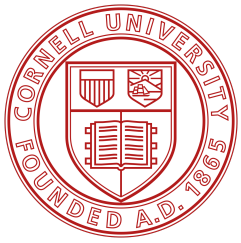

CornellEngineering
Nancy E. and Peter C. Meinig
School of Biomedical Engineering

Observing Pathological Differences due to Menopause Following an Ischemic Stroke in a Mouse Model of Alzheimer's disease

Ashmitha Sivakumar

Supervised by Dr. Lianne Trigiani

Laidlaw Research and Leadership Scholarship

Summer 2022 Research Report

Abstract

Alzheimer's Disease (AD), a devastating form of dementia, is the sixth leading cause of death in the United States. Two hallmark features of AD include the deposition of amyloid-beta peptides into plaques and neurofibrillary tangles, however, vascular health is emerging as an important cause of morbidity and mortality. Females are protected against strokes before menopause. After the onset of menopause, females face an increased risk of experiencing strokes compared to men. Observing pathological differences between premenopausal and menopausal APP-KI female mice as well as male mice is important to understanding the role of menopause in the exacerbation of Alzheimer's. The photothrombic occlusion of penetrating arterioles is a vascular injury model used to detect changes in morphology methoxy-XO4 labeled Aβ plaques. The maximum intensity projection images of a select plaque near the occluded vessel over a seven day period revealed that the area of the plaque increased as it became more diffused in shape over time. Also detecting the change in the number of plaques over time before and after the stroke demonstrates that there is an increase in plaque numbers up till day 4 before it dips slightly on day 7.

1 Introduction

Alzheimer's disease is a neurodegenerative disorder and accounts for 60 to 80% of dementia cases in older adults. Symptoms include memory loss, cognitive decline, and impaired reasoning. The loss of cognitive function and other abilities leads to decreased quality of living. A key event in AD is the deposition of amyloid-beta plaques and intracellular neurofibrillary tangles which are associated with synaptic damage and neurodegeneration (Marongui 2019). Vascular health is emerging as another important factor in disease progression. AD can be initiated or worsened by events that prevent amyloid beta from being cleared. Studies on animal models have shown that strokes and reduced blood flow in the brain can increase the presence of Aβ plaques. Ischemic strokes, a type of vascular injury, are known to increase inflammation and the presence of reactive oxygen species, reducing clearance or increasing production of Aβ (Zhang et al., 2020). Recent works suggest that the sex dimorphism plays a major role in the onset and progression of Alzheimer's disease. Women with AD experience a broader range of symptoms and more cognitive deterioration compared to men (Marongui 2019). Menopause especially increases the risk of dementia sometimes by 2-fold and increases the incidence/severity of strokes (Shekhar et al., 2017). Considering that women make up 70% of AD patients, exploring the effect of vascular injury before and after menopause is important to understanding the biological mechanisms involved in increased stroke incidence in menopausal women. Using the pre-menopausal, menopausal, and male AD mice, it is possible to correlate the presence of Aβ plaques and blood flow in vessels with AD progression following a microstroke.

Mouse models have been used to replicate elements of human menopause. In this study, Alzheimer's mouse models will be treated with the 4-vinylcyclohexene diepoxide (VCD) to accelerate ovarian failure. This preclinical model mimics hormonal changes that occur in human menopause including components of the perimenopause and postmenopause.

Following the onset of menopause, male and female mice were subjected to the photothrombotic stroke model to determine how Aβ plaque morphology and blood flow is affected following a microvascular occlusion. Occlusions of penetrating arterioles are predicted to affect a large number of surrounding vessels and result in 400-mm cortical infarcts (Zhang et al., 2020). Two-photon microscopy was an important tool used to perform in-vivo imaging and determine much of the findings of this study.

2 Materials and Methods

2.1 APP-KI mouse model

The APP-KI mouse model is a newer mouse model that carries the humanized Aβ sequence and was created using CRISPR/Cas9.

