

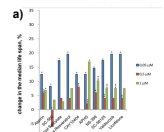
Research Objective

Determine the relationship between NSAIDs and neuroinflammatory diseases and the possibility of NSAIDs as a long-term treatment.

Introduction

Neuroinflammatory diseases, including Alzheimer's disease, pose significant challenges to global healthcare. Nonsteroidal anti-inflammatory drugs (NSAIDs) have emerged as potential candidates for therapeutic intervention due to their anti-inflammatory properties. Based on the readings, consistent usage of NSAIDs has shown possible therapeutic effects on neuroinflammatory diseases. There have been signs of increased longevity in *Drosophila* and more mutated displays of neuroinflammatory phenotypes after consistent usage (Danilov et al., 2015). Most studies, however, were written after a correlation was noticed among people. As a result, many need help understanding the long-term effects of taking NSAIDs or what happens when NSAIDs are taken before phenotypes are displayed.

The goal is to explore and conclude the relationship between NSAIDs, specifically Naproxen, and neuroinflammatory diseases, specifically Alzheimer's Disease, and whether it can become a possible therapeutic treatment in a *Drosophila* model. *Drosophila* with a mutation that presents itself in the form of Alzheimer's Disease phenotypes as well as a stock of control *Drosophila* will consume Naproxen incorporated into their food from the beginning of their life cycle to track the progress or lack thereof of the disease. The consistent usage of NSAIDs will be analyzed through locomotor and memory assays. While there is uncertainty if this will result in the phenotypes being displayed later in the cycle or if this can possibly act as a cure for the disease, the phenotypes are expected to be repressed.



- a) The influence of NSAIDs on the median lifespan in male *Drosophila*. Shows a drastic increase in median life span as a result of NSAID intake.
- b) Illustration of the *Drosophila* life cycle.

Using Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) as a Possible Treatment for Neuroinflammatory Diseases

Madison Lee

Methods & Materials

Drosophila Strains

- Wild-type D. *Melanogaster* *Drosophila*
- Alzheimer's Model: APP C99 Fragment mutated D. *Melanogaster*
- Stocks received from the Bloomington Stock Center and Rainbow Mealworms

Drug Distribution

- Add 5 milligrams of Naproxen to 500 microliters of water and aliquot accordingly so the ultimate food concentration is 1 mg Naproxen/1 L of food

Locomotor Activity/Bang Sensitivity Assay

- Place 15-25 adult *Drosophila* into an empty vial.
- Let the vial sit for 5 minutes to ensure the flies are familiarized with the new environment.
- Gently flick the bottle so all of the flies are on the bottom.
- Start a timer for 10 seconds and once 10 seconds pass, count the number that climbed above the 6 cm mark.
- Repeat steps 3-4 three more times.
- Take the average of all three trials and record.

Lifespan analysis

- Count the number of dead flies every time a new food is added.
- Calculate survival functions
 - The mean, median, minimum, maximum lifespans, the age of 90% mortality, and other parameters were counted.
- Compare the results to the average lifespan of a fly (40-50 days)

Olfactory Association Assay – Memory Assay

- Place a single adult *Drosophila* on a 2M fructose agarose plate with a cap of 1:25 Methylcyclohexane (MCH) on the right side (see below) for 5 minutes.
- Transfer *drosophila* onto a clean agarose plate for 3 minutes.
- Repeat steps 1 and 2 again.
- Place flies onto the left side of a fresh agarose plate of the same layout. On the right side, place a cap of 1:25 MCH. After 5 minutes, record the position of the fly.
 - If the fly moved towards the right side of the plate where the scent is, means it is capable of remembering and associating the scent with fructose.
- Compare the number of Alzheimer's model flies that moved to the right of the plate and compare the value to the control.

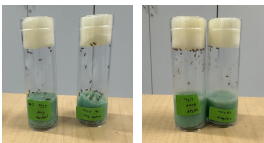


Results & Findings

Visual Differences

(Left: Control strain on drug food, Right: Alzheimer's strain on drug food)

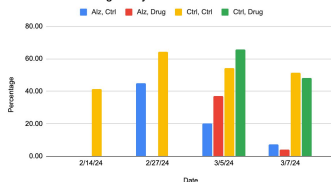
- Control
 - 0 larvae along the walls
 - All of the larvae were at the bottom
 - Seemed to be in 1st or 2nd instar
 - Visible tremors
 - Jerky movement
- Alzheimer's
 - Both vials had 24-25 larvae along the walls
 - Seemed to be in 2nd or 3rd instar
 - Very active; climbing the walls



Locomotor Activity/Bang Sensitivity Assay

- Alz, Drug produced worse results than Alz, Ctrl, suggesting NSAIDs are not an effective solution to neuroinflammatory diseases
- Drug doesn't seem to affect control flies at all
 - Success rate similar for both Ctrl, Ctrl and Ctrl, Drug

Successful Climbing Assays



Conclusions

- Current results point toward negative results of long-term use of NSAIDs as a therapeutic of neuroinflammatory diseases, however, more trials will be needed to determine the relationship

Future Directions

- Perform the Olfactory Association Assay to determine any differences in memory
- Perform the Lifespan analysis to determine any changes in life expectancy
- Standardize the number of flies for each assay
- Perform experiments at a more consistent rate
- Perform experiments using larvae to determine whether NSAIDs impact development in any way

Acknowledgements

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