Professional Development Grant - April 2018

"Travel support for grant proposal development"

Final Report

Dr. Mariusz P. Gajewski
mgajewski@atu.edu
Arkansas Tech University
Department of Physical Sciences

September 26, 2018

Purpose of the project

The purpose of this project was to strengthen my application for external support for my research. In January 2018, I applied for Arkansas INBRE Summer Project Grant, which was rejected. To address reviewers' comments, with support from this grant, in the summer of 2018, I worked closely with my INBRE mentor (Dr. Steven Barger, UAMS) in order to obtain additional statistically significant preliminary results. I have also consulted with Dr. Helen Benes (Associate Director of Arkansas INBRE, and Director of Arkansas INBRE Developmental Research Project Program, UAMS) about the approach to modifications of my proposed research. The new molecules developed in my lab are capable of growth inhibition of human brain cancer (glioblastoma), *in vitro.* One of the very fortunate "side effects" that these compounds produce is inhibition of glutamate efflux in the brain, which might prove useful in reduction of neuroinflammation and therefore, therapy. The rejected proposal addressed research on this "side effect" but lacked strength in the preliminary results. The results obtained in the summer of 2018 are now included in the newest version of the external grant proposal application (due in January 2019) as preliminary findings. These findings will increase the chances of my resubmission being funded. At Arkansas Tech, I have no access to highly advanced instrumentation, such as in Dr. Barger's lab and lack expertise in working with live cell cultures. Support from this grant made my summer commuting to UAMS, and therefore, conduction of research and collection of results possible. I needed to evaluate my new molecules' ability to inhibit transport of glutamic acid, a neurotransmitter involved in neurotoxicity related to conditions and diseases such as traumatic brain injury, stroke, Alzheimer's, and numerous others. The process involved screening my molecules' activity by conducting bioassays, utilizing Dr. Barger's lab infrastructure, and his license to handle radioactive materials, required in tritium glutamate assays.

Review of the professional development opportunity

The support from this grant made my commuting to UAMS possible. It was my goal to complete the collection of the preliminary results for my external grant application.

Summary of experiences

The methodology used involved harvesting rat brains, isolation of microglia and growing the microglial cells in cell cultures. The cell cultures were then exposed to lipopolysaccharide (LPS), a common agent that induces neuroinflammation. In the state of neuroinflammation, microglia release excitotoxic levels of glutamate, which kills neighboring neurons in the brain. The exposure of microglia to LPS was executed against controls (no LPS) and contrasted against microglia exposed to both LPS and the molecules developed in my lab. Comparison of the three groups (control, LPS, LPS + inhibitors) provided the results regarding neuroprotective properties of my molecules. The molecules assayed proved to be very efficient inhibitors of glutamate efflux. Figure 1 illustrates the potency of their action by summarizing all results collected in the summer of 2018.

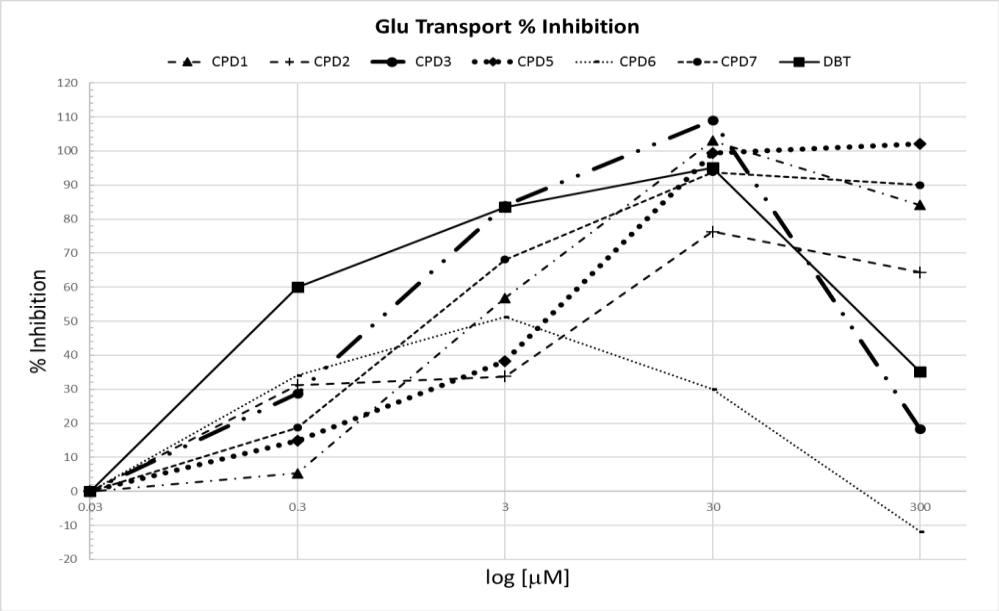

Figure 1. *Microglial Glu efflux inhibition by CPD 1-7 and 3,5-DBT.*

In brief, at relatively low concentrations (3 μM) the seven screened compounds inhibited the glutamate transport by 33%-83%, which is remarkable. At 30 μM, five out of seven compounds practically shut the transport down (~100% inhibition). These sound preliminary findings are more than convincing to justify extension of this research over animal models (mice), in *in vivo* studies, which are the primary scope of the external grant proposal submission.

Conclusion

In conclusion, as evidenced by the glutamate assays, the new molecules are potential therapeutic agents against neuroinflammation, the PI's primary area of research interests. The preliminary results collected over the summer of 2018 are now included in the updated version of the Arkansas INBRE Summer Project grant proposal, which will be submitted by the end of year 2018. The PI is grateful for the support from this Professional Development Grant, without which the collection of these results would not have been possible.