2.2 Preparation

Injections were administered to the 24 male and female mice over a 4-week period (5 days a week). Six of the male were APP-PS1 and the other 6 male mice were APP-KI. All of the 12 females were APP-KI. Of these, 6 of them were treated with VCD. The dosage for each mouse was determined by its weight multiplied by 160 mg/kg/day. A solution of sesame oil and VCD at 10 mg VCD per mL of oil was prepared daily for the VCD injections. The remaining 18 mice received sesame oil injections according to their weights. This serves as the standard of comparison.

2.3 Multiphoton microscopy for in-vivo imaging

Cranial (glass) windows are implanted on adult mice and allow us to perform in-vivo imaging. Texas red dextran in saline is used to fluorescently label the microvasculature (including blood vessels). To label the Aβ plaques, methoxy-X04, a dye that can cross the blood-brain barrier, is used and injected into the mice before each imaging session. Glucose is used to prevent dehydration by injecting about 100 μL of 5% glucose in saline at the start. The mice are anesthetized using 1-2% isoflurane in oxygen during the imaging and the breathing rate is maintained at around 1 beat per second. The mouse is placed on a heating pad attached to the stereotax to maintain its temperature at 37°C. Using the stereotaxic tool, the head is firmly secured in place to help with image acquisition. The images were captured using a custom-built multiphoton microscope and ScanImage software. Images of the site of the stroke and nearby plaque were taken before and right after the stroke as well as 1, 4, and 7 days after the stroke.

2.4 Photothrombic occlusion using rose bengal

Photothrombosis was used to occlude the vessels during the imaging and cause an ischemic stroke in a select penetrating arteriole in the mouse. Images of the right and left hemisphere of the brain were taken using a speckle microscope. After analysis, vessels from one or both of the hemispheres were chosen to occlude. Micro were injected retro-orbitally (or in the tear duct) with 50 μL of rose bengal. Rose bengal is a photoactive dye that will react when excited at the right wavelength. A green laser light was focused through the microscope objective on to the selected penetrating arteriole activating the rose bengal and causing it to form a blockage in the

vessel. The laser irradiation took place for about 3000 ms and right away it was possible to see that the arteriole and its surrounding vessels were occluded. A select plaque near the site of stroke was chosen to track its morphology over time (whether it would become smaller or larger). A control area (where no stroke was placed) with a penetrating arteriole and nearby plaque in the same hemisphere the stroke was placed were chosen for comparison.

2.5 Image analysis of plaques

The maximum intensity projections of the selected plaque images were taken to characterize the size and morphology of plaques. The mean intensity can be used to find the area of the methoxy-X04 labeling and thus the area of the plaque. Over time, we can track whether the plaque has increased/decreased in size or if its shape appears cloudier in the image. The number of plaques present around the vessel will also be counted to see if there is an increase or decrease in the amount. Speckle image analysis can be used to measure the blood flow changes over the seven days using the intensity of the image.

