2015 Vision Award: Interim Progress Report

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I would like to thank the Preeclampsia Foundation for awarding a Vision Award to my research proposal, “The role of efflux transporters on the blood-brain barrier in preventing seizure during pregnancy.”

Project Goal: The goal of this project is to evaluate the modulation in expression and activity of efflux transporters on the blood-brain barrier throughout gestation. Efflux transporters, located on the blood-brain barrier, are integral to preventing passage of damaging seizure-provoking factors into the brain. We hypothesized that efflux transporters are involved with seizure prevention during pregnancy and are modulated throughout gestation, limiting exposure of neurons to seizure-provoking serum. Our goal was to characterize activity and expression of p-glycoprotein, a commonly expressed efflux transporter on the BBB, throughout gestation. Additionally, we planned to evaluate the effect of pre-eclamptic serum on the modulation of p-glycoprotein. We believe that this will help elucidate a mechanism impaired in pre-eclampsia, increasing the seizure risk by allowing seizure-provoking factors to more readily enter the brain.

Specific Aim #1: *To characterize the activity and expression of efflux transporters within the BBB over the course of normal gestation.*

Progress: In order to evaluate the changes in expression of p-glycoprotein on the blood-brain barrier throughout gestation, we quantified the variation of expression in the cerebral vasculature via confocal immunohistochemistry. After evaluating efflux transporter families known to be active at the blood-brain barrier that could play a role in preventing seizure, we determined that the multidrug resistance-associated protein (MRP) was an important transporter group to evaluate. Therefore, we also utilized confocal microscopy to evaluate the expression of MRP-2, the subgroup most commonly associated with the luminal side of the endothelial cells that compose the blood-brain barrier.

In order to evaluate the activity of these efflux transporter families at different times of gestation, we developed a method of evaluating P-glycoprotein and MRP activity in rat cerebral capillaries isolated by microdissection. In this technique, we were able to utilize a fluorescent substrate and an Ion Optix fluorescence photometry system to evaluate changes in transporter activity throughout gestation. To our knowledge, this is the first study to evaluate both transporter expression and activity at the blood-brain barrier throughout gestation.

These data are going to be submitted for presentation at an international meeting in the next coming year and will then be submitted for publication.

Specific Aim #2: *To determine the effect of exposure of pre-eclamptic serum on efflux transporter expression and activity within the BBB.*

Progress: This aim has been separated into two sets of experiments. Firstly, we isolated cerebral capillaries from non-pregnant rats via microdissection. These vessels

were then exposed to rat serum from either non-pregnant, late pregnant rats, or rats with experimental preeclampsia (reduced uterine perfusion pressure model with high cholesterol diet). Following exposure to the serum, we utilized the activity assay, described above, to evaluate the activity of both p-glycoprotein and MRP in these experimental conditions. In a second set of experiments, we exposed isolated cerebral capillaries from non-pregnant rats to 4 groups of human serum: normal pregnant women, women with preeclampsia, women with eclampsia (serum from prior to seizures and after seizures). These experiments have been completed, and data analysis is ongoing. We hope to present this work at an international conference next year.

Another aspect of study that we have pursued has included interrogating other organs to evaluate if gestational transporter modulation is organ specific or uniform throughout the entire animal. We are currently in the process of evaluating transporter expression in the liver, placenta, and choroid plexus. These experiments are ongoing.