

SUMMARY OF FINAL REPORT

Time dependence and dose response of HDAC inhibitors of the pimelic diamide family on the chromatin structure of the frataxin gene

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Background and aims:

This project is aimed at the development of HDAC inhibitors as an effective treatment for Friedreich's ataxia (FRDA). FRDA is caused by the *frataxin* gene, which encodes the essential mitochondrial protein frataxin, being abnormally 'switched off', or repressed.

We recently identified a novel class of histone deacetylase (HDAC) inhibitors that relieve repression of the *frataxin* gene in white blood (lymphoid) cells derived from people with FRDA.

A library of HDAC inhibitors, all based on the structure of the commercially available HDACi BML-210, was developed at Repligen corporation. These small molecules were tested for pharmacokinetic, cell permeability, cytotoxicity and other properties. The most active HDAC inhibitors were identified and tested for cytotoxicity and ability to increase *frataxin* gene expression. From this initial screening a group of 7 lead compounds was selected at Repligen Corporation for further analysis (compounds **44, 106, 109, 123, 136, 526, 531**). One of these molecules, compound **106**, is able to increase frataxin transcription in a mouse model of FRDA.

To gain further understanding of the effects of our HDAC inhibitors, we analyzed the dose and time of exposure necessary to restore function of the *frataxin* gene and the duration of this effect. The experiments were carried out in cells; in a FRDA lymphoblastoid cell line and in FRDA primary lymphocytes. We also investigated the cellular target(s) and specificity of effective HDAC inhibitors.

Results:

The HDAC inhibitors were analyzed for their ability to overcome repression of the *frataxin* gene in primary lymphocytes taken from people with FRDA. The levels of frataxin mRNA and the acetylation state of the gene were measured as indicators of its function (FRDA-causing mutations in the *frataxin* gene are associated with a low level of acetylation whereas higher acetylation levels are associated with a functional gene).

Of the compounds tested, **106** and **109** achieved the highest increase in frataxin mRNA and this was seen at low doses; 5µM for both molecules. As compound **109** was consistently better than **106** at increasing frataxin transcription, it was used in further experiments to determine the timing of induction, the duration of effect and the minimum exposure time required to achieve *frataxin* activation.

Primary lymphocytes from an FRDA donor were treated with 5 and 10 µM **109** for 5 to 46 hours and frataxin mRNA levels were measured as an indicator of the function of the gene. Maximum induction of frataxin mRNA was seen after 46 hours of treatment with 10 µM **109**, and 10 hours of treatment with 5 µM **109** was sufficient to cause a two-fold increase in frataxin mRNA. An increase in the acetylation of the *frataxin* gene was seen after 12 hours of treatment.

In terms of the duration of this response, frataxin mRNA was still detectable at therapeutically significant levels 12 hours after the inhibitor had been removed from the media. Four hours of treatment was sufficient to induce a two-fold increase of frataxin mRNA levels.

In summary, the minimum effective dose of compound **109** for the treatment of FRDA primary lymphocytes is 5µM. The results show that a 10 hour treatment at this dose is necessary to achieve a two-fold increase in *frataxin* transcription; such an increase is considered therapeutically significant for most people with FRDA. To achieve such activation in 10-12 hours, the inhibitor needs to be present for a minimum of 4 hours, and after removal of the inhibitor the effect lasts for 12 hours.

To investigate the cellular targets of effective HDAC inhibitors, two compounds with different HDAC specificity were investigated; compounds **3** and **106**. Both compounds were found to inhibit two types of class I HDACs; HDAC1 and HDAC3. However, compound **3** was more selective for HDAC1 enzyme whilst compound **106** was more selective for HDAC3 enzyme. Only compound **106** was able to induce a therapeutically significant increase of frataxin expression. These results show that HDAC3 has an important role in frataxin repression and targeting this enzyme is crucial to restore expression of the mutant *frataxin* gene.

Lay Summary of results:

The different HDAC inhibitor compounds were analyzed for their ability to 'switch on' the *frataxin* gene, in cells taken from people with FRDA. This was done by measuring the 'message' from the frataxin gene (frataxin mRNA), and assessing the acetylation state of the gene (which is known to decrease when the gene is mutated).

Of the compounds tested, **106** and **109** were the best at switching the frataxin gene back on, although **109** was more effective. Therefore, compound **109** was used in further experiments to determine what dose and timing for delivery of the drug is necessary to achieve maximum activation of the *frataxin* gene. This was done by taking cells from a person with FRDA and treating them with different concentrations of compound **109** for different lengths of time.

The results showed that the minimum effective dose of compound **109** is 5µM and that a 10 hour treatment at this dose is necessary to achieve a two-fold increase in frataxin mRNA. Such an increase is considered therapeutically significant for most people with FRDA and the effects of the inhibitor lasted for over 12 hours.

There are different types, or classes, of HDAC enzymes and the specific targets of HDAC inhibitors that are effective at switching on the *frataxin* gene were investigated. To do this, two HDAC inhibitor compounds targeting different HDAC enzymes were examined; compounds **3** and **106**. Both compounds were found to inhibit two types of HDAC enzyme; HDAC1 and HDAC3. However, compound **3** was more selective for HDAC1 enzyme and compound **106** was more selective for HDAC3 enzyme. Only compound **106** was able to induce a significant increase of frataxin expression, showing that improved therapeutics can be obtained in the future by targeting specifically this enzyme.

Benefits to people with ataxia arisen/likely to arise from this research:

Results from these experiments support the idea that development of HDAC inhibitors of the pimelic diamide family with HDAC3 specificity can lead to new improved therapeutics for Friedreich's ataxia. Specifically, the results are an important contribution to the establishment of a dose regimen for clinical trials that will be performed by Repligen Corporation in the future.

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