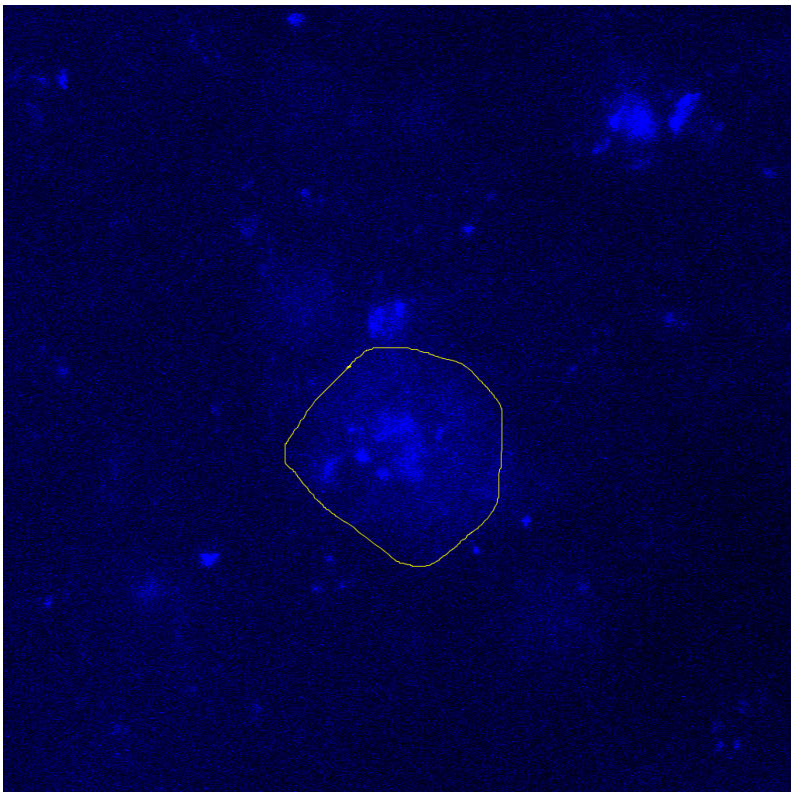

Fig. 1 This is an example of an image of a select plaque near the occluded vessel. These images were analyzed using the maximum intensity profile to find the area of the plaque and characterize them.

3 Preliminary Results

The results presented are the images and image analysis performed on them before statistical analysis. In-vivo multiphoton microscopy was used to capture images of the chosen penetrating arteriole and nearby plaque before and after the stroke and 1, 4, and 7 days after stroke. To observe changes in Aβ plaque deposition in male and female (perimenopausal and menopausal) mice, we used maximum intensity projections. The number of plaques present around the occluded vessel was observed as well to track the appearance/disappearance of plaques around the site of vascular injury. The images of the vessel were also analyzed at the site and around the stroke to measure changes in blood flow. After occluding a targeted vessel, the morphology of nearby plaques began to change. For all 3 groups (menopausal female, female, and male), there was a general upward trend in the number of plaques that appeared up till day 4 after which the number of deposits decreased slightly. It can be noted that especially with the menopausal female mice the plaque selected near the occluded vessel appeared cloudier over time. Sex-related hormones are known to influence the inflammatory response following vascular injury (Shekhar et al., 2017). One possible explanation for differences in the results between menopausal and non-menopausal mice includes varying levels of estrogen. Females experience a reduction in the production of estrogen during menopause which is known to be neuroprotective and reduce the incidence of stroke. In the future, we could possibly measure hormone levels for male and female mice to further explore this relationship

