GT-02329, a structurally targeted allosteric regulator of GCase, restores GCase activity, reduces microgliosis and improves fine locomotor skills in the CBE model of neuronopathic Gaucher's disease

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Objective

To investigate the ability of the structurally targeted allosteric regulator, GT-02329, a candidate for the treatment of neuronopathic Gaucher disease (nGD), to protect against relevant pathophysiological and behavioral features in a chemically-induced mouse model derived from the intraperitoneal (i.p.) injection of conduritol β epoxide (CBE).

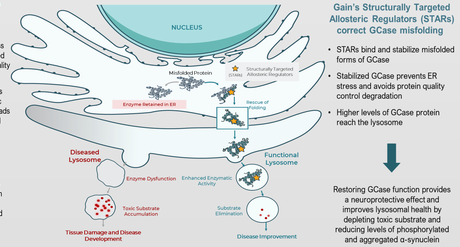
Background

Gaucher disease (GD) is a lysosomal storage disease characterized by deficiency in the glucocerebrosidase (GCase) enzyme encoded by mutated forms of the GBA1 gene. This causes the accumulation of toxic lipid substrates in the liver, spleen and bone marrow, and in nGD type II and type III, also in the nervous system. Current standard of care for GD, namely enzyme replacement therapy, does not cross the blood-brain barrier, leaving an unmet medical need for the development of novel therapies for nGD patients. Gain Therapeutics has applied its proprietary computational drug discovery platform, Site-directed Enzyme Enhancement Therapy (SEE-Tx®), to the development of small-molecule structurally targeted allosteric regulators (STARs) that bind to GCase, stabilize it, and restore its function. SEE-Tx® is a fast and cost-effective solution that has allowed us to discover STARs of the GCase enzyme that are orally bioavailable and brain-penetrant.

Misfolded GCase leads to tissue damage and disease development

- Misfolded GCase causes ER stress and tissue damage, and is targeted for degradation by the protein quality control system in the ER
- GCase protein levels in lysosomes are not sufficient to eliminate toxic substrates (*e.g.* GlcCer), which leads to build-up of phosphorylated and aggregated α-synuclein

Accumulation of toxic substrates and α-synuclein is associated with cell death, including death of dopaminergic neurons, decreased production of dopamine and increased risk of PD



Gain's Structurally Targeted Allosteric Regulators (STARs) correct GCase misfolding

- STARs bind and stabilize misfolded forms of GCase
- Stabilized GCase prevents ER stress and avoids protein quality control degradation
- Higher levels of GCase protein reach the lysosome

Restoring GCase function provides a neuroprotective effect and improves lysosomal health by depleting toxic substrate and reducing levels of phosphorylated and aggregated α-synuclein

CBE is a covalent inhibitor that reacts with the catalytic site of GCase and inactivates the enzyme, causing a reduction in GCase activity. Therefore, CBE represents an optimal and rapid tool to develop nGD-like symptoms in the mouse, since it is crosses the blood-brain barrier and has been shown to replicate important neurological features that occur in nGD patients.

Methods

We have used a nGD model in which C57BL/6 male wild type mice were i.p. injected once daily with CBE (100 mg/kg) for 14 days. Simultaneously, mice were orally treated with GT-02329 at 60 mg/kg q.d. or 90 mg/kg q.d. At day 14, GCase activity and glycosphingolipids were quantified by the 4-methylumbelliferyl-β-D-galactopyranoside assay and ultra high-performance liquid chromatography tandem mass spectrometry, respectively. Neuroinflammation in the brain was assessed by Iba-1 western blot and motor performance by the wire hang test.

Results

We show that Gain Therapeutics' orally bioavailable, brain penetrant candidate molecule GT-02329: (1) restored GCase activity, (2) reduced accumulation of GCase substrates (glucosylceramide and glucosylsphingosine), (3) reduced a marker of microgliosis (Iba-1), and (4) improved fine locomotor skills in this CBE mouse model.

Conclusions

GT-02329 restores GCase activity, depletes accumulation of toxic lipid substrates, reduces neuroinflammation and improves fine locomotor skills in a mouse model of neuronopathic Gaucher disease. These results support the potential of GT-02329 as a disease-modifying, first-in-class, orally available and brain penetrant therapy for the treatment of neuronopathic Gaucher disease.

