**Amyloid Beta (1-42) and Alzheimer’s Disease**

**I. Introduction**

Alzheimer’s Disease (AD) is the 6th leading cause of death in the United States affecting 5.5 million people in America and striking 5 in 10 people over 65 (1). The dementia characteristic of AD is associated with the over-production and aggregation of amyloid beta (Aβ). Aβ is a 40-42 amino acid peptide, which in healthy brains modulates synaptic activity and is recycled, while in AD, Aβ forms neurotoxic aggregates in the brain leading to dementia. Research into molecules that prevent the aggregation of Aβ is ongoing and could lead to new treatments for AD.

**II. Amyloid Beta in Alzheimer’s Disease**

In Alzheimer’s Disease (AD), Aβ forms plaques to clump together to form senile plaques, which damage synapses, the connections between neurons. The spread of these plaques is accomplished by the destruction of neurons, affecting memory, language abilities, problem solving, emotions, coordination, and finally, breathing and heart rate. Some research suggests that Aβ might cause the death of neurons by triggering the breakdown of microtubules in the axons of neurons. Microtubules are long protein strands, along which motor proteins walk carrying materials essential for the life of the neuron. However, in AD the tau protein stops carrying out its function of binding to and stabilizing microtubules, causing them to disassemble. Tau proteins clump to form neurofilamentary tangles typical of AD.

**III. Amyloid Precursor Protein to Aβ with Secretases**

Amyloid precursor protein (APP) is a large, single pass, trans-membrane, signaling protein important for neural growth and repair; which is found in many tissues and organs including the brain. Although not all of the functions are known, APP may also help cells attach to one another. Three secretases (proteases) are active in processing the APP protein; each makes cuts to produce biologically active fragments of APP. When APP is cut by β-secretase and γ-secretase, Aβ is not produced. When APP is cut by β-secretase and γ-secretase the Aβ peptide is formed. Over production of Aβ can lead to early AD, as it does in many Down’s Syndrome patients who have three copies of the APP gene. Some mutations of APP can change where γ-secretase cleaves APP, producing an hydrophobic, insoluble, sticky form of Aβ (42 amino acids) that aggregates to form the senile plaques of AD.

**IV. Structure of Amyloid Beta**

In a healthy brain, Aβ is recycled; while in AD, Aβ forms neurotoxic aggregates forming senile plaques. Aβ fibrils are homodimers consisting of two protofilaments and are a major component of plaques. The protofilaments consist of 18-30 amino acids, and α-amyloid Aβ long peptide Aβ peptides aggregate with each other in a cross-beta sheet structure. Hydrophobic sidechains on Aβ strands maintain the N-terminal L shape and the C-terminal S shape of each strand of each protofilament. The two protofilaments are held together by hydrophobic interactions as well as salt bridges.

**V. Amyloid Beta (1-42) Mutations**

Over 50 mutations in the APP protein are associated with early onset Alzheimer’s Disease (AD) but these account for only 5% of cases. The model of an Aβ(1-42) fibril forming from 35-42 amino acids of the precursor shows aggregation when Aβ(1-42) is incubated with ThioflavinT. The fibril highlights the positions of six pathogenic mutations located in the Aβ portion of APP, causing cerebral amyloid angiopathy (CAA) and early onset AD. CAA is characterized by amyloid deposits in the walls of cerebral blood vessels, weakening them and leading to strokes. The dementia characteristic of AD also develops. Three pathogenic mutations occur at position 22 (the Aβ(1-42) peptide). They are the Dutch mutation (Glu22Val), the Italian mutation (Glu22Lys), and the Arctic mutation (Glu22Asp). The mutations Ala21Gly, Leu34Val, and Asp23Asn cause the Flemish, Piedmont, Iowa types of this condition, respectively. The Italianic mutation (Ala21Thr) is thought to be protective against AD. It may be protective because a small hydrophobic sidechain, alanine, is replaced with a polar sidechain, threonine, that could disrupt aggregation of Aβ.

**VI. Current Treatments**

According to the Alzheimer’s Association, there are currently only five FDA approved drugs for Alzheimer’s Disease (AD). None of the drugs can prevent the death of neurons in the brain or stop the progression of the disease, although they can improve memory for a time. All of the drugs except memantine are cholinesterase inhibitors. Cholinesterase is an enzyme that breaks down neurotransmitter acetylcholine released from the axon of neurons sending a message. When receptors on the dendrite of the receiving neuron bind enough transmitter an electrical signal moves down its axon. Inhibiting cholinesterase increases the amount of acetylcholine in the brain of AD patients who have a shortage. Memantine increases the amount of a different neurotransmitter (glutamate) by a different mechanism.

**VII. Current Research**

**Inhibiting Aβ(1-42) Aggregation In vitro**

Peptides that can prevent the aggregation of amyloid beta (Aβ) into the senile plaques associated with the death of neurons in AD are currently being investigated by individual researchers and by drug companies. In the Scaglione lab the ability of one such peptide to inhibit Aβ aggregation in vitro was analyzed with positive results. In the experiment, a 40 micromolar solution of Aβ(1-42) peptide was incubated with shaking at 37°C and the fluorescence intensity caused by the binding of Thioflavin T to amyloid fibrils was measured in real time. Thioflavin T is a dye that produces increased fluorescence when it binds to the beta sheet structure formed when Aβ aggregates. Then a 1 to 1 molar ratio of a peptide designed to inhibit Aβ aggregation was added and the peptide partially suppressed Aβ aggregation as measured by a decrease in fluorescence intensity (dark blue) compared to the positive control (orange). The negative control is light blue (8).

**Inhibition of Aβ(1-42) Aggregation Measured Using Thioflavin-T Fluorescence**

**Figure 13.** The dark blue line shows partial inhibition of aggregation when Aβ(1-42), Thioflavin-T and the inhibitor are present in solution. The positive control (orange line) shows aggregation when only Thioflavin-T plus Aβ(1-42) are in solution but no inhibitor is present. The negative control (light blue line) does not show the aggregation of Aβ(1-42) because no peptide inhibitor is present in solution with Thioflavin-T.

**Figure 14.** Structure of the fluorescent dye Thioflavin-T (41)

**VIII. Summary**

The St. Dominic Middle School SMART (Students Modeling a Research Topic) Team designed a model of an Aβ(1-42) fibril using 3D printing technology to investigate its structure/function relationships to Alzheimer’s Disease (AD) and to Aβ(1-42) aggregation. The placement of Aβ's many hydrophobic sidechains promotes folding and stacking into β-sheet protofilaments that dimerize into the fibrils which clump together to form the senile plaque characteristic of AD. The 3D model of AD is not known and there is no cure, but over 50 mutations in the gene for amyloid precursor protein (APP), the protein from which Aβ is clipped, have been associated with rare autosomal dominant early onset AD. These mutations are helping researchers understand more aspects of the disease and research into molecules that prevent the aggregation of Aβ(1-42) is ongoing.

**IX. References**


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