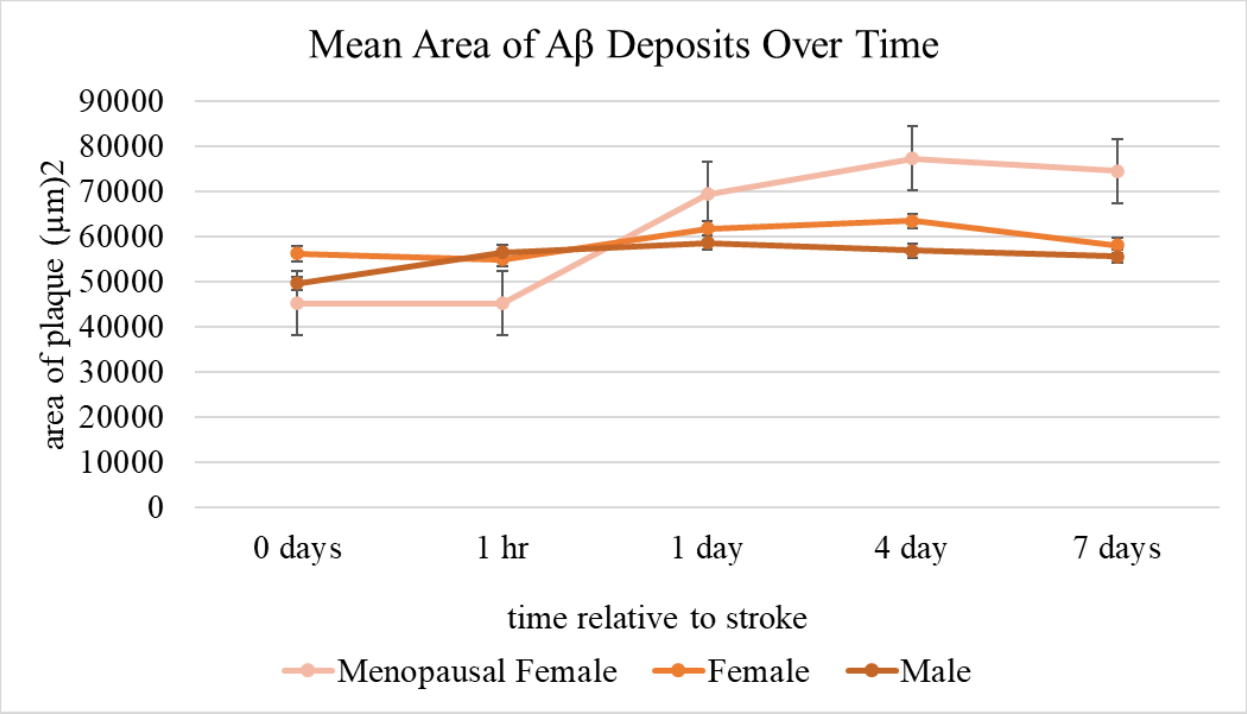

Fig. 2 Mean Area of Amyloid Beta Deposits

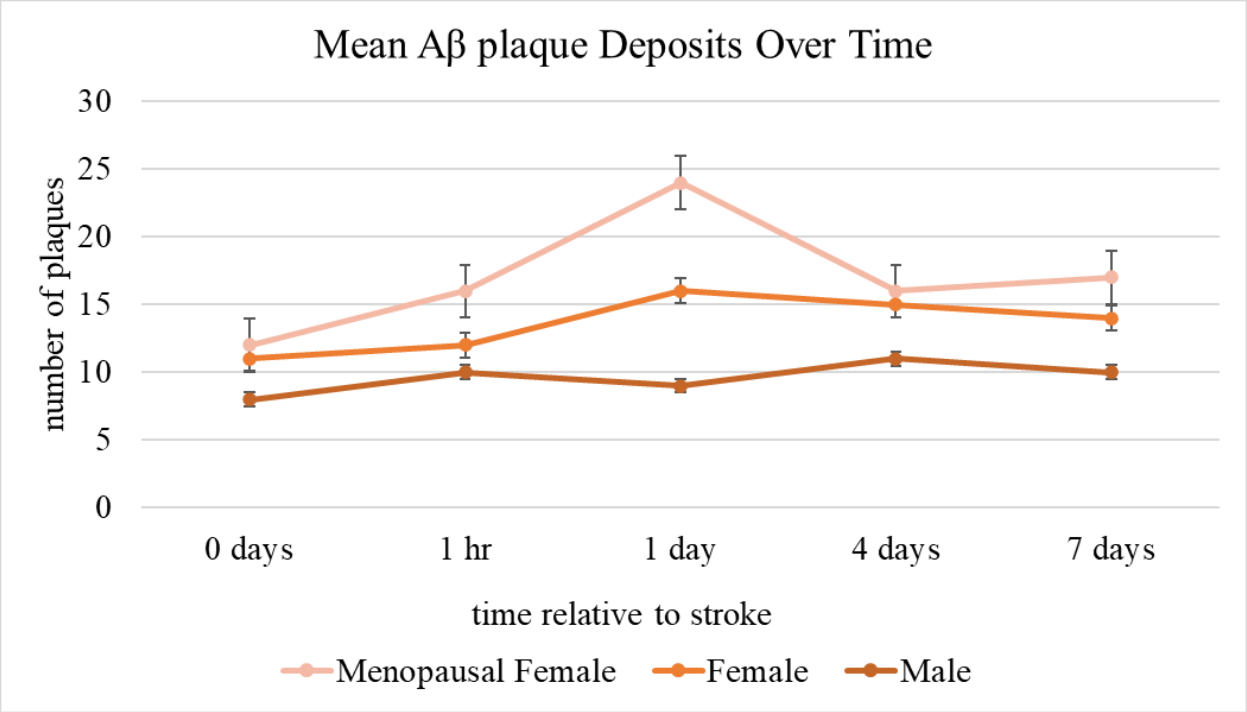

Fig. 3 Mean Number of Amyloid Beta Plaques

4 Conclusion

As AD remains a debilitating disease with very few treatment options available, more insight on its pathogenesis and causes can be brought out through this study. Worldwide nearly 50 million people suffer from this disease and its effects extend far beyond the patients; caring for patients places a great financial and emotional burden on families. It remains a significant burden on healthcare in many countries as worldwide the spending for treatment and hospice care is more than $1 trillion US dollars and is set to rise. Considering all these factors, it is of great public concern and more realistic models incorporating late onset AD risk factors and genes need to be studied. The findings from this study could be valuable in increasing available knowledge about progressive neurodegenerative diseases and has important clinical implications.

Works Cited

Nilsson, Per, et al. “New Mouse Model of Alzheimer's.” ACS Chemical Neuroscience, 16 July 2014, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4102956/.

Chen, Guo-fang, et al. “Amyloid Beta: Structure, Biology and Structure-Based Therapeutic Development.” Acta Pharmacologica Sinica, vol. 38, no. 9, 17 July 2017, pp. 1205–1235, 10.1038/aps.2017.28.

