

Project Title

Student name, major; Mentor name, department

Background

Current Alzheimer's disease (AD) research has largely focused on one of the primary hallmarks of AD – the self-assembly of amyloid- β ($A\beta$) monomers into soluble aggregates and later insoluble fibrils that deposit plaques. The "Amyloid Cascade Hypothesis" suggests that increased deposition of $A\beta$ in the brain parenchyma is fundamental to the development of AD and leads to synaptic and neuronal loss (Fonesca et. al, 2014). Modifications of the amyloid hypothesis have suggested that smaller soluble aggregates are the primary neurotoxic species (Karran, Mercken&Strooper, 2011); these aggregates, in the form oligomers, protofibrils, and $A\beta$ -derived diffusible ligands, are implicated in AD pathology (Gonzalez-Velasquez&Moss, 2008). Evidence supports that these plaques promote mitochondrial dysfunction with the increased expression of reactive oxidative species (ROS) and inhibition of generation of cellular ATP, leading to cell death (Xu et. al, 2009).

Polyphenols are micronutrients and secondary metabolites found in plants involved in defense against pathogens, and are characterized by multiple hydroxyl groups on aromatic rings (Manach et. al, 2004). Polyphenols have been extensively studied as neuroprotective agents and possible therapeutics, due in part to their promotion of endogenous antioxidant properties and free-radical scavenging. Polyphenols have also been shown to inhibit β A fibril formation, which has been linked to its antioxidant characteristics (Kim et. al, 2005). The therapeutic potential of these compounds is also studied due to their ability to cross the blood-brain-barrier (BBB). Evidence has shown polyphenol compounds have suppressed inflammatory responses in the hippocampus and cortical neurons with reduction of NF κ B activation, warranting further study of these compounds and their neuroprotective potential (Choi et. al, 2012).

One of subclasses of polyphenols, flavonoids, are the most common group of polyphenols, found in fruits, vegetables, tea, cereals, cocoa, and wine (Baptista et. al, 2014). Flavonoids are further classified into flavonols, flavones, isoflavones, flavanones, flavanols, and anthocyanidins which are found from various dietary sources.

Research Statement

To evaluate the ability of polyphenols to alleviate $A\beta$ -induced toxicity in cerebrovascular endothelial cells.

Project Objectives

- Determine dose-dependent response of HMBECs to $A\beta$ oligomers to establish treatment concentration.
- Assess the effectiveness of polyphenol compounds in reducing $A\beta$ -induced apoptosis in HBMECs

Project Significance

AD is a neurodegenerative disorder and the most common type of dementia, characterized by behavioral changes, memory impairment, and impairment of bodily functions such as walking and swallowing. AD consists of 60% to 80% of dementia cases, with approximately 5.2 million afflicted with AD in the United States alone (Alzheimer's Association, 2014). A large proportion of dementias are a mix of AD and vascular cognitive impairment, with vascular impairment possibly aggravating AD pathology. Aggregation and toxicity have been found to lead to apoptosis, a marker for severe and prolonged stress on cells and is consistently seen in AD pathology, with $A\beta$ toxicity increasing endothelial monolayer permeability and compromising BBB integrity (Gonzalez-Velasquez&Moss, 2011). Reduction of this response can be used to identify potential treatments and their effectiveness in alleviating harmful effects of $A\beta$, and thus AD pathogenesis.

Methodology

The [mentor] lab has already developed assessing A β -induced toxicity. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) is used to detect programmed cell death – apoptosis – by binding to nicks in exposed termini of DNA – a hallmark of apoptosis - through the enzyme TdT. These nicks indicate severe internucleosomal DNA fragmentation and damage, with fluorescent markers allowing for visual identification of fragmentation (Loo, 2011).

