Elizabeth Chun

Ardsley High School

Combating Familial Alzheimer's Disease: A Study of Resveratrol's Effects on a Presenilin Model of *Drosophila melanogaster*

Abstract

Currently the sixth overall leading cause of death in the United States, Alzheimer's Disease (AD) is an irreversible, progressive neurodegenerative disease that destroys memory and learning. With only five medical treatments of limited effectiveness that are FDA-approved to treat AD, a major gap in current research is the lack of medications that are able to not only target symptoms but actually alter the course of the disease. To this end, one drug that displays potential as a possible treatment is resveratrol ($C_{14}H_{12}O_3$), a polyphenol found in red grapes and wine. In this study, I use presenilin *Drosophila* as a model of AD in order to confirm whether resveratrol is an effective treatment for AD. The first phase is a climbing assay to determine resveratrol's effects on the behavioral symptoms of AD, and the second phase is an anatomical study to determine whether resveratrol is able to alter the neurological course of AD. The climbing essay showed that presenilin flies have significantly decreased climbing ability compared to 2u flies and that resveratrol treatment is able to improve the climbing ability of these flies. For flies that were fed resveratrol starting at birth, it was also found in the anatomical study that resveratrol leads to brighter, healthier-looking cells -- this indicates slowed neurod. These results reveal that, when taken starting early on, resveratrol can both improve the symptoms of AD and preserve brain structure by slowing the course of the disease.

Introduction

Alzheimer's Disease (AD) is an irreversible, progressive neurodegenerative disease that destroys memory and learning. Most individuals with AD have the late-onset form of the disease, so symptoms become noticeable in their mid-sixties (Bekris et al., 2010). The effects of AD become increasingly more severe over time, and patients ultimately lose their ability to carry out the simplest of day-to-day tasks. As the sixth overall leading cause of death in the United States (Atri, 2019), AD is by far the most common cause of dementia, or severe cognitive impairment. Additionally, the cost for healthcare related to AD is estimated at nearly $500 billion annually (Weller & Budson, 2018). One of the major gaps in current research, however, is the lack of successful medications.

AD causes brain cells to degenerate and die; therefore, it halts the processing, storing and retrieving of information among cells. Scientists are uncertain as to the clear cause for this neurodegeneration, but one common explanation is beta-amyloid, a toxic protein fragment whose accumulation in the brain is a key characteristic of AD (Thinakaran, 1999). Beta-amyloid interrupts communication between brain cells and eventually kills them; in the amyloid hypothesis, scientists propose that errors in the production, accumulation or disposal of beta-amyloid are the cause of AD. Another significant hallmark of AD is the presence of neurofibrillary tangles, which contain the protein tau. These tau tangles prevents transport between nerve cells and, like beta-amyloid peptide, ultimately causes cell death.

There are currently only five medical treatments, involving merely two classes of drugs, that have been FDA-approved to treat AD: three cholinesterase inhibitors (donepezil, rivastigmine, and galantamine), memantine, and memantine/donepezil. Furthermore, these

approved treatments only target symptoms, rather than truly change the course of the disease (Yiannopoulou & Papageorgiou, 2013), and they have had a 99.6% failure rate in past clinical trials (Briggs et al., 2016). Given the prevalence of AD and its serious effects, further research on alternative treatment options a pressing need.

One drug that has received attention as a possible treatment is resveratrol ($C_{14}H_{12}O_3$), a polyphenol found in red grapes and wine. In particular, resveratrol's anti-inflammatory properties and ability to mimic caloric restriction have been suggested as factors that may make it successful in combating AD. When mouse models of AD were used to test resveratrol's impact on amyloid beta plaques in the brain, it was found that the mice that were given resveratrol treatment exhibited less plaques across several parts of the brain (Karuppagounder et al., 2009). Resveratrol has even been tested in some preliminary clinical trials; in one of these studies, resveratrol was found to stabilize the progressive decline in cerebrospinal fluid Aβ40, plasma Aβ40, and cerebrospinal fluid Aβ42 levels, thereby combatting AD (Moussa et al., 2017).

