Targeted Chaperone Therapy Agents for Gaucher Disease

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Introduction
Defects in lysosomal enzymes have been identified as being associated with a number of genetic diseases. These include mutations within the GBA gene encoding acid beta-glucosidase involved in Gaucher Disease. One of the potential therapeutic routes for treatment of Gaucher Disease is the use of small molecule chaperones that can bind to the misfolded enzyme and provide sufficient levels of conformational correction to allow passage through the QC mechanisms of the Endoplasmic Reticulum (ER) and Golgi apparatus with afflicted cells. Several small molecules have shown promise as molecular chaperones including N-butyl-deoxynojirimycin (NBu, miglustat) a mild inhibitor of glucosidase.

We have developed several small molecule chaperones, based on NBu and its analogs, that can be targeted to the ER and Golgi to improve their activity at the site of processing and degradation of the misfolded protein, potentially increasing the amount of enzyme that can be delivered to the lysosome and allowing increased clearance of unprocessed glucosides in the cells. These new compounds have been shown to increase enzyme activity by up to 60% in fibroblast and immortalized leukocyte patient cell lines including GM4434, GM10870, GM44394 and F0971988 compared to untreated cells.

Changes in enzyme activity were measured using standard lysis based assays as well as live cell analysis utilizing novel cytometric enzyme probes. The efficacy of the new targetted chaperones was also measured by measuring the ability of the compounds to stabilize purified enzyme under sub-optimal thermal conditions as well as by measuring substrate clearance levels of glucosidases in live cells. This work was supported in part by NIH Grant SRA45010372S25-05.

Methods and Materials
• Primary Human Fibroblasts from Gaucher I (GM00372A) patients were obtained from the Coriell Institute for Medical Research.
• Primary Human Fibroblasts from the fibroblast donor (AG06173) and Gaucher I (GM00372A) patients were obtained from the Coriell Institute for Medical Research (Camden, NJ). Cells were cultured in Minimum Essential Medium Eagle supplemented with 10% FCS.

Figure 1. Following drug treatment cells were washed with PBS prior to lysis with buffer at pH 5.2 containing 0.2% sodium lauryl sarcosine. Protein content of lysates was measured by BCA assay and this was used to normalize amount of protein present in assay. Lysates were incubated with 3μM 4-methylumbelliferyl-β-D-glucoside for 2 hours and the reaction stopped by the addition of 400μM nuclease, pH10.8. Fluorescence was measured using Tecan Infinite M200 Pro plate reader and enzyme activity is shown as a percentage of that of non-disease cells cultured.

Live Cell Analysis via Targeted Fluorescent Substrate
• Cells treated with M2091 or M2092 have shown an increase in GCase enzyme activity when compared to untreated cells as demonstrated by live cell staining with specific lysosomal targeted glucosidase enzymatic substrates.

Figure 2. After drug treatment cells were stained with 200nM LysoTracker® Green in PBS and incubated in the dark at 37°C for 1 hour. Staining media was then removed and cells bathed in 10μM M2091® Fluorescent Imaging Buffer containing 20μM Hoechst 33342 and imaged on AMG EVOS Auto FL microscope. The staining intensity of the resulting images was then analyzed using CellProfiler. For flow cytometry analysis (B) cells were reseeded in Stelara™ 10μM Harding and Storung Buffer for Non-Adherent Cells containing 3μM DRAQ7™ and 10,000 singlet events were recorded and the median fluorescence plotted.

Discussion and Conclusions
• The ability of M2091 or M2092 to stabilize purified GCase has been shown by a shift in denaturation temperature of approximately 2°C in thermal shift assays at pH 7.0, demonstrating their ability to bind to the enzyme and protect it from conformational changes.

CONCLUSIONS: Targeting small molecule Pharmacological Chaperones to the ER or Golgi may act to both stabilize GCase under sub-optimal pH and thermal conditions and increase GCase levels or activity in vitro. Gaucher I cells treated with potential new therapeutics may be useful in developing a new method of cellular targeting may represent a new avenue to improve current treatment regimens.

References