SOAR Research Proposal – Summer 2018

Submitted by March 12, 2018

Title of Proposed Project: The Neuroprotective Effect of Curcumin in the Striatal 6-Hydroxydopamine Model of Parkinson's disease

Faculty Advisor: Cecilia M. Fox, Professor of Biological Sciences and Director of Neuroscience Program

Name of Student: Adriana Facchiano

Purpose of Project: To determine whether intraperitoneal administration of cucumin, a biphenolic compound derived from turmeric and known to have antioxidant/anti-inflammatory properties, may protect substantia nigra dopamine neurons in the striatal 6-hydroxydopmine (6-OHDA) rat model of Parkinson's disease.

Background and Relevance of Project:

Parkinson's disease is a progressive neurodegenerative disorder in which resting tremor, muscular rigidity, bradykinesia (slowness of movement) and impaired postural reflexes predominate. It is observed in approximately 1 % of the American population over the age of 55. Within ten years of onset, 60 % of patients diagnosed with Parkinson's disease are severely disabled or deceased (Yokoyama, Uchida, Kuroiwa, Kasahara, and Araki, 2010).

The primary pathology of this disease is degeneration of the nigrostriatal pathway. This pathway originates in the substantia nigra of the midbrain and projects anteriorly to the striatum. As degeneration of this pathway progresses, there is a loss of substantia nigra dopamine neurons as well as depletion of dopamine and dopamine metabolite levels in the striatum. Current available therapy relieves many of the symptoms in the early to middle stages of the disease but does not arrest the advancement of the disease. Therefore, it is of significant benefit to identify possible alternative therapies in alleviating or inhibiting the progression of this debilitating neurodegeneration.

The Rat Model of Parkinson's disease:

Many advances in our understanding of the cause of Parkinson's disease as well as insights into its treatment (for example, L-dopa therapy) have been derived from animal studies. The discovery of neurotoxins that selectively destroy dopamine neurons, such as 6-OHDA has played an important role in the study of this disorder. The discriminating effects of 6-OHDA in the rat midbrain are a result of its structural similarity to dopamine and its ability to efficiently bind to receptors on the dopamine cell membrane for subsequent entry into the neuron. Once inside the dopamine neuron, 6-OHDA undergoes a rapid auto-oxidative process resulting in the formation of several highly reactive oxygen species such as hydrogen peroxide, the superoxide radical and the hydroxyl radical. These oxidative products initiate a series of events that lead to the destruction of DNA and proteins as well as deterioration of cell membranes. In the past, my lab has focused on an intranigral 6-OHDA lesion which is capable of destroying neurons within minutes. For this study, we intend to use the more chronic model of an intrastriatal lesion. 6-OHDA would be administered in the striatum (also known as the target tissue for substantia nigra neurons) that would lead to a slowly progressive type of cell death that is analogous to the human condition (Sauer and Oertel,1994).

Curcumin:

Curcumin, a biphenolic compound derived from turmeric, has been shown to have antioxidant and anti-inflammatory properties in animal models of neurodegeneration. It is known that inflammation is present in the parkinsonian brain, which may be the result of enhanced glial cell responses that contribute to the destruction of dopamine neurons (Chinta and Anderson, 2004). The activation of microglia and astrocytes (types of glial cells) results in an increase production of toxic proteins, such as chemokines and cytokines. Research has shown that activated microglia also produce reactive oxygen species (ROS) and nitric oxide (NO), which are inflammatory mediators (Peterson and Flood, 2012).

Through extensive research it has been found that curcumin displays unique antioxidant and anti-inflammatory properties in treating a multitude of disorders ranging from brain cancer to Alzheimer's disease to traumatic brain injury (Hu et al, 2015). As an antioxidant, curcumin effectively protects against DNA oxidative damage in the Tg2576 mouse and lipid peroxidation in an Alzheimer rat model. It conserves glutathione (an antioxidant) levels and reduces the concentration of free radicals within Alzheimer mouse models as well as humans (Hu et al, 2015). Through a reduction of neuroinflammation, curcumin has been identified as a novel protectant.

However, there is not much research focusing on the protective effects of curcumin in parkinsonian animal models. A study examining the administration of curcumin in a mouse model of Parkinson's disease has shown to not only provide protection against a 6-hydroxyopamine lesion, but also reduce the response of astrocytes and microglia within the brain (Tripanichkul and Jaroensuppaperch, 2011). There was a significant correlation between the loss of dopamine neurons and the increased amount of glial response. At this time, there are no documented studies examining whether curcumin provides neuroprotection through the anti-inflammatory properties as a consequence of a diminished glial cell response in a rat parkinsonian model. Therefore, this study aims to investigate this mechanism of protection. It is hypothesized that curcumin will function in protecting dopaminergic neurons by specifically inhibiting glial cell responses that will, in turn, provide a reduction in neuroinflammation.