GT-02329 increases GCase activity and reduces toxic lipid substrates in a nGD mouse model

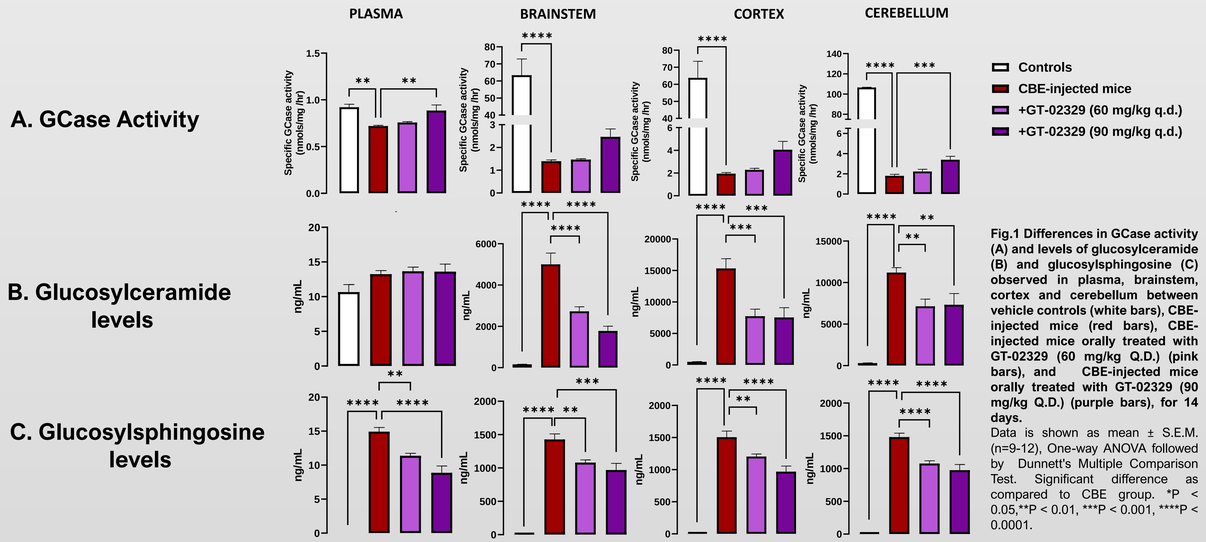


Fig.1 Differences in GCase activity (A) and levels of glucosylceramide (B) and glucosylsphingosine (C) observed in plasma, brainstem, cortex and cerebellum between vehicle controls (white bars), CBE-injected mice (red bars), CBE-injected mice orally treated with GT-02329 (60 mg/kg Q.D.) (pink bars), and CBE-injected mice orally treated with GT-02329 (90 mg/kg Q.D.) (purple bars), for 14 days. Data is shown as mean ± S.E.M. (n=9-12), One-way ANOVA followed by Dunnett's Multiple Comparison Test. Significant difference as compared to CBE group. *P < 0.05,**P < 0.01, ***P < 0.001, ****P < 0.0001.

GT-02329 reduces neuroinflammation in a nGD mouse model

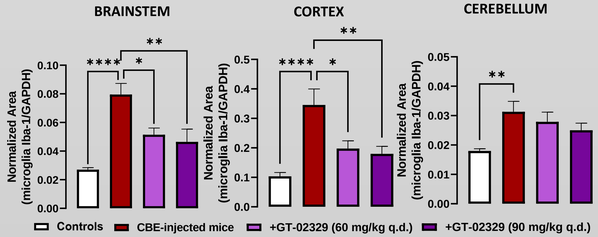


Fig.2 Differences in the levels of Iba-1 – a marker of activated microglia – were observed in the brainstem, cortex and cerebellum between vehicle controls (white bars), CBE-injected mice (red bars), CBE-injected mice orally treated with GT-02329 (60 mg/kg Q.D.) (pink bars), and CBE-injected mice orally treated with GT-02329 (90 mg/kg Q.D.) (purple bars), for 14 days. Data is shown as mean ± S.E.M. (n=10-12), One-way ANOVA followed by Dunnett's Multiple Comparison Test. Significant difference as compared to CBE group. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

GT-02329 improves fine locomotor skills in a nGD mouse model

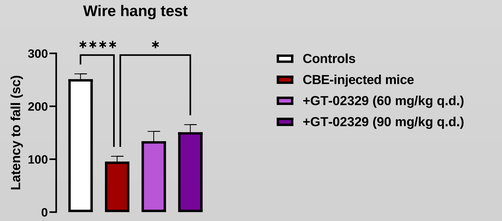


Fig.3 Differences in fine locomotor skills performance in the wire hang test were observed between vehicle controls (white bar), CBE-injected mice (red bar), CBE-injected mice orally treated with GT-02329 (60 mg/kg Q.D.) (pink bar), and CBE-injected mice orally treated with GT-02329 (90 mg/kg Q.D.) (purple bar), for 14 days. Data is shown as mean ± S.E.M. (n=10-12), One-way ANOVA followed by Dunnett's Multiple Comparison Test. Significant difference as compared to CBE group. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

References

1. Vardi et al., (2016). Delineating pathological pathways in a chemically-induced mouse model of Gaucher disease: Characterization of a chemically induced model of Gaucher disease. The Journal of Pathology. 239. 10.1002/path.4751.