This study will explore the application of this assay for treatment of human brain microvascular endothelial cells (HBMECs). HBMECs will be cultured up to a passage of 10 and treated with varying concentrations of A β ₁₋₄₂ oligomers to simulate the effects of toxic soluble oligomers in the neurovasculature. Experiments will initially determine minimum levels of A β treatment to activate significant neuroinflammatory and apoptotic activity. These experiments will be replicated to confirm dose-dependency responses. After determining the optimal level of A β treatment, cells will also be treated with flavones and flavonols, including kaempferol, luteolin, quercetin, and apigenin, to assess ability of these compounds to reverse the effects of A β oligomers.

For the TUNEL assay, endothelial cells will be seeded and treated with A β ₁₋₄₂, salt, DMSO, and inhibitor, with cells treated without A β ₁₋₄₂, inhibitor or completely untreated serving as controls. After 24 hours, cells are fixed using PBS and 4.0% paraformaldehyde washes. Cells are then prepared for staining through PBS and Triton-X washes. Equilibration buffer, rTdT Enzyme, and fluorescent Nucleotide Mix from a TUNEL assay kit is then mixed to prepare for staining, with cells incubated following staining at 37°C before mounting cells with DAPI. The level of apoptotic activity is determined and compared to control groups using confocal microscopy and a specialized Matlab program to quantify activation in individual cells.

Progress in data acquisition and analysis will be presented weekly to [mentor] at lab group meetings. I will work under the supervision of a graduate student mentor in the lab, however, I am already proficient in both the TUNEL assay for SHSY5Y cells and maintenance of HBMECs, so experiments will be performed independently.

Project Timeline

October-November: Finalize dose-dependency response for TUNEL assay treatments of A β .

December-March: Perform TUNEL experiments (three trials) for various polyphenol compounds.

February-April: Prepare poster for Discover USC and conferences, begin write-up of results

September: Present poster as the Southeast Regional Biomedical Engineering Career Conference

February: Present poster at Biophysical Society Conference

Anticipated Results/Final Products and Dissemination

Reduction of cerebrovascular endothelial cell death due to toxic A β oligomers as a result of treatment with polyphenols would support research exploring the therapeutic potential of these natural compounds as complimentary therapeutics or as lead compounds for drug discovery. The results of these TUNEL experiments will be incorporated into several published papers concerning the therapeutic potential of different polyphenol compounds. This data will also be presented at two conferences: the Biophysical Society Conference and the Southeast Regional Biomedical Engineering Career Conference. An oral presentation will also be given at Discover USC.

Personal Statement

I have worked in [mentor]'s lab since the start of my sophomore year. [Mentor]'s research interests focus on assessing the impact of different stages of Amyloid β aggregation and its significance in AD pathology, along with potential therapeutic targets to alleviate neuroinflammatory response. Through [mentor]'s Lab, I have worked on projects relating to assessment of neuroinflammation and apoptosis in human neuroblastoma cells, as well as worked under a graduate mentor studying neuroinflammation in HBMECs. This project would be a culmination of all of my past work. Working in this lab has allowed me to further define and explore both my research interests as a Biomedical Engineering major and Neuroscience minor, providing me skills in critically analyzing scientific publications, practicing sterile techniques, and working with cell culture. This research has motivated me to pursue a Ph.D. either in a neuroscience or biomedical engineering graduate program to study neurodegenerative or mood disorders.