Proposed Project:

Overall Design:

Animals- 20 Sprague Dawley young adult male rats will be randomly divided equally into the following 2 groups:

a) DMSO (vehicle) + intrastriatal 6-OHDA lesion
b) Curcumin (20mg/kg dissolved in DMSO/day) + intrastriatal 6-OHDA lesion

Curcumin and DMSO – Animals will receive an intraperitoneal injection for 45 days

Behavior – All animals will be evaluated for motor function using the rotometer and footfault tasks every week for 10 weeks post-surgery. Cylinder test will be administered once pre-surgery and twice post-surgery.

Tissue Processing- Animals will be euthanized at 11 weeks post-surgery and then the brain tissue will be stored until the start of the fall 2018 term.

Due to the expansive nature of this project, this SOAR study will focus on the surgical procedures and behavior testing of these experimental animals. When the fall 2018 term begins, this study will then be continued as an *Honors Project*. Immunocytochemical staining, cell counting via stereology and data analysis will take place in the second half of this project.

In the past, the Moravian College Institutional Animal Care and Use Committee has approved my research protocols. We will be sure to file to appropriate paperwork for project approval.

Student Involvement and Faculty Responsibilities:

Adriana is a strong student who will become more familiar with the implementation of the scientific method, acquisition and interpretation of relevant primary literature and data analysis through this SOAR experience. As with all my research students, I will work with her as a colleague. I will be responsible for personally teaching her the background information expressed in this proposal as well as the techniques for successfully completing this project. Adriana will learn stereotaxic brain surgery, animal care, behavior testing, euthanasia and brain extraction. Since many of these techniques are not commonplace in our teaching laboratories, I will assist her in every stage of this research process. As she becomes more comfortable, Adriana will perform each of the procedures described in this proposal.

Proposed Project Timetable:

Week 1: Literature searches, familiarization with neuroanatomy and techniques of study
Tour of animal facility, review of the guide for care and use of animals in research
Baseline behavior testing

Week 2: 6-OHDA Surgery and curcumin/DMSO administration

Week 3-12: Extensive post lesion behavior testing and continued curcumin/DMSO administration
Since this is a progressive model of neurodegeneration, weekly testing is needed to determine the extent of the lesion at given time points.

Week 12: Euthanasia via intracardiac perfusion and brain tissue extraction

Future Time Commitment:

Adriana is aware that this project will take the length of her summer. Yet, due to the timing of procedures, I will ensure she does not exceed the "10 week" limit. There will be some weeks when she will not work 40 hours. Yet, the overall amount of time working on this project will be the equivalent of what is expected in this program.

Adriana is committed to completing her project and preparing a poster for presentation at the spring 2019 Lehigh Valley Society for Neuroscience Research Symposium and Moravian College Scholars Day.

Impact of the Project – Benefits for Student, Faculty and Moravian College:

Student:

Since Adriana hopes to pursue a career in medicine, this SOAR experience will help her develop skills of surgical care, critical thinking, data analysis and research presentation. I am confident this research endeavor as well as the academic opportunities at Moravian College will offer her the foundation she needs to be a competitive candidate for medical school. I intend to have her present her work at the Lehigh Valley SfN conference as well as the NCUR conference.

Faculty:

This SOAR project will have a positive impact on my professional development as a neuroscientist as well as a professor at Moravian College. I have been studying the neuroprotection of dopamine neurons in rodent models of Parkinson's disease for over twenty years. Though I have focused on growth factors and certain antioxidants, this study on curcumin is a follow up to a previous SOAR/Honors project that generated some very promising results. I am looking forward to see whether curcumin may be able provide a more robust protective effect than some other antioxidants that I have studied in the past. Successful completion of this project will ensure the presentation of the results at the annual Society for Neuroscience Conference as well as the Lehigh Valley Society for Neuroscience Research Symposium. Furthermore, it is my hope to publish this work and any future meaningful data gathered from my lab in peer reviewed neuroscience journals. Finally, as a college professor, I look forward to incorporating the results of this research into my Neuroscience and Physiology courses.

Moravian College:

The benefits to the college are the following –

- Increased biology/neuroscience faculty participation in research programs
- More opportunities for science majors to engage in scientific research
- Enhanced student interest in my Neuroscience and Human Physiology courses
- Continued growth of the Neuroscience research program
- Future opportunities for collaborative research with other institutions
- Dissemination of these research findings via conference presentations and peer reviewed journals

Budget Request:

$3000.00	Student stipend
$1000.00	Faculty stipend
$ 500.00	Supplies and expenses - $400 for rats - $100 for Isoflurane anesthesia

Additional expenses for the project will be covered by the Department of Biological Sciences.