In order to study AD, animal models are often employed; one organism that is frequently used is *Drosophila melanogaster*, also known as the common fruit fly. *Drosophila* is advantageous for research because it is easy to handle and culture in laboratory conditions, has short life cycles, is inexpensive, and can be genetically modified (Jennings, 2011). *Drosophila* is especially ideal for the study of neurodegenerative diseases, including AD, because it has a complex nervous system, conserved neurological function, and human disease-related loci (Stephenson & Metcalfe, 2013). There also exist specific strains of *Drosophila* that can serve as models of AD; these strains include presenilin, AB40, and AB42, which involve modifications to

express AD genes at a molecular level. Using *Drosophila*, both behavioral and anatomical studies can be conducted. In the study of AD, behavioral studies bring out any symptomatic effects that are present, while anatomical studies and fluorescent observation of *Drosophila* brains contribute to the identification of important structural alterations. Structural observations are of particular importance because they may provide insight into changes to the molecular path of the disease. The mushroom bodies (MBs) in specific are a key structure of the *Drosophila* brain that play a role in memory and learning (McBride et al., 2010).

There are several studies indicating that resveratrol shows promise in treating AD. In this study, I use presenilin *Drosophila* as a model of AD in order to confirm whether resveratrol is an effective treatment for AD. Two generations of flies undergo the same experiment and are compared to one another: a first generation of flies is raised on its experimental diet starting on day 8 after eclosion, and a second generation of flies is raised on its experimental diet starting at birth. For both generations, there are four different groups: the control groups are the wild-type flies that are not given resveratrol and the presenilin flies that are not given resveratrol, and the experimental groups are the wild-type flies that are given resveratrol and the presenilin flies that are given resveratrol. The first phase of this experiment aims to determine resveratrol's effects on the symptoms of AD: for this purpose, the climbing assay, a behavioral test, is administered on all of the flies. The second phase of this experiment aims to determine whether resveratrol is able to alter the neurological course of AD: for this purpose, an anatomical study is performed in which flies' brains are dissected, stained with antibodies, then analyzed under a fluorescence microscope. The results reveal that, when taken as a supplement starting early on, resveratrol

can not only improve the symptoms of AD but preserve brain structure by slowing the course of the disease.

Statement of Purpose

The purpose of this study is to (1) determine the effect of resveratrol on the brain function of wild-type and presenilin *Drosophila*, and (2) determine the effect of resveratrol on the brain matter and structure of wild-type and presenilin *Drosophila.*

Methods & Materials

***Drosophila* Strains and Drug Administration.** The wild-type and presenilin *Drosophila* strains used in this study were obtained from Indiana University's Bloomington Drosophila Stock Center. Specifically, a 2u strain of wild-type flies were used. Flies were cultured in bottles at 25℃ in 60% humidity in a 12 hr:12 hr light:dark cycle, on standard cornmeal medium. 9 days after living in the bottles, the flies were transferred into vials. Two of the vials, which served as the controls, were filled with 1.5 g of Fisher BioReagants Instant Fly Food, 5 mL of plain reverse osmosis (RO) water, and two gentle taps of yeast. The other two vials, which were the experimental groups, were also filled with 1.5 g of Fisher BioReagants Instant Fly Food and two taps of yeast. However, these experimental vials also received 0.4 g of resveratrol that was mixed into the 5 mL of RO water using a vortex mixer. 30-35 2u flies were put into one vial with plain RO water and one vial with resveratrol-incorporated RO water, and 30-35 presenilin flies were put into the other two vials (Generation 1). When the larvae and pupae became visible

in the vials (Generation 2) after around a week, the grown flies were transferred into fresh vials to make way for the flies that would newly hatch.

Climbing Assay. The procedure that was described by Manjila & Hasan and originally used in a 2015 study by Pathak et al. was roughly followed. 16 days after eclosion (1 week after being in vials for the 1st generation flies, 16 days after being in the vials for the 2nd generation flies), ten flies were placed in a vial with a diameter of 2.5 cm and a height of 10 cm that had a mark at the 6 cm point. The vial was gently tapped to get the flies to the bottom of the vial, and the number of flies that climbed to the 6 cm mark after 10 seconds was counted. For each of the four fly groups, this process was conducted for 3 batches of 10 flies each, and each batch of flies underwent 3 trials. The statistical significance between different genotypes and flies that received treatment was calculated using One-way analysis of variance (ANOVA) for $P < 0.05$.