D’Andrea, MR, and RG Nagele. “Morphologically Distinct Types of Amyloid Plaques Point the Way to a Better Understanding of Alzheimer’s Disease Pathogenesis.” Biotechnic & Histochemistry, vol. 85, no. 2, Jan. 2010, pp. 133–147, 10.3109/10520290903389445. Accessed 22 Apr. 2022.

Marongiu, Roberta. “Accelerated Ovarian Failure as a Unique Model to Study Peri-Menopause Influence on Alzheimer’s Disease.” Frontiers in Aging Neuroscience, vol. 11, 6 Sept. 2019, 10.3389/fnagi.2019.00242. Accessed 25 Sept. 2019.

Mosconi, Lisa, et al. “Menopause Impacts Human Brain Structure, Connectivity, Energy Metabolism, and Amyloid-Beta Deposition.” Scientific Reports, vol. 11, no. 1, 9 June 2021, p. 10867, www.nature.com/articles/s41598-021-90084-y, 10.1038/s41598-021-90084-y. Accessed 27 June 2021.

Saito, Takashi, et al. “Single App Knock-in Mouse Models of Alzheimer’s Disease.” Nature Neuroscience, vol. 17, no. 5, 13 Apr. 2014, pp. 661–663, 10.1038/nn.3697. Accessed Feb. 2020.

Shekhar, Shashank, et al. “Menopause and Ischemic Stroke: A Brief Review.” MOJ Toxicology, vol. 3, no. 4, 10 Aug. 2017, 10.15406/mojt.2017.03.00059. Accessed 7 Aug. 2020.

Zhang, Yuying, et al. “Microvessel Occlusions Alter Amyloid-Beta Plaque Morphology in a

Mouse Model of Alzheimer's Disease." Journal of Cerebral Blood Flow & Metabolism, vol. 40, no. 10, 19 Nov. 2019, pp. 2115–2131, 10.1177/0271678x19889092. Accessed 13 Mar. 2022.