On-campus housing for research student is requested.

Works Cited:

Chinta, S., & Andersen, J. (2004). Cell in focus: Dopaminergic Neurons. The International Journal of Biochemistry & Cell Biology, 37, 942-946.

Hu, S., Maiti, P., Ma, Q., Zuo, X., Jones, M., Cole, G., & Frautschy, S. (2015). Clinical development of curcumin in neurodegenerative disease. Expert Review of Neurotherapeutics, 15(6), 629.

Peterson, L., & Flood, P. (2011). Oxidative Stress and Microglial Cells in Parkinson's Disease. Mediators of Inflammation, 1, 1-12.

Sauer, H. and Oertel, W.H. (1994). Progressive degeneration of nigrostriatal dopamine neurons following intrastriatal terminal lesions with 6-hydroxydopamine: a combined retrograde tracing and immunocytochemical study in the rat. Neuroscience, 59 (2), 401-415.

Tripanichkul, W., & Jaroensuppaperch, E. (2011). Curcumin Protects Nigrostriatal

Dopaminergic Neurons and Reduces Glial Activation in 6-Hydroxydopamine Hemiparkinsonian Mice Model. International Journal of Neuroscience, 122, 263-270.

Yokoyama, H., Uchida, H., Kuroiwa, H., Kasahara, J., & Araki, T. (2010). Role of glial cells in neurotoxin-induced animal models of Parkinson's disease. Neurological Sciences, 32(1), 1-7.

Student Statement of Purpose

Student Name: Adriana Facchiano

Major: Neuroscience- Cellular Track

Expected Graduation: May 2019

Faculty Mentor: Dr. Cecilia Fox

On-Campus Housing Request: Yes

Project Title: The Neuroprotective Effect of Curcumin in the Striatal 6-Hydroxydopamine Model of Parkinson's disease

Participation Rationale and Expected Outcomes:

I have been interested in going to medical school since I was a freshman. My initial impression of being a pre-medical student was that clinical observation was the most important attribute of a strong applicant. However, faculty mentors at Moravian such as Dr. Fox encouraged me to explore other aspects of healthcare. One of the areas of healthcare that I most recently became exposed to is clinical research. My first experience was participating in the Lehigh Valley Health Network Research Scholar Program. This opportunity helped me to understand that research is a critical experience for pre-medical students, and more importantly, the advancement of clinical practice. Not only could other opportunities in research strengthen my application, but would help me to strengthen valuable skills such as data acquisition, data analysis, and communication of results to help me be successful in my graduate studies. Such research would also help me learn much more about my chosen field and contribute to the neuroscience community. I recognize that participating in a SOAR project this summer would serve each of these purposes.

As I have progressed through the neuroscience curriculum, I have come to appreciate the value of laboratory research in the field. I have seen the benefits of such research firsthand during my experiences shadowing and assisting in clinical research at local hospitals. Additionally, through critical analysis of various empirical studies, I have become familiar with pertinent subjects in neuroscience, especially the 6-hydroxydopamine rat model of Parkinson's disease. My Neuroscience Methodology (NEUR 367) course allowed me to learn about many of the procedures described in these papers. For example, I have learned about stereotaxic surgeries to induce parkinsonism in rats. I have learned about the proper care of laboratory animals before, during, and after experimentation. Additionally, I have been exposed to rodent motor function and behavior with the use of various tests. This proposed SOAR project would allow me to improve all these skills, and provide me with further opportunities to learn about other neuroscience lab techniques.

Conducting this SOAR project will also allow me to gain firsthand experience in neuroscience research. I believe this should be the ultimate goal of all students in the neuroscience curriculum since it brings many of the topics and studies discussed in class to life. Additionally, the work done in labs like Dr. Fox's is critical to the advancement of the understanding of neurological diseases, especially Parkinson's disease. This project will contribute to Parkinson's disease research by continuing a study previously conducted by Dr. Fox and student, Loukya Kanakemedala. The study aims to look examine possible neuroprotective effects of curcumin, found in turmeric, in parkinsonian rat models. It has been found that curcumin has protective effects on dopaminergic neurons, but the mechanism of protection is not yet fully understood. The potential impacts of this study are promising, and I would be honored to continue this project.

Most importantly, this project will provide me with insight about whether I wish to pursue other research opportunities in the future as a clinician. I have witnessed the symbiotic relationship between

clinical practice and clinical research at St. Luke's Hospital and I have considered pursuing such opportunities when I begin practicing medicine. I would be fortunate to work under the supervision of Dr. Fox, who has helped me discover a passion for my major. If given the opportunity to participate in this project, I would be able to further explore this passion and find a newfound appreciation for the field of neuroscience.