**Everolimus Not Neuroprotective in R6/2 mice**

*Dr. Hersch*

Dr. Steven Hersch and colleagues have investigated the effect of the rapamycin derivative, everolimus, on the R6/2 mice. While there was improvement in rotarod performance and the drug did penetrate the brain, it failed to protect neurons. Everolimus reduced the HD protein in skeletal muscle tissue but not in the brain where it did not activate autophagy.

Everolimus is an inhibitor of mTOR (mammalian target of rapamycin). Inhibiting mTOR has been previously found to lead to an increase in macroautophagy (commonly referred to as autophagy) which is of interest to HD researchers as a possible way to clear away the HD protein which accumulates in cells. Autophagy is a very old cellular house cleaning process found in organisms from yeast to mammals. Damaged parts of the cell, pathogens, and large proteins are surrounded by autophagosomes. The autophagosomes deliver their cargo to the lysosomes by fusing with them. The lysosomes then consume the material.

Everolimus is approved for advanced kidney disease and as an immunosuppressant for transplant patients. The researchers were interested in finding out if this drug would be an effective treatment for Huntington's disease since it is already available for these other purposes.

Everolimus was found to have penetrated the brain as measured by decreased phosphorylation of the mTOR target protein S6 kinase. However, autophagy was not upregulated in the brain as it was in the muscles as measured by normalized levels of the cystolic protein LC3BII which is associated with autophagosomes. Levels of soluble HD protein were reduced in muscle tissue but not in the brain. No signs of neuroprotection were found as measured by brain weight, striatal volume, or striatal neuronal cell body volume. Rotarod performance, a standard measure of motor ability was improved on everolimus. The authors suggest that the positive results in rotarod performance were due to increased autophagy in the muscle tissues. Survival data was not reported.

These results point to the need to closely examine the potential of autophagy as a treatment which has looked promising since a 2004 study by Rubinsztein and colleagues. In that study, aggregate load was reduced and survival time increased in a cell model and in a drosophila model. Improvement was found in rotarod performance, grip strength, the wire maneuver test, and tremors in an N171-82Q HD mouse model. Aggregates were also reduced. However, because the study was conducted in the UK where regulations require the mice to be euthanized before end stage disease, there were no survival data.

In 2010, Rubinsztein and colleagues investigated the effects of rilmenidine on the N171-82Q mice. Rilmenidine, a drug which is FDA approved for hypertension, gets into the brain, has been safe for long term use, and was previously shown in a screening to induce autophagy through an M-tor independent pathway. The researchers found that rilmenidine reduced levels of the solutble HD protein but not aggregates in the brain. Mice treated with the drug had improved grip strength, did better on the wire maneuver

test and had less severe tremors but did not improve on the rotarod test. Again, survival data could not be obtained.

The mouse modes are different in the Rubinsztein studies and in the Hersch study. Rubinsztein's N171-82Q HD mice are slower to develop symptoms and were treated before symptom onset while the R6/2 mice which have rapid onset of symptoms were treated after symptoms began in the Hersch study. The lack of survival time data and the discrepancies between the studies suggest that more questions need to be answered before autophagy inducers go into clinical trials.

Additional research is needed to determine whether autophagy inducers can increase survival time. Also raising concerns is the recent research by Dr. Ana Maria Cuervo and colleagues who found that that cargo recognition was impaired in Huntington's disease and cellular garbage wasn't getting to the lysosomes. Does autophagy clear away the HD protein or is the process itself impaired in Huntington's Disease? If so, at what point in the disease process does the impairment occur? Is there a window of time in which autophagy inducement would be an effective treatment?

We will continue to report on preclinical studies of drugs which induce autophagy as they are published.

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