



## **Cannabinoid therapeutic testing of a Friedreich's ataxia mouse model**

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**Dates of project:** 1<sup>st</sup> October 2005 – 3<sup>rd</sup> November 2008

### **Background and aims:**

Medicines that are derived from the cannabis plant are currently being developed by the company GW Pharmaceuticals for the treatment of a number of human diseases. The medicines contain two main compounds, called cannabinoids;  $\Delta$ -9-tetrahydrocannabinol (THC) and cannabidiol (CBD). These two compounds can be mixed in different ratios to give potentially different therapeutic effects. Both cannabinoids have been shown to protect nerve cells from dying in a number of cell culture and animal model studies, including studies of neurodegenerative diseases similar to Friedreich's ataxia (FRDA). However, there is currently no information available on how useful these cannabinoids may be for the treatment of FRDA or other ataxia disorders.

The aims of this PhD project were to examine the potential neuroprotective effects of cannabinoids in Friedreich's ataxia (FRDA) by performing trials on our FRDA mouse model. At the same time, other types of potential FRDA therapy would also be investigated in a similar manner using the FRDA mouse model. Two cannabinoid preparations, with different ratios of the cannabinoid compounds, were provided by GW Pharmaceuticals and tested on the FRDA mice. The "G5" preparation, which has a high cannabidiol ratio (95% CBD: 5% THC) and is considered to be the most useful for neuroprotection, was tested at two concentrations; 10mg/kg and 20mg/kg. The "BDS" preparation, which has a lower cannabidiol ratio (47.5% CBD: 52.5% THC), was tested at one concentration; 20mg/kg.

Additionally, two other types of compounds were tested on the FRDA mice; an antioxidant called CTMIO and two HDAC inhibitors (initially developed by J. Gottesfeld, The Scripps Institute, La Jolla, USA, and currently licensed by Repligen Corporation, Waltham, PA, USA). The HDAC inhibitors inhibit a particular kind of enzyme called histone deacetylase, which may contribute to the "silencing" of the frataxin gene in FA, thereby promoting the disease.

FRDA mice were tested for co-ordination by their ability to balance on a rotating rod (the "rotarod" test). Their movement was assessed by a locomotor activity test and their weight was also monitored. After sacrificing, tissues from the mice were obtained for histological and biochemical analyses.

**Results:**

No overt change in behaviour (neither improvement nor deterioration) was observed in the FRDA mice with any of the cannabinoid treatments. This was reflected by the results of the biochemical analysis, which revealed no improvement in the level of oxidised proteins within brain samples. However, there are further histological or biochemical analyses that we could carry out to look for a perhaps more subtle beneficial effect of the cannabinoids on our FRDA mouse model. To this effect, we have collected and stored fixed and frozen tissue samples that can be analysed in the future.

Treatment with the novel antioxidant CTMIO also did not reveal any overt improvement or deterioration in the behaviour of FRDA mice, or any alterations to the oxidised protein levels.

In preliminary studies, the treatment of mice with HDAC inhibitors did result in positive effects on locomotion. Specific details about the HDAC inhibitor results await the complete analysis of tissues from experimental animals. Mice were treated either orally or subcutaneously with one of two different HDAC inhibitors. Behavioural analysis did not detect any overt beneficial or detrimental effects for one of the HDAC inhibitors when it was given either orally or subcutaneously. However, our preliminary studies using the second HDAC inhibitor show that subcutaneous administration produces a statistically significant beneficial effect on the rotarod coordination performance and the locomotor activity of the FRDA mice. Additionally, the weight gain that is seen in FRDA mice was reduced in mice treated with this compound.

Overall, our results show that none of the cannabinoid treatments, or the CTMIO treatment or treatment with one of the HDAC inhibitors has produced any overt beneficial effect on the FRDA mice. However, the results from the other HDAC inhibitor are very interesting as this is the first time that we have observed such beneficial effects of any compound tested on our FRDA mice. Therefore, these studies will now be pursued further, including the molecular biological, histological and biochemical analysis of fixed and frozen FRDA mouse tissue samples.

**Publications arisen from this project:**

Throughout the 3-year period of this grant, FRDA (and SCA3) mouse model research investigations have been taking place in the Pook lab. These have been partly funded by Ataxia UK and papers arising from the work, which have recently been published in peer-reviewed journals, are listed below (1-6).

Potential future publication of the cannabinoid FRDA mouse model results will be considered after further discussion with GW Pharma. Any publication of the CTMIO results will be considered after consultation with Nuri Gueven, and future publication of the HDAC inhibitor results will be considered after consultation with, and agreement from, Repligen Corporation.

1. Al-Mahdawi, S., Pinto, R.M, Varshney D, Lawrence L, Lowrie MB, Hughes S, Webster Z, Blake J, Cooper JM, King R and **Pook MA**. (2006) GAA repeat expansion mutation mouse models of Friedreich ataxia exhibit

oxidative stress leading to progressive neuronal and cardiac pathology. *Genomics* **88**: 580-590.

2. Clark, R., De Biase, I, Malykhina, A.P., Al-Mahdawi, S., **Pook, M.** and Bidichandani, S. (2007) The GAA triplet-repeat is unstable in the context of the human FXN locus and displays age-dependent expansions in cerebellum and DRG in a transgenic mouse model. *Human Genetics* 120: 633-640.

3. De Biase I., Rasmussen A., Endres D., Al-Mahdawi S., Monticelli A., Coccozza S., **Pook M.** and Bidichandani S.I. (2007) Progressive GAA expansions in dorsal root ganglia of Friedreich ataxia patients. *Ann. Neurol.* 61: 55-60.

4. De Biase, I., Rasmussen, A., Monticelli, A., Al-Mahdawi, S., **Pook, M.**, Coccozza, S. and Bidichandani, S.I., (2007) Somatic instability of the expanded GAA triplet-repeat sequence in Friedreich ataxia progresses throughout life. *Genomics*, 90: 1-5.

5. Al-Mahdawi, S., Pinto, R.M., Ismail, O., Varshney, D., Lymperi, S., Sandi, C., Trabzuni, D. and **Pook, M.** (2008) The Friedreich ataxia GAA repeat expansion mutation induces comparable epigenetic changes in human and transgenic mouse brain and heart tissues. *Hum. Mol. Genet.* 17: 735-746.

6. Chen, X., Tang, T-S., Tu, H., Nelson, O., **Pook, M.**, Hammer, R., Nukina, N. and Bezprozvanny, I. (2008) Deranged calcium signaling and neurodegeneration in spinocerebellar ataxia type 3. *J. Neurosci.* (In Press)

**Conferences/ meetings where this research has been presented:**

The FRDA mouse model research has been publicised by presentations at the 3<sup>rd</sup> International Friedreich's Ataxia Scientific Conference in Bethesda 2006 and the European Society of Genetics Conference in Nice 2007. The results of the cannabinoid testing of our FRDA mouse model have been presented to GW Pharma, and with their agreement, these results were presented by the PhD student, Ricardo Mouro Pinto, at the American Society of Human Genetics San Diego October 2007.

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