Restoration of β-Hexosaminidase A deficiency through the use of molecular chaperones

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Abstract

Tay-Sachs disease (TSD, also known as GM_2-gangliosidosis) is an autosomal-recessive neurodegenerative lysosomal storage disease caused by a mutation in the HEX A gene which codes for the lysosomal enzyme β-hexosaminidase A (Hex A)[7,8]. For patients with TSD, GM_2-gangliosides cannot be properly broken down, and, as a result, accumulate in their neurons, causing severe neurological complications[5].

Currently, there is no cure for TSD. Although all past treatment options have been ineffective, the novel pharmacological chaperone therapy has shown promise as a means for effective treatment by improving the folding and transportation of Hex A. Here, pharmacological chaperone therapy for TSD was investigated using both chemical chaperones and pharmaceutical chaperones. This study set out to reduce the amount of GM2 gangliosides in cells by increasing Hex A activity. To stop the accumulation of GM2 gangliosides, only about 10 percent of enzyme activity is actually needed[2].

This study set out to ameliorate Hex A activity in TSD cells and decrease the GM_2 ganglioside buildup in a TSD derived cell line, GM00502, using four factors. Each of these factors; DMSO, glutamic acid, Pyrimethamine, and a decrease in temperature, have been shown to improve Hex A activity in *in vitro* cell culture models of TSD[3,4]. Pyrimethamine is currently the leading pharmacological chaperone for treating TSD and is currently in its Phase I and II clinical trials[1].

It is important to note that the fate of mutated Hex A enzymes depends on their specific mutation. Of the 74 mutations for Hex A, three mutations (1278ins4, 1421+1G→C, and Gly269Ser) account for *98 percent* of all TSD cases[8]. The human kidney fibroblast cell line, GM00502, used in this study contained two of these mutations (1278ins4 and 1421+1G→C)[6]. The effects of the molecular chaperones outlined in this project have yet to be tested on a cell line with *both* of these major mutations. I hypothesized that temperature reduction, DMSO, glutamic acid, and pyrimethamine would rescue Hex A activity in the TSD derived human kidney fibroblast cell line GM00502.

A study in cytotoxicity was conducted to find the optimum concentration of DMSO, glutamic acid, and Pyrimethamine to treat both GM00502 and HEK293 (control) human kidney fibroblasts. After confirming proposed treatment concentrations were not cytotoxic, a gradient of three concentrations of each factor was then used in the subsequent testing phase. Differences in GM2 ganglioside levels can be quantified through the use of LysoTracker DND-26 staining and fluorescence microscopy. GM00502 TSD derived cells were shown to contain an increased accumulation of GM2 gangliosides when compared to the unafflicted HEK293 control cells. After treating GM00502 cells with DMSO, glutamic acid, Pyrimethamine, and a temperature reduction it was found that all treatments were able to reduce the overall GM2 ganglioside level. Likewise, each treatment was also able to further reduce the GM2 ganglioside levels in HEK293 cells. Of the four factors tested, 250 mM glutamic acid was shown to be the most effective in reducing lysosomal accumulation in HEK293 cells whereas 1.5 ug/mL pyrimethamine was most effective in GM00502 cells.

