Allison Hamill

St. Louis, MO

Su20-003

Senior
Biomedical Engineering

Faculty Mentor: Dr.Shinghua Ding, Biomedical, Biological & Chemical Engineering

The Role of Astrocytes in Combatting Oxidative Stress After Stroke

Allison Hamill and Shinghua Ding

Astrocytes play an important role in the response to oxidative stress after stroke. While astrocytes respond both positively in an antioxidant capacity as well as negatively in a pro-oxidant capacity, research shows that astrocytes' antioxidant functions are necessary to combat oxidative stress. One such antioxidant function is astrocytic stimulation of the Pentose Phosphate Pathway (PPP), which produces a reducing and antioxidizing agent, NADPH, as its main byproduct. In the PPP, G6PDH plays a role in reduction of oxidative stress by converting NADP to NADPH to promote the production of GSH, a neuroprotective molecule, in astrocytes. Our goal is to genetically engineer a viral DNA plasmid with an astrocyte-specific promoter to express G6PDH selectively in astrocytes to increase the reducing compound NADPH and decrease oxidative stress. We designed DNA primers including a DNA sequence of the G6PDH gene and two restriction enzyme sites. After polymerase chain reactions using the designed primers, we amplified the G6PDH gene which we ligated to the DNA plasmid with an astrocyte-specific promoter gfaABC1D. Finally, the ligated DNA product was transformed into E. Coli., which was cultured for preparation of the recombinant DNA plasmid. The last step was to sequence the DNA plasmid to confirm it was correct. Unfortunately, our first trial did not produce the correct DNA sequence; however, we believe that after a second endeavor, we will successfully generate the correct DNA sequence. We ultimately intend to prepare viral vectors to express G6PDH in astrocytes in mice, and test whether astrocytic G6PDH can reduce oxidative stress after ischemic stroke is induced. Our goal is to determine that overexpression of G6PDH in astrocytes is an effective approach to reducing brain damage through its antioxidant function after stroke.