Acid ceramidase inhibition as a mechanism to treat lysosomal storage disorders

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Background

- Lysosomal storage disorders (LSDs) are complex metabolic disorders characterised by an abnormal build-up of toxic materials within lysosomes as a result of lysosomal enzyme defects and deficiencies[1]. Due to the complex nature of these disorders, there is currently no available treatment for the majority of LSDs.
- Acid ceramidase (ACase) is a lysosomal hydrolase that hydrolyses ceramide into sphingosine and a free fatty acid[2]. However, ACase can also hydrolyse accumulating glycosphingolipids into lyso-glycosphingolipids (Fig. 1), a biomarker of LSDs[3].
- Studies have shown that inhibition or genetic removal of ACase prevents the formation of lyso-glycosphingolipids in Gaucher, Fabry[4] and Krabbe disease[5], thus ameliorating lyso-glycosphingolipid induced pathology.
- ACase therefore makes a promising therapeutic target as a potential treatment for LSDs.

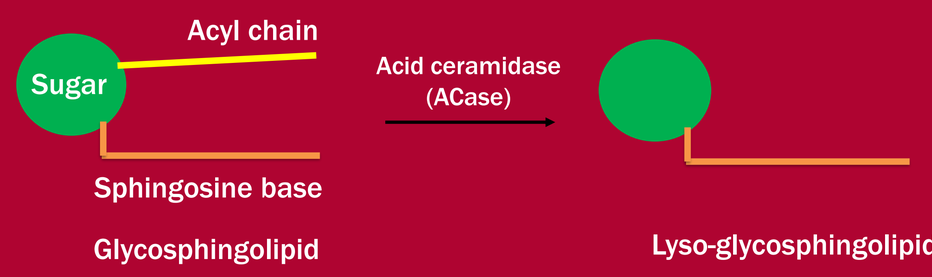

Figure 1. Simplified overview of the ACase mechanism in LSDs. In LSDs, glycosphingolipids accumulate as a result of lysosomal enzyme defect or deficiency. ACase deacylates accumulating glycosphingolipids into lyso-glycosphingolipids, a biomarker of LSDs.

Why a non-covalent inhibitor?

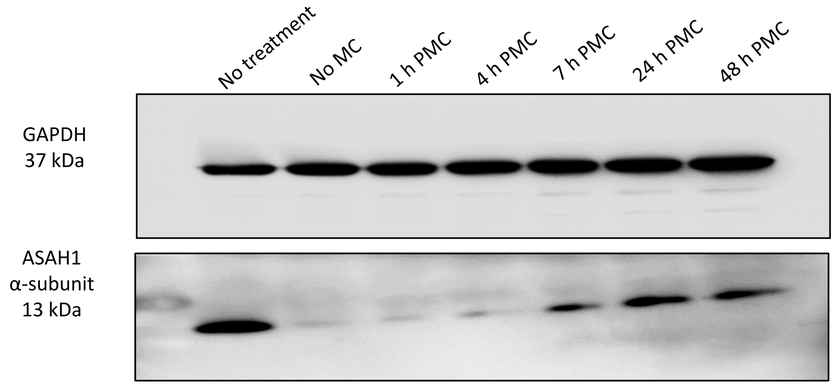

Figure 2. ACase has a turnover time of 24 h. Cells were treated with 15 µM tamoxifen for 24 h. After 24 h, the media was removed and replaced with fresh media. Cells were collected 1, 4, 7, 24 and 48 h post-media change. **Media change (MC). Post-media change (PMC).**

- Over-inhibition of ACase causes the development of Farber disease, an aggressive LSD.
- Our data shows that ACase has a relatively slow turnover time of 24 h (Fig. 2), corroborating our aim to find a non-covalent inhibitor of ACase.

Project aim

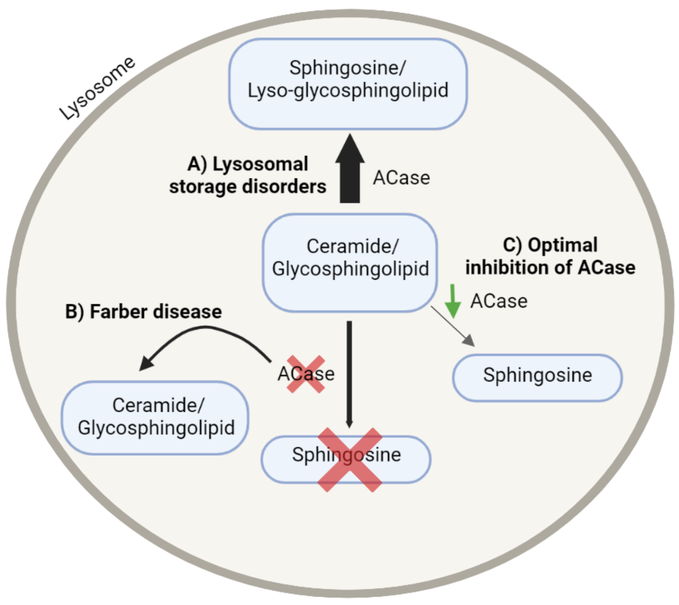

Figure 3. Simplified overview of the overall project aim. Our aim is inhibit ACase enough to alleviate lyso-glycosphingolipid induced pathology, but not enough to induce Farber disease. Image created using BioRender.