References

1. "2014 Alzheimer's Disease Facts and Figures." *Alzheimer's & Dementia* 10.2 (2014): E47-92. *Science Direct*. Web.
2. F. I. Baptista et. al. "Flavonoids as Therapeutic Compounds Targeting Key Proteins Involved in Alzheimer's Disease." *ACS Chemical Neuroscience* 5 (2013): 83-92. Web.
3. A. C.Fonseca et. al. "Loss of Proteostasis Induced by Amyloid Beta Peptide in Brain Endothelial Cells." *Biochimica Et Biophysica Acta (BBA) - Molecular Cell Research* 1843.6 (2014): 1150-161. *Science Direct*. Web.
4. F. J. Gonzalez-Velasquez and M. A. Moss. "Soluble Aggregates of the Amyloid- β Protein Activate Endothelial Monolayers for Adhesion and Subsequent Transmigration of Monocyte Cells." *Journal of Neurochemistry* 104 (2008): 500-13. Web.
5. F. J. Gonzalez-Velasquez, J. A. Kotarek, and M. A. Moss. "Soluble Aggregates of the Amyloid- β Protein Selectively Stimulate Permeability in Human Brain Microvascular Endothelial Monolayers." *Journal of Neurochemistry* 107.2 (2008): 466-77. Web.
6. F. J. Gonzalez-Velasquez et. al."Activation of Brain Endothelium by Soluble Aggregates of the Amyloid-B Protein Involves Nuclear Factor- KB." *Current Alzheimer Research* 8.1 (2011): 81-94. Web.
7. E. Karran, , M. Mercken, and B. D. Strooper. "The Amyloid Cascade Hypothesis for Alzheimer's Disease: An Appraisal for the Development of Therapeutics." *Nature Reviews Drug Discovery* 10.9 (2011): 698-712. *Nature Reviews*. Web.
8. H. Kim et. al. "Effects of Naturally Occurring Compounds on Fibril Formation and Oxidative Stress of β -Amyloid." *Journal of Agricultural and Food Chemistry* 53.22 (2005): 8537-541. Web.
9. D.T. Loo. "In Situ Detection of Apoptosis by the TUNEL Assay: An Overview of Techniques." *Methods In Molecular Biology* 682 (2011): 3-13. *Springer*. Web.
10. C. Manach et. al. "Polyphenols: Food Sources and Bioavailability." *The American Journal of Clinical Nutrition* 79 (2009): 727-47. Web.
11. S. Xu et. al. "Overexpression of Tfam Protects Mitochondria against β -amyloid-induced Oxidative Damage in SH-SY5Y Cells." *FEBS Journal* 276.14 (2009): 3800-809. Web.

Budget Form

Student's Name:

	Estimated number of hours student will work	Enter the hourly wage	
Research hours during semesters when enrolled in classes	150	\$8.00	\$1,200.00
Research hours during semesters when NOT enrolled in classes			\$0.00
Fringe: Student salary * student fringe rate (What is fringe? See budget instructions or guidebook)			
Enrolled in classes	\$1,200.00	0.55%	\$6.60
NOT enrolled in classes	\$0.00	8.29%	\$0.00
Materials/Supplies	Enter sub-total from below:		\$1,442.00
Travel	Enter sub-total from below:		\$760.00
	TOTAL:		\$3,408.60
	Amount requested for MGS award:		\$3,000.00

Budget Justification/Description

Student Salary: Indicate estimated number of student research hours per week and hourly rate separated by semesters when student is enrolled in classes or not enrolled in classes (generally fall or spring vs summer semesters).

The majority of the project will be implemented during the Spring semester.
Taking classes: 10 hours/week for 15 weeks at \$8.00 per hour.

Materials/Supplies: Indicate items, quantity, and estimated price. *Be sure to include taxes on all purchases.*

Amyloid-beta protein (10 mg at \$77 per mg, Anaspec): \$770
Luetolin (10mg at \$19, Cayman Chemical): \$19
Kaempferol (25 mg at \$38, Cayman Chemical): \$38
Quercetin (5 g at \$12, Cayman Chemical): \$12
Apigenin (25 mg at \$48, Cayman Chemical): \$48
DeadEnd™ Fluorometric TUNEL System (1 kit at \$555, Promega): \$555

*Additional supplies, including HBMECs, cell media, PBS, paraformaldehyde, other reagents, and disposable labware will be provided by [mentor].

Travel: Indicate location, purpose of travel, estimate itemized costs (transportation, lodging, registration, etc.).

Airfare (Charlotte, NC to Los Angeles, CA, round trip, U.S. Airways): \$600
Registration Biophysical Society Conference (Student Nonmember rate): \$120
Registration for Southeast BME Regional Career Conference (Student BMES member rate): \$40

*Additional funding may be sought through the Magellan Voyager Award or Magellan Mini-Grant.