Fluorescence Microscopy

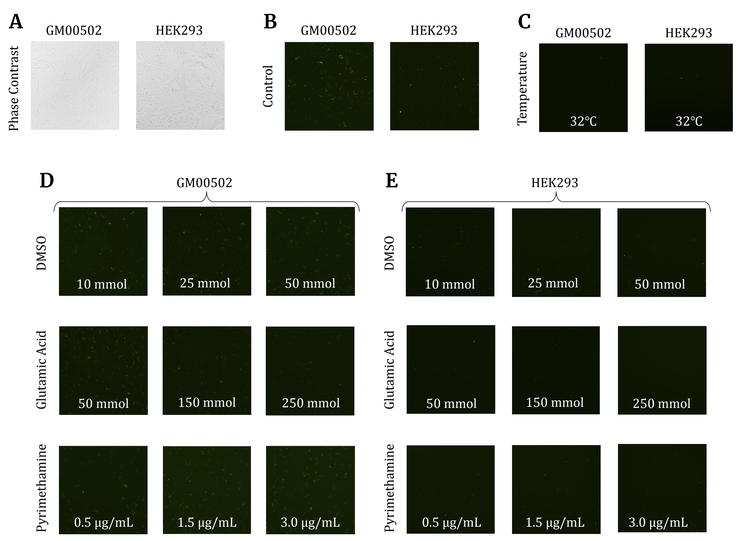

Figure 1: Fluorescent Microscopy. Cells were cultured and treated as described in the methods. Images were captured using a Nikon NIS Element and representative images were reported. **A)** Phase contrast images of control GM00502 and HEK293 cells. **B-E)** Cells were treated and then stained with Lysotracker DND-26. Acidic bodies fluoresce green and their relative fluorescence intensity can be captured. **B)** Representative image of Control GM00502 and HEK293 cells. **C)** Cells were grown at 32℃ for the duration of experiment. **D)** GM00502 cells treated with a gradient of DMSO, glutamic acid, and Pyrimethamine. **E)** HEK293 cells treated with a gradient of DMSO, glutamic acid, and Pyrimethamine.

Question

Can temperature reduction, DMSO, glutamic acid, and pyrimethamine rescue Hex A activity in the TSD derived human kidney fibroblast cell line GM00502?

Results

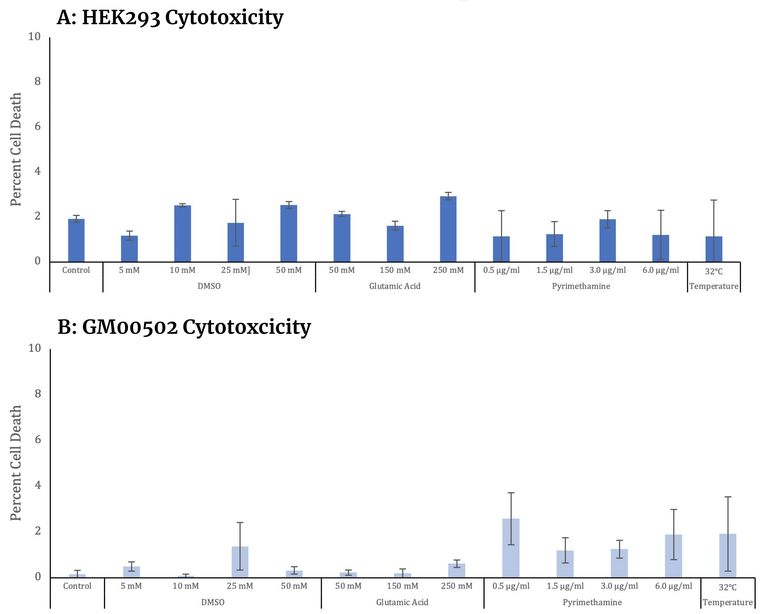

Figure 2: Treatment compounds do not significantly impact cell viability. Total cell count and number of dead cells was quantified using Cell Counter Version 1.2.1. Cell death did not exceed 5% for any reported concentrations. **A)** Cytotoxicity testing of HEK293 cells treated with various concentrations of DMSO, glutamic acid, pyrimethamine and decreased growth temperature. **B)** Cytotoxicity of GM00502 cells treated with various concentrations of DMSO, glutamic acid, pyrimethamine and decreased growth temperature. Values report average cell viability ± SEM (n=3).

