Wake Forest School of Medicine

Department of Neurobiology and Anatomy
ALS Translational Science Unit

Blazeman Foundation for ALS

Request for Continuation of Project: Administration of Hsp70 maintains muscle innervation in the SOD1 mouse- a new therapeutic approach?
Principal Investigator: Carol Milligan, Ph.D.
Institution: Wake Forest School of Medicine
Dates of Proposed Continuation: April 15, 2015 to December 31, 2016

Project Summary:
Researchers at Wake Forest School of Medicine have found a new treatment that may delay the onset of symptoms and increase the lifespan for those who are afflicted with ALS, or Lou Gehrig's disease. The researchers have determined that injections of a particular type of protein called heat shock protein (Hsp) 70 may benefit ALS suffers. The study was conducted in the mutant SOD1 mouse model of ALS. When the mice were given treatments of Hsp70, their survival was increased. This study focused on protecting the motor neurons in the mice that had ALS. The injected protein was not detected in the nervous system of the mice. Rather, this treatment appeared to work where the neurons and muscles contact each other. When the neurons and muscles loose contact, muscle weakness occurs, the prominent symptom of ALS. In a second study, the group found that the contact between neurons and muscle was maintained much longer in treated mice as compared to untreated mice. While these studies have shown positive signs, researchers caution that many more studies are needed before they can begin to conduct clinical trials in people. The group has a smaller fragment of the protein that also shown positive effects and is currently being more intensely tested. The group is determining the best way to efficiently produce the proteins to assure they will function properly. This is the first step to develop the protocols for protein production and develop GMP large-scale production strategies that will facilitate IND application and clinical trials. With support from the Blazeman Foundation, Wake Forrest School of Medicine is the only medical center that is engaged in researching this potential ALS treatment.

Thus far we have:

1. Developed protein production, isolation and purification protocols that allow us to isolate recombinant Hsp70 and its substrate binding domain derivative (SBD) with greater than 95% purity.
2. We have completed treating mice with the first dose of SBD and are finishing the first dose of full-length protein cohort. Tissue is being processed to determine effect of treatment on maintaining NMJ innervation.

During the upcoming 15 months we will:

1. Continue to modify our purification protocols to increase the amount of protein we collect without compromising purity. Our goal is to increase yield by two fold.
2. Complete dose-response studies in mice with two different doses of the SDB and full- length protein.
3. Confirm that administered protein reaches its predicted target, i.e., muscle.
4. If the original results are confirmed, we will send the protein to Dr. Terry Heiman-Patterson for independent confirmation of effect.

Below are three series of experiments that will be completed in the Milligan Lab during the continuation of the study:

1. Increase protein yield without compromising purity.

The methods we have developed produce pure and reliable full-length and SBD-proteins. At this point, however, yield/purification is relatively low. Dr. Milligan had discussed this with a colleague at the NEALS meeting last fall and he suggested growing and inducing cells to produce protein at a lower temperature (25 vs. 36^{o}C). In his experience, this modification dramatically increased the yields of his protein production and purifications. We will modify our protocols to determine if this relatively simple alteration will result in significantly increased protein production by bacteria cells. We do not anticipate any change in our purification protocols as the modification will occur prior to purification.

2. Complete dose-response studied of full-length and SBD proteins in the SOD1G93A mouse model of ALS.

Both the SBD and full-length Hsc70 preparations are now being tested in SOD1 mice to determine if treatment reduces/delays early denervation of the neuromuscular junction. The initial experiments are testing the dose that we initially showed to be effective (20 ug/day). To date tissue from 18 animals is being processed and counted to determine potential effects on NMJ innervation. Three animals are completing the injections. Breeding cages are underway to generate the additional animals necessary for the experiments. We are proposing to test at least 3 doses, and 16-20 animals are needed for each dose. We anticipate experiments with the first dose of SBD and full-length protein to be complete within 3-5 months. While we do not anticipate difficulty with these experiments, the time necessary to complete proposed experiments may be extended because of Limited numbers of animals at any one time.