Anatomical Study. Flies were submerged in ethanol, immediately transferred to 1 X PBS, then immediately transferred into fixative, which was previously prepared with 10 mL of 16% Paraformaldehyde, 4 mL of 10 X PBS, and 26 mL of milliQ water. To dissect a fly brain in fixative, the head of the fly was detached from the body using a pair of forceps. The head capsule (including the eyes) was then carefully ripped open and removed, isolating the brain. Any remaining tracheae/other debris was removed from the brain. This dissecting process was performed on at least 6 flies from each of the four groups, and after dissection, fly brains were placed into a fresh well of fixative, with each fly group receiving its own well. After 50 minutes had passed since the final brain was dissected, the brains underwent three 5-minute washes in 150 uL of 1 X PBS; during each of the 5-minute intervals, the dissection wells were put on a rocker in the lab room. The brains were then stained with Fasciclin II, placed on a rocker in the

4℃ cold room overnight, and stained with Gam the next day. Then, 4 microscope slides were prepared (one for each fly group) by sticking two reinforcement labels on top of each other and dropping 16 uL of 80% glycerol into the center of the labels. The fly brains were transferred into the glycerol using a pair of forceps, then a coverslip was gently placed on top of the reinforcement labels. Clear nail polish was used to seal the coverslip securely onto the slide. Once the nail polish had tried, the slides were observed under the Keyence Fluorescence Microscope.

Results

Brain function of Psn *Drosophila* rescued by resveratrol. A climbing assay was performed for all fly groups of both generations in order to determine the effect of resveratrol treatment on brain function, as evidenced as the behavioral symptoms of AD. The climbing assay used climbing ability, an objectively measurable attribute, as the means of assessing brain function. In both generations, more than half of the wildtype flies were able to complete the climb within 10 seconds, and there was no difference when compared to the number of wildtype flies, given resveratrol, to complete the climb. There was a significant decrease in the number of presenilin flies to complete the climb. However, there was a significant increase in the number of presenilin flies given resveratrol to complete the climb, when compared to the untreated presenilin flies. In fact, there was no significant difference between the climbing ability of the presenilin flies given resveratrol treatment and the wildtype flies.

Neurodegeneration of 1st gen. Psn *Drosophila* not slowed by resveratrol. To determine the effect of resveratrol treatment on the neurodegeneration (especially in the mushroom bodies) of

1st generation presenilin *Drosophila* brains, immunofluorescent staining was performed. After being dissected and stained, all *Drosophila* brains were observed under a fluorescence microscope. At the location of the mushroom bodies, towards the center of the brain, the 2u wildtype brain showed distinction of two lobes -- likely part of the mushroom bodies. The 2u + resveratrol brain showed similar distinction in the same location of the brain. However, the presenilin brain did not show distinction at the mushroom bodies; furthermore, distinction was not rescued in the presenilin + resveratrol brain. The presenilin and presenilin + resveratrol brains looked very similar to one another with respect to the lack of bright neuronal distinction.

Neurodegeneration of 2nd gen. Psn *Drosophila* slowed by resveratrol. Immunofluorescent staining was also performed on 2nd generation *Drosophila* brains. When observed under a fluorescence microscope, the 2u wildtype brain showed distinction of two lobes at the location of the mushroom bodies, just like the 1st generation 2u wildtype brain. Again, as with the 1st generation, the 2u wildtype + resveratrol brain of the 2nd generation was very similar in appearance to the 2u wildtype brain. The presenilin brain did not show distinction at the mushroom bodies; however, distinction was rescued in the presenilin + resveratrol brain, where areas of bright neuronal lobes were once again present near the center of the brain. To this end, the 2nd generation presenilin + resveratrol brain resembled the 2u wildtype and 2u + resveratrol brains more than it did the presenilin brain.

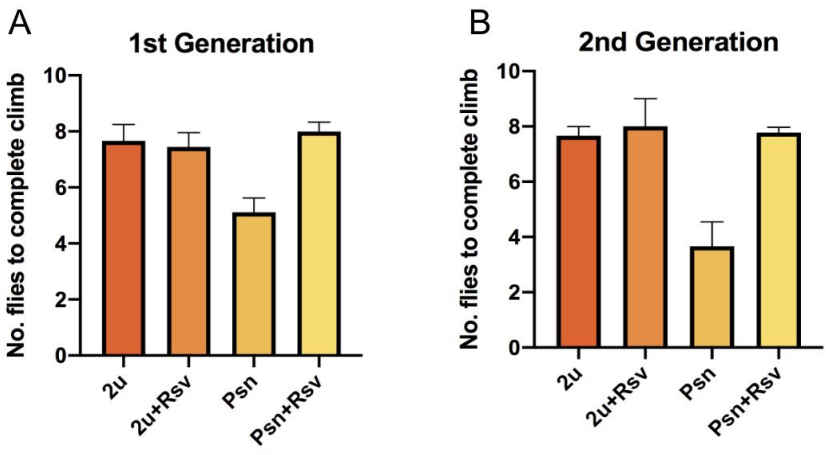

Figure 1: Climbing Assay. Brain function of flies as reflected by climbing assay. Bars show the number of 2u, 2u + rsv, Psn, and Psn + rsv flies in the (A) 1st and (B) 2nd generations to successfully complete climb. For both generations, $p<0.001$ when analyzed with ANOVA; Student *t* tests performed between two groups at a time indicate that significant differences exist between 2u (+ rsv)/ Psn flies and Psn/Psn + rsv flies for both generations.