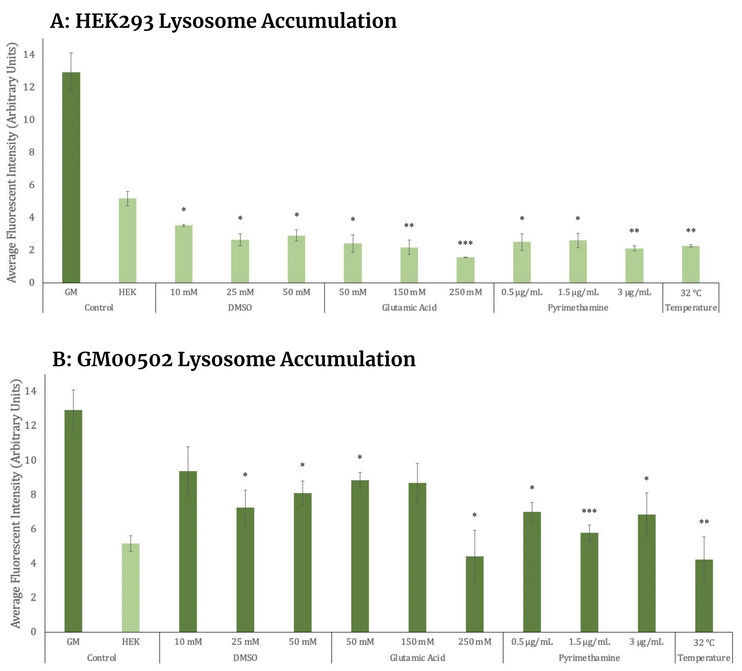

Figure 3: DMSO, glutamic acid, pyrimethamine, and decreased growth temperature ameliorate the accumulation of GM2 gangliosides. Cells were grown and treated as described in the methods. Cells were then stained with Lysotracker DND-26 for visualization of lysosomes by fluorescent microscopy. Relative fluorescent intensity was quantified using NIH Image J software. **A)** Average fluorescent intensity of HEK293 cells treated with various concentrations of DMSO, glutamic acid, pyrimethamine, or a temperature change. **B)** Average fluorescent intensity of GM00502 cells treated with various concentrations of DMSO, glutamic acid, pyrimethamine, and a temperature change. and GM00502 cell lines treated with various concentrations of DMSO, glutamic acid, pyrimethamine and a temperature change. Values report average fluorescent intensity ± SEM (n=3). *p<0.05, **p<0.01, ***p<0.005 compared to HEK293 (A) or GM00502 (B) control cells

Methods

TSD derived human kidney fibroblast GM00502 cells and wild-type human kidney fibroblast HEK293 cells were cultured in Eagle's Minimum Essential Medium supplemented with 15% fetal bovine serum and non-essential amino acids. Cells were seeded in 24-well plates and grown to 70% confluence before exposure to treatment compounds DMSO, glutamic acid, or pyrimethamine for 2.5 hours. Cells treated with a reduction in temperature were seeded in 24-well plates and grown at 32℃ for the duration of the experiment. Following exposure to these experimental factors, treatment media was aspirated, cells were rinsed with PBS, stained with either propidium iodide and Hoechst 33342 nuclear stain or Lysotracker DND-26 and Hoechst 33342 solution for ten minutes, and then rinsed. Cells were then imaged by fluorescent microscopy. Cell cytotoxicity was quantified using Cell Counter Version 1.2.1 (Evan Dexter, ONU), and Lysotracker fluorescent intensity was quantified using NIH Image J.

Model

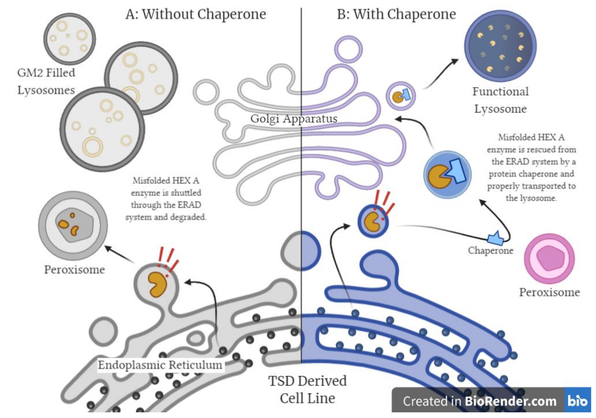

Figure 4: Pathology of TSD and intervention of tested chaperone variables. This illustration depicts the fate of HEX A enzymes with and without protein chaperones. **A)** In a TSD cell line, misfolded HEX A is marked for peroxisomal degradation via the endoplasmic reticulum associated degradation system (ERAD). As a result, GM2 gangliosides accumulate causing a build up of lipid filled lysosomes within the cell. **B)** When a protein chaperone is added, HEX A proteins are able to be transported properly through the golgi apparatus and then to the lysosome where it can degrade GM2 gangliosides. As a result, lysosome accumulation is prevented.

Conclusions

- None of the tested variable concentrations showed significant cytotoxicity.
- In HEK293 cells, all tested variable concentrations significantly decreased lysosomal accumulation. 250 mM glutamic acid appeared the most effective in decreasing lysosomal accumulation ($p<0.005$).
- In GM00502 cells, all tested variables, with the exception of 150 mM glutamic acid and 10 mM DMSO, significantly lowered fluorescent intensity. 1.5 ug/mL pyrimethamine appeared the most effective in decreasing lysosomal accumulation ($p<0.005$).
- This study set out to ameliorate Hex A activity in TSD cells and decrease the GM_2 ganglioside build up in a double mutant TSD derived cell line, GM00502, using four factors. We found that the leading protein chaperone treatment, pyrimethamine, is the most effective treatment for decreasing lysosomal build up in GM00502 cells. In future studies, novel compounds should also be tested in attempts to find an even better treatment option for TSD.

References

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