3. Develop an assay to reliably detect if recombinant Hsc70 and/or SBD proteins are present in muscle of treated animals.

"How are we going to determine if the protein gets to "site of action" (i.e., muscle) in patients?" This is a critical question to answer if we are able to proceed to clinical trial. We hypothesize that Hsp70 acts at the NMJ. It will be important to demonstrate localization of the protein. We are proposing to treat with recombinant human protein. Because this is a protein normally expressed, we do not expect to have adverse effects in patients. However, this raises a significant issue with regard to detection because it can be difficult to distinguish the recombinant protein from the naturally produced protein. This is not a trivial question to answer, but essential to answer. Many previous preclinical and clinical trials in ALS have failed to address this issue and therefore it is impossible to attribute negative results to failure of the therapeutic or failure of appropriate delivery. We will begin to develop a reliable method to distinguish between the two. Our initial approach will utilize the His-Hsc70 protein. Mice will be treated with different doses of protein. At 1, 6, 12, 24, and 48 hours muscles will be collected and protein isolated. The isolated muscle protein will be run over a Nickel column to which the His-tag will bind. Bound protein will be eluted and assayed by Western Blot, and 6xHis and human Hsc 70 ELISA to measure concentration of Hsc70. An important aspect of these experiments is that we will determine if we can measure actual, full-length protein and not simply freed or metabolized His tag. Additionally, these experiments will allow us to determine half-life of the protein.

Expectations

Our goal in this project is to conduct sufficient pre-clinical studies whose results, together with those already published will serve as the foundation for an investigational new drug (IND) application from the FDA. While there is no guarantee of FDA approval, we are proceeding with a reasonable expectation that approval will be granted when we can demonstrate efficacy in the mutant SOD1 ALS mouse model, no adverse effects and delivery to expected site of action. Other issues that may contribute to FDA approval are as follows. The protein to be used in treatment is an endogenously expressed protein in human subjects, therefore, significant adverse effects are not expected as might be observed with novel drugs or molecules not usually expressed. Additionally, while we have not yet determined the specific half-life of the protein (see experiment 3 above), like all proteins, ours will be degraded and therefore have a limited time of exposure. If the IND application is approved, the basic science contributions to this project will lessen and the clinical expertise of Dr. Caress and the ALS Clinic will take on a greater role.

The ALS Clinic at Wake Forest participates in many NEALS-initiated clinical trials. As such, the collaborations are in place to help move forward for Hsp70 in a Phase 1 safety trial. If the FDA grants an IND for recombinant Hsc70, Drs. Milligan and Caress will be responsible for submitting grant applications to private foundations and NIH to initiate the Phase 1 clinical trial. We believe that positive results from gradual incremental steps of this project will ultimately provide a foundation for the necessary investment for full scale Phase 3 trial and market production.

Publications

The goal of this project is to develop SOPs and confirm earlier results. Because of potential proprietary methods of protein production, the results, and associated documents in reference to protein production and treatment regimes will not be available for immediate publications. However, the results of treatment of mice with recombinant full-length Hsc70 and the SBD proteins are not proprietary, and we expect that we will be able to publish these results in a timely manner. As experiments will be completed throughout the third year, a publication will not be available until the following year.

Changes in personnel

Effective April 1, 2015, Dr. Mac Robinson will assume the Principal Investigator position for independent government contracts. He will maintain his position in the Department of Neurobiology and Anatomy. His primary role was to develop the protein production and purification protocols. Having completed these tasks, he will no longer devote significant effort to the project. However, he will remain a non-paid consultant and will be available to assist Ms. Forbes should problems arise with the protocols. Dr. Robinson will also remain associated with the project to assist in part with analysis of data, preparation of manuscripts and preparation of the IND application.

We are fortunate to be able to bring on Elizabeth Forbes to assist with the project. As described below, Ms. Forbes has extensive experience and expertise to conduct the proposed experiments. Additionally, she has worked in industry on preclinical studies for the development of novel therapeutics for neurological disorders.