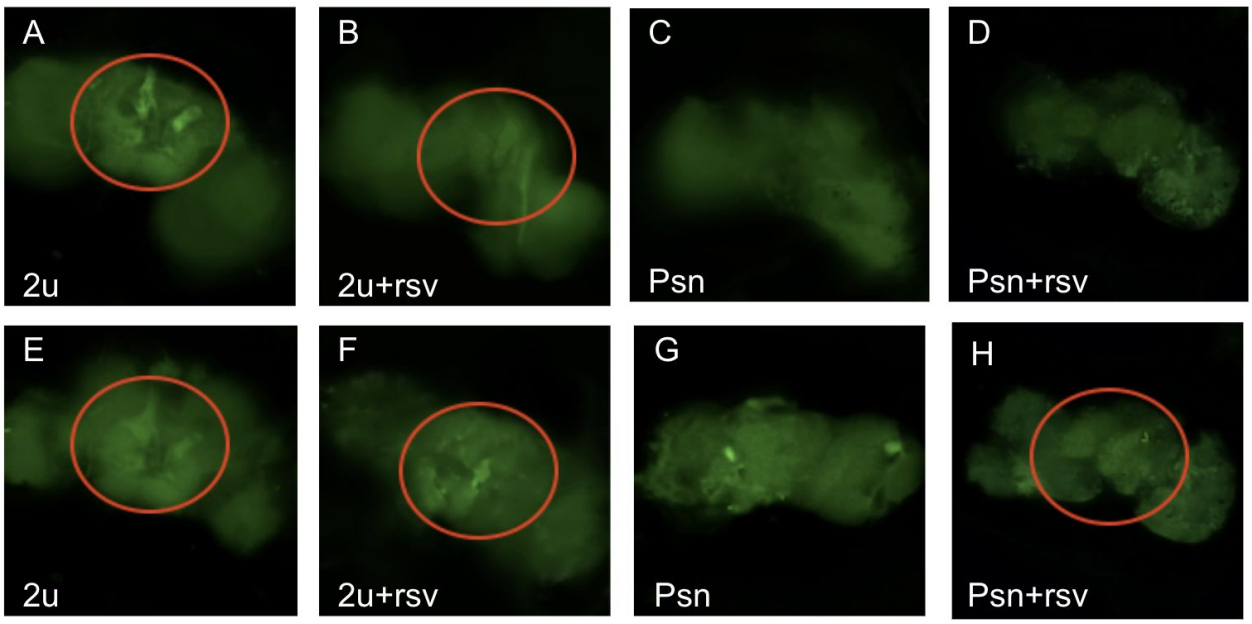

Figure 2: Anatomical Study. Analysis of neurodegeneration, with focus on the mushroom bodies. Red circles indicate presence of visibly distinct mushroom bodies. (A-D) 1st generation *Drosophila* brains dissected and stained with Fasciclin II primary antibody and Gam594 secondary antibody; equally visible distinction of mushroom bodies in 2u and 2u+rsv brains, but no distinction in Psn brain and no rescue of distinction in Psn+rsv brain. (E-H) 2nd generation *Drosophila* brains also dissected and stained with Fasciclin II and Gam594; equally visible distinction of mushroom bodies in 2u and 2u+rsv brains, no distinction in Psn brain, but notable rescue of distinction in Psn+rsv brain.

Discussion

In this study, it is established that the decreased climbing ability of presenilin *Drosophila* is restored by resveratrol treatment. Presenilin *Drosophila* also exhibited darker, less defined brain structures than their wildtype counterparts; interestingly, bright brain structure was restored only for flies that were given resveratrol immediately from birth.

Based on the results of the climbing assay, it can be determined that resveratrol is able to reverse behavioral symptoms of AD. More striking, however, resveratrol's role in brightening the brain structure of presenilin *Drosophila* indicates that if resveratrol is taken for a long time, it slows the neurodegeneration that is typically present in presenilin *Drosophila* and characterizes AD. Therefore, resveratrol may actually be involved in altering the neurological course of AD.

