

## GM1 restores motor function in the YAC128 mouse

Researchers at the University of Alberta and McMaster University have reversed motor symptoms in the YAC128 HD mice by a continuous infusion of the ganglioside GM1 into the ventricles of the brain. Other treatments in the pipeline have delayed onset or slowed progression in the HD mice; this is the first treatment to actually bring about improvement.

GM1 (monosialotetrahexosylganglioside) is a lipid or fat that is normally found in the brain and is part of the outer covering or membrane of neurons. By influencing and interacting with membrane proteins, gangliosides affect the activity of a number of tyrosine kinase and neurotransmitter receptors and ion channels and are involved in cell to cell interaction. GM1 plays an especially important role in cell signaling and cell to cell interaction.

In a 2010 study, Dr. Simonetta Sipione and colleagues from the University of Alberta showed that the synthesis of GM1 is reduced in a variety of models of HD as well as in fibroblasts (cells found in connective tissue) from HD patients. They also showed that striatal cells with reduced GM1 are more vulnerable to apoptosis (programmed cell death) and that restoring levels of GM1 reversed the vulnerability. Administration of GM1 to striatal cells resulted in the activation of the prosurvival kinase AKT. The PI3K/AKT pathway is known to be impaired in HD. They also found that the HD protein experienced increased phosphorylation but exactly how and where could not be determined because of the methodology used. Given these findings and the known importance of GM1 in the brain, restoration of GM1 thus appeared to be a promising therapeutic strategy.



**Simonetta Sipione, PhD**  
Assistant Professor

In the current study, principal investigator Dr. Sipione and her team worked with Dr. Ray Truant and a colleague from McMaster University as well as others to test GM1 as a potential treatment in five month old YAC128 mice. At that point, the mice have marked motor impairment. GM1 was continuously infused into the ventricles of the mice for four weeks. After two weeks, improvement in motor skills began to be noted with a complete reversal of the impairment occurring, as measured by challenging rotarod, ladder climbing and narrow balance beam tests. The improvements lasted an additional two weeks after discontinuation of the treatment but by four weeks, motor skills had declined to pre-treatment levels.

"We didn't expect to see such dramatic changes after administering this therapy," said Dr. Sipione. "We expected to see improvement, but not complete restoration of motor skills. When we saw this, we were jumping with excitement in the lab. This is very promising and should give hope to those with Huntington disease. I think it's a treatment that deserves to go to clinical trials because it could have huge potential."

The researchers found that treatment with GM1 restores normal levels of DARPP-32 (dopamine and cAMP-regulated neuronal phosphoprotein) expression. DARPP-32 is a protein involved in dopamine signaling. Decreased DARPP-32 expression is an early sign of neuronal dysfunction in both the R6/2 and the YAC128 mouse models of HD. The restoration of normal motor behavior in the mice treated with GM1 correlated with the increased striatal expression of DARPP-32.

There are likely other mechanisms involved in the positive results but the primary mechanism is likely to be the phosphorylation of serines 13 and 16 on the HD protein. Research that we covered two years ago identified phosphorylation of these two places on the HD protein as a potential therapeutic strategy.

Phosphorylation is a post-translational (ie, after the protein is made) modification of a protein in which a phosphate group is added to a serine, threonine, or tyrosine residue by a kinase (an enzyme that transfers phosphate groups). Proteins are commonly regulated through phosphorylation.

In a landmark study, Dr. William Yang and colleagues in his lab engineered two types of BAC transgenic (HD) mice and followed them for a year. In one type of HD mouse, serines 13 and 16 were mutated to aspartate. This mimics phosphorylation and essentially makes it efficient and permanent throughout the life of the mouse. In the other type of HD mouse, the serines were mutated to alanine which cannot be phosphorylated.

The mice with the mutation that mimics phosphorylation did not demonstrate the motor and behavioral problems that indicate early neuronal dysfunction nor did they experience neurodegeneration. In addition, no large aggregates were detected as would normally be found. These aggregates are a hallmark of the disease so their absence can be a good sign. However, if the disease process is present and the HD protein begins accumulating in the cells, the aggregated form appears to be less toxic than the soluble form.

"Our study identified a critical molecular switch which lies next to the polyQ mutation in the huntingtin protein," Dr. Yang said. "We were surprised to find that subtle modification of only two serine residues in this very large protein can prevent the onset of disease. This finding suggests an exciting new avenue to develop therapeutics for Huntington's disease."

The current study by Dr. Sipione and colleagues takes a major step forward by finding a brain chemical which phosphorylates serines 13 and 16 in addition to its other actions. "I think it is really exciting to know that there are evidence for chemicals that can boost phosphorylation of the mutant huntingtin at serines 13 and 16 and markedly improve the behavioral deficits in HD mice. This is the first proof-of-concept that such molecular switch could be altered in a beneficial way in vivo by a drug-like molecule." said Dr. Yang about the current study.

## Next Steps

In a study covered two years ago, Dr. Joan Steffan and colleagues raised a concern about phosphorylation as a potential treatment. They speculated that such a treatment would need to be offered early, while the protein clearance machinery is still working at top form since phosphorylation enhances nuclear localization. When the machinery becomes less efficient with aging and exposure to the HD protein, the treatment could actually become harmful by speeding up the accumulation of the HD protein in the nucleus of the cell. This hypothesis is based on research with cell models and drosophila (fruitfly) models of HD and needs to be investigated in mammals. Dr. Yang recommends that a longer study be completed in the YAC128 or BACHD mice to examine the effects of long term administration, see if GM1 administration prevents neurodegeneration and learn whether or not it could exert any harmful effects at a certain point in the disease process.

Dr. Sipione and her group are interested in seeing GM1 go to clinical trials as soon as possible and expect this to happen in the next year or two. They are hoping to do a pilot study at the University of Alberta.

Administration of GM1 would require the use of a brain infusion pump or an intrathecal pump. These devices are available through Medtronics.

GM1 has been in clinical trials for Parkinson's disease for some time. A five year open label study reported a good safety profile and possible benefits. In 2002, a pilot study involving ventricular infusion into the brains of five Alzheimer's patients was published. The patients did well and some benefits were reported.

Dr. Ray Truant's group will be looking for drugs with the safe effect as GM1 but which might be easier to administer. In an interview with the *Hamilton Spectator* Dr. Truant said, "I'm optimistic in that I think there's going to be, in the three-to-five-year timeframe, a major advancement in clinical trials as a result of this," he said. "It may not be GM1, but it may be a drug that acts like GM1."

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- Marsha L. Miller, Ph.D., February 14, 2012