Investigating the Effects of Amyloid Plaques on Oxidative Stress throughout the Life Cycle of Caenorhabditis elegans

According to an estimate by the World Health Organization, the number of people who will suffer from dementia in 2030 is 78 million. Alzheimer's Disease (AD) is currently the most common form of dementia. AD is a neurodegenerative disease that is most often found in older adults, especially in people above the age of 60 (Zhang et al., 2024). This means that as the average life expectancy increases, so does the incidence of Alzheimer's. Alzheimer's can lead to memory loss, personality changes, difficulty with thinking, and dysfunctions in the motor skills and executive functions of the patient.

Patients with Alzheimer's are known to have large accumulations of amyloid-β (Aβ) plaques, neurofibrillary tangles (NFTs), neuroinflammation, synaptic dysfunction, and mitochondrial and bioenergetic disturbances. There are various hypotheses explaining the mechanisms of Alzheimer's, yet no hypothesis can explain every aspect of Alzheimer's disease pathology. Due to the current lack of understanding of the underlying pathology, most cases of Alzheimer's are diagnosed at late stages, which lowers the chance that treatments will work. The treatments are also less effective because they can only treat the effects of the underlying pathology, which will never stop the progression of the disease and have limited effectiveness. The current hypothesis is that a number of factors, such as Aβ, Tau proteins, acetylcholine deficiency, neuroinflammation, oxidative stress, biometal dyshomeostasis, glutamate imbalance, insulin resistance, gut microbiome abnormalities, cholesterol homeostasis disruption, mitochondrial dysfunction, genetic factors, and autophagy abnormalities all combine to cause Alzheimer's (Zhang et al., 2024). Amyloid plaques and oxidative stress are two hypotheses that are highly connected. There has been much debate over which mechanism initially causes the other. It has been very difficult to determine this because Amyloid plaques cause Oxidative Stress and Oxidative Stress causes more Amyloid plaques (Tamagno et al., 2021).

Amyloid Plaques are a hallmark of AD pathology and many of the genetic causes of AD have some relation to the overexpression of Amyloid beta. Amyloid beta is a protein produced from the Amyloid Precursor Protein as it is broken down by beta- and gamma- secretases. Amyloid plaques are formed when many Amyloid beta proteins combine to form fibrils, which further combine into the plaques (Chen et al., 2017). Reactive Oxygen Species are naturally produced during Oxidative Phosphorylation, but an excess of ROS is damaging to the cell and can contribute to cell death. The oxidative stress and ROS produced by the mitochondrial dysfunction can in turn influence the pathology of Amyloid beta. (Gao & Ma, 2022). Amyloid Plaques and oxidative stress are both found naturally throughout the process of aging. High levels of amyloid plaque pathology do not always indicate Alzheimer's. Some patients with high levels of amyloid beta, an integral part of the pathology of Alzheimer's, do not actually have the disease or its symptoms. This proves that these two mechanisms do not work alone (Zhang et al., 2024). However, the scope of this study is not wide enough to consider the other factors that play into the pathology of Alzheimer's.

In previous research, *Caenorhabditis elegans* had been used as a model organism to study the pathology of Alzheimer's disease. These worms are transparent, which makes them easy to use for fluorescence imaging, most commonly with GFP (Hutter, 2012). While *C. elegans* cannot generate Amyloid beta peptides naturally, transgenic lines of *C. elegans* have been created to express the human versions of Amyloid beta (Alvarez et al., 2022) through the mutation of genes known to help produce Aβ that are associated with Alzheimer's, such as the Amyloid Precursor Protein (Bellenguez et al., 2022). Transgenic *C. elegans* that express amyloid beta have been known to have decreased locomotion and paralysis as a result. Current research on the mechanisms of AD ties mitochondrial dysfunction and oxidative stress to Amyloid beta, showing that Amyloid plaques can cause oxidative stress, damage to mitochondrial DNA, and excess Reactive Oxygen Species (ROS).

There is a lack of knowledge on the interactions between the different mechanisms of AD. Understanding the interactions and relationships between the different mechanisms of the disease will help to uncover the underlying mechanism, which in turn will help with prevention, early detection, and more effective treatment of Alzheimer's. As Amyloid plaques and oxidative stress are found in both healthy and Alzheimer's patients, research on the relationship of these two mechanisms throughout the process of aging can have implications for the differentiation of patients with Alzheimer's and healthy patients with high levels of amyloid plaques and oxidative stress that would usually indicate AD. The goal of this project is to understand the difference in the impact of Aβ on oxidative stress based on the age of the *C. elegans*. The MO1 strain of *C. elegans*, which overexpress human amyloid beta in neuronal cells when exposed to heat and continuously express GFP as a reporter for ROS was created. The CL2355 and CL691 strains that were crossed to create MO1 were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, MN, USA) and maintained with the standard agar and E. coli (Kittimongkolsuk et al., 2021). We exposed the MO1 transgenic *C. elegans* to heat at different stages of their life cycles, causing them to produce amyloid plaques. After being heated, we stained the worms with Congo red to determine the levels of amyloid beta. Then, we observed the levels ROS produced in response to the production of Amyloid plaques using flashlights of specific wavelengths and filters. We found that as the age of the *C. elegans* increases, the levels of ROS produced in response to amyloid plaques will also increase.