Differences in neuronal distinction are clear, but the process of dissecting and staining brains lends itself to potential limitations. Firstly, 10 brains from each fly group were dissected, and dissections were performed by hand. Given the small sample size, potential imperfections in individual dissections may have swayed the results. Additionally, the staining of the mushroom bodies is still not strong enough to clearly make out the structure of the mushroom bodies; based on the general vicinity of the differences in brightness of staining, it is assumed that the mushroom bodies have been altered. In future research, using a higher concentration of the primary antibody Fasciclin II would allow for more detailed visualization of the mushroom bodies (McBride et al., 2010). By moving beyond just the brightness of the staining and identifying specific structural differences between the fly groups, resveratrol's impact could be better detailed. This, paired with a western blot, could ultimately lead to an understanding of the mechanism by which resveratrol works.

Additionally, presenilin *Drosophila* is highly valuable but nevertheless only study one aspect of AD. Presenilin models do not address, for example, amyloid-beta plaques or tau tangles, two structures which are key characteristics of AD. Hence, resveratrol may have other significant effects on different mechanisms of facets of AD for which presenilin models do not account. AB42 *Drosophila* are one line of flies that show both the behavioral and neuronal manifestations of AD as they relate to amyloid-beta plaques. To this end, it would be valuable to administer resveratrol on AB42 *Drosophila* and identify how it influences the brain function as well as the brain matter of these flies (Iijima et al., 2004). By identifying resveratrol's impact on various aspects of AD, a more well-developed understanding of its effects can be obtained.

Conclusion

This study addresses the lack of effective treatments for AD by testing the effect of resveratrol on the brain function and brain matter of presenilin *Drosophila*. Through the climbing assay, it was found that resveratrol improves the brain function of presenilin *Drosophila* given resveratrol starting both immediately upon eclosion and 8 days after eclosion; through the anatomical study, it was found that resveratrol improves the brain matter only of presenilin *Drosophila* given resveratrol starting immediately upon eclosion.

These results indicate that resveratrol shows great potential in not only treating the symptoms of AD, but actually influencing the course of the disease. Further research to gain a complete understanding of resveratrol's effects on the varying aspects of AD could potentially lead to resveratrol entering the market as a treatment in the future.

References

Atri, A. (2019, March). The Alzheimer's Disease Clinical Spectrum: Diagnosis and Management. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/30704681

Bekris, L. M., Yu, C., Bird, T. D., & Tsuang, D. W. (2010, December). Genetics of Alzheimer's disease. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3044597/#__ffn_sectitle

Briggs, R., Kennelly, S. P., & O'Neill, D. (2016, June). Drug treatments in Alzheimer's disease. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5922703/

Iijima, K., Liu, H.-P., Chiang, A.-S., Hearn, S. A., Konsolaki, M., & Zhong, Y. (2004, April 27). Dissecting the pathological effects of human Abeta40 and Abeta42 in Drosophila: a potential model for Alzheimer's disease. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/15069204.

Jennings, B. H. (2011, April 23). Drosophila – a versatile model in biology & medicine. Retrieved from https://www.sciencedirect.com/science/article/pii/S1369702111701134#bbib2

McBride, S. M., Choi, C. H., Schoenfeld, B. P., Bell, A. J., Liebelt, D. A., Ferreiro, D., . . . Jongens, T. A. (2010, July 14). Pharmacological and genetic reversal of age-dependent cognitive deficits attributable to decreased presenilin function. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/20631179

Moussa, C., Huang1, X., Rissman3, R. A., Aisen4, P. S., & Turner5, R. S. (2017, January 3). Resveratrol regulates neuro-inflammation and induces adaptive immunity in Alzheimer's

disease. Retrieved from https://jneuroinflammation.biomedcentral.com/articles/10.1186/s12974-016-0779-0

Stephenson, R., & Metcalfe, N. H. (2013). Drosophila melanogaster: A fly through its history and current use. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/23516695

Thinakaran, G. (1999, November 15). The role of presenilins in Alzheimer's disease. Retrieved from https://www.jci.org/articles/view/8728

Weller, J., & Budson, A. (2018, July 31). Current understanding of Alzheimer's disease diagnosis and treatment. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6073093/#__ffn_sectitle

Yiannopoulou, K. G., & Papageorgiou, S. G. (2013, January). Current and future treatments for Alzheimer's disease. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3526946/