Currently, 5.7 million people in America suffer from Alzheimer’s disease (AD), with this number expecting to rise to 14 million by the year 2050. (3) AD, an ager-related neurodegenerative disease characterized by a decrease in grey matter and loss of memory, can be classified as a tauopathy. Tauopathies are characterized by intracellular accumulations of the microtubule-associated protein tau that stabilizes and regulates microtubule assembly. (2) Tau, normally unfolded, has 85 possible phosphorylation sites within its amino acid sequence, in which hyperphosphorylation can reduce its affinity for microtubules and enhance neurofibrillary tangle (NFT) formation. (5) To date, no therapeutic interventions for tauopathies have been discovered. Although AD approved drugs have been shown to improve cognitive function and quality of life for late-stage AD patients, these drugs do not treat the underlying cause of the disease. Despite intense efforts to understand AD, determination of the mechanism of disease progression remains crucial to the development of novel therapeutics to prevent and cure AD and other tauopathies. (4)

Brains of AD patients typically show a distribution of extracellular amyloid plaques, composed of Aβ aggregates, and NFTs, composed of post-translationally-modified and abnormally aggregated tau proteins. Expression of NFTs correlates best with impairment of neuronal function and cognitive decline associated with AD and other tauopathies. Recent evidence indicates that tau can propagate between neurons in a prion-like manner to further seed tau aggregates, however the mechanism of propagation is unknown. (5) Evidence also suggests that tau protein is transported between cells via bulk endocytosis or micropinocytosis. (6)

The significance of this research is to determine the basic mechanism of cellular tau uptake and cellular spread by the groundwork for future therapeutic interventions. Specifically, this project focused on determining the rate of cellular uptake of wild-type (WT) tau protein and phosphorylated tau protein. Flow cytometry and microscopy were used to analyze cellular uptake of the two forms of the tau protein and provide the basis for determining the physiological effects of tau uptake and cellular transmission as a function of cell type.

Flow Cytometry

- Enables segregation of different populations and analysis of cells based on size, shape, and internal complexity within a heterogeneous mix.
- Heterogeneous mixture of cells pass through a laser beam in a single file line scattering light.
- Emitted light is detected by corresponding wavelengths to detect the presence of fluorophores.
- This project used flow cytometry to detect tau-positive vs. tau-negative cells using tau monomer conjugated to a FITC (Fluorescein) dye.

Cell Types Tested

- C6 Glioma: Rat-derived glial tumor cell line
- HEK 293 Cells: Human, embryonic kidney cells
- SH-SY5Y Cells: Female, human-derived neuroblastoma cell line
- Adherent, astrocyte cells
- Adherent, fibroblastic cells
- Adherent, neuronal cells

References


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