Results

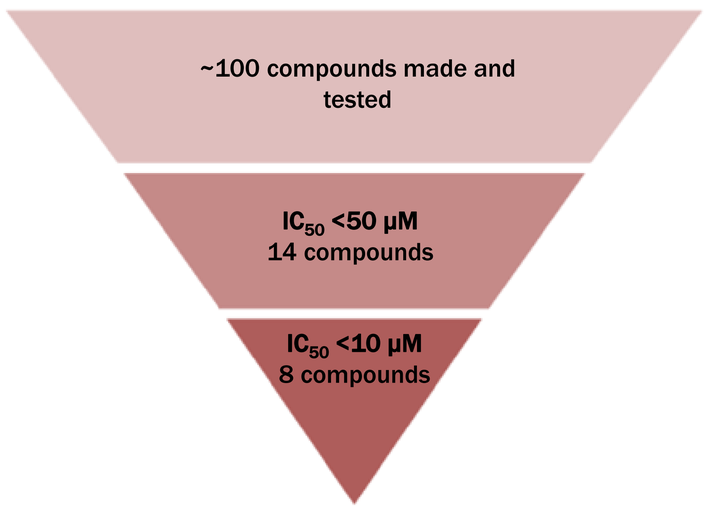

Figure 4. Brief overview of the number of compounds tested, and the number of compounds that have an IC_{50} less than 50 µM and 10 µM.

- A fluorescent-based biochemical assay[6] has been established in-house to measure ACase activity.
- Approximately 100 compounds have been made, and their effectiveness as ACase inhibitors have been tested (Fig. 4).
- 14 and 8 compounds can inhibit ACase activity with an IC_{50} of <50 µM and <10 µM respectively.
- Compound A is our most promising compound with an IC_{50} of 1.4 µM (Fig. 5A).

Compound A: biochemical assays

- With an IC_{50} of 1.4 µM, compound A is our lead compound (Fig. 5A).
- Exploratory enzyme kinetic experiments have showed that compound A is a non-competitive inhibitor of ACase (Fig. 5B).

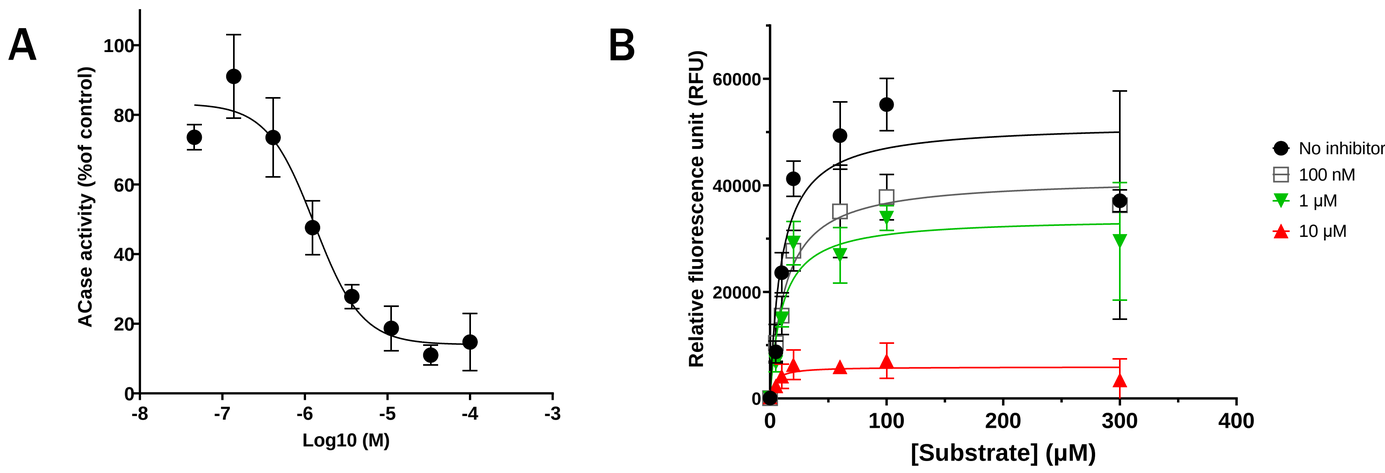

Figure 5. Compound A is an non-competitive inhibitor of ACase with an IC_{50} value of 1.4 µM. (A) Effects of compound A on ACase activity. **(B)** Michaelis-Menten analysis of ACase in the presence of 10 µM, 1 µM and 100 nM of Compound A. Results are presented as mean ± SD (*n* = 3).

Conclusions

- Compound A provides a suitable and promising starting point to find a compound that will inhibit ACase as a mechanism to treat LSDs.

Future directions

- Establish crystallisation conditions for ACase to aid drug design.
- Determine which LSDs are likely to benefit from ACase inhibition.

Compound A: biophysical assay

- A biophysical assay was performed to help our understanding of the interactions between ACase and our compounds at a molecular level.
- Using the WAVE, we found that Compound A binds non-covalently to ACase (Fig. 6).
- Further assay optimisation is needed to obtain association and dissociation rates of Compound A and ACase.

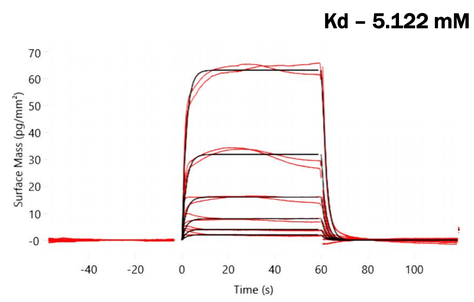

Figure 6. Compound A binds non-covalently to ACase and has quick on/off rates with a Kd of 5.122 mM.

References

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