GT-02287, a brain-penetrant structurally targeted allosteric regulator for glucocerebrosidase shows evidence of pharmacological efficacy in an animal model of Parkinson's disease

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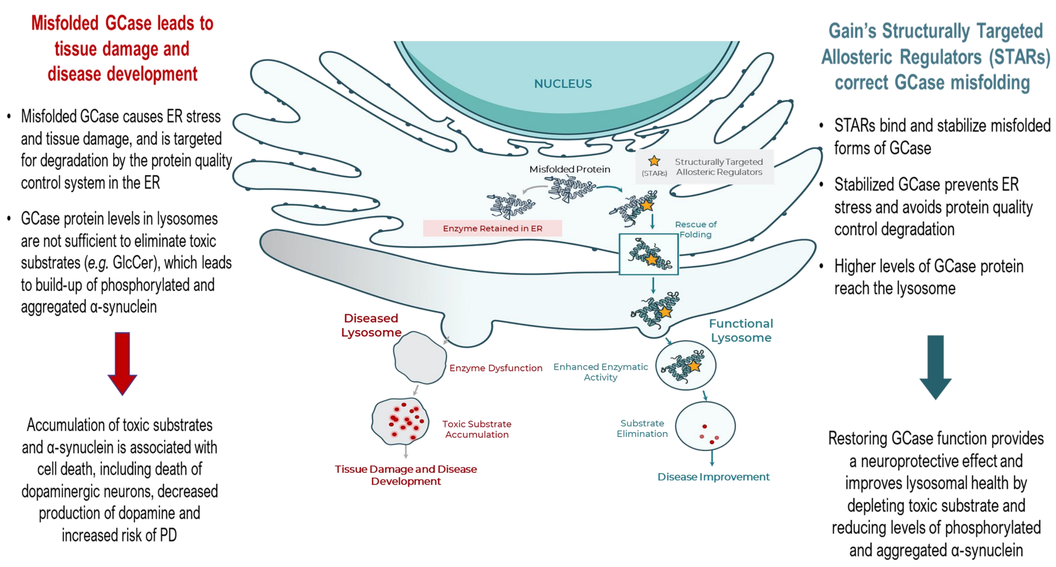
Objective

To investigate the effect of the structurally targeted allosteric regulator and drug candidate for Parkinson's disease (PD), GT-02287, on rotenone-induced neurotoxic effects in a PD animal model.

Background

Mutations in the *GBA1* gene, which encodes the lysosomal enzyme glucocerebrosidase (GCase), represent the most significant genetic risk factor for Parkinson's disease (PD). Misfolded and dysfunctional GCase expressed by mutated GBA1 is linked to impaired lysosomal function and α-synuclein accumulation. Sporadic PD patients also exhibit GCase deficiency linked to α-synuclein and lysosomal pathology, along with ER stress. The rat rotenone model is widely used to model PD. *Rocha et al., 2020*, demonstrated that rotenone administration impairs GCase and alters its glycolipid substrate levels. The rotenone model thus represents an optimal tool to study therapeutic interventions in the context of GBA1 PD pathophysiology. Gain Therapeutics applied its proprietary computational drug discovery platform, SEE-Tx®, to discover GT-02287, a small molecule allosteric GCase modulator.



GT-02287 stabilizes GCase, protects it from degradation, facilitates its trafficking to the lysosome and restores its function.

Methods

Rats were treated with rotenone (2.5 mg/kg, i.p.) and GT-02287 (60 and 90 mg/kg p.o.) once a day (Q.D.) for ten days. Glucosylceramide and glucosylsphingosine levels in the CSF were assessed by UHPLC-MS/MS. GCase protein, LAMP-1, aggregated α-synuclein, TNF-α and tyrosine hydroxylase (TH) levels were assessed by immunostaining and confocal microscopy quantification.

Results

GT-02287 restored GCase levels, improved lysosomal health, reduced aggregated α-synuclein and TNF-α levels, as well as increasing TH immunostaining (a marker of dopaminergic neurons) in the brains of rotenone-injured rats. It also reduced accumulation of toxic GCase substrates glucosylceramide and glucosylsphingosine in the CSF.

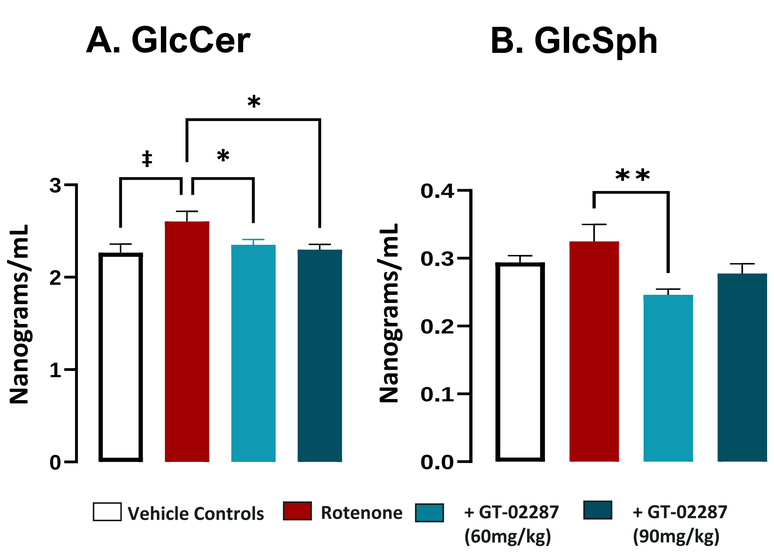


Fig.1 Glucosylceremide (GlcCer) (A) and glucosylsphingosine (GlcSph) (B) levels in the CSF of vehicle controls (white bars), rotenone-injected rats (red bars) and rotenone-injected rats orally treated with GT-02287 at 60 mg/kg Q.D. (light blue bars) and 90 mg/kg Q.D. (dark blue bars) for 10 days. Data is shown as mean ± S.E.M. (n=10-21), One-way ANOVA followed by Dunnett's Multiple Comparison Test. *Significant difference as compared to rotenone group. ‡Significant difference as compared to vehicle control. ‡*P < 0.05, ‡‡**P < 0.01, ‡‡‡***P < 0.001, ‡‡‡‡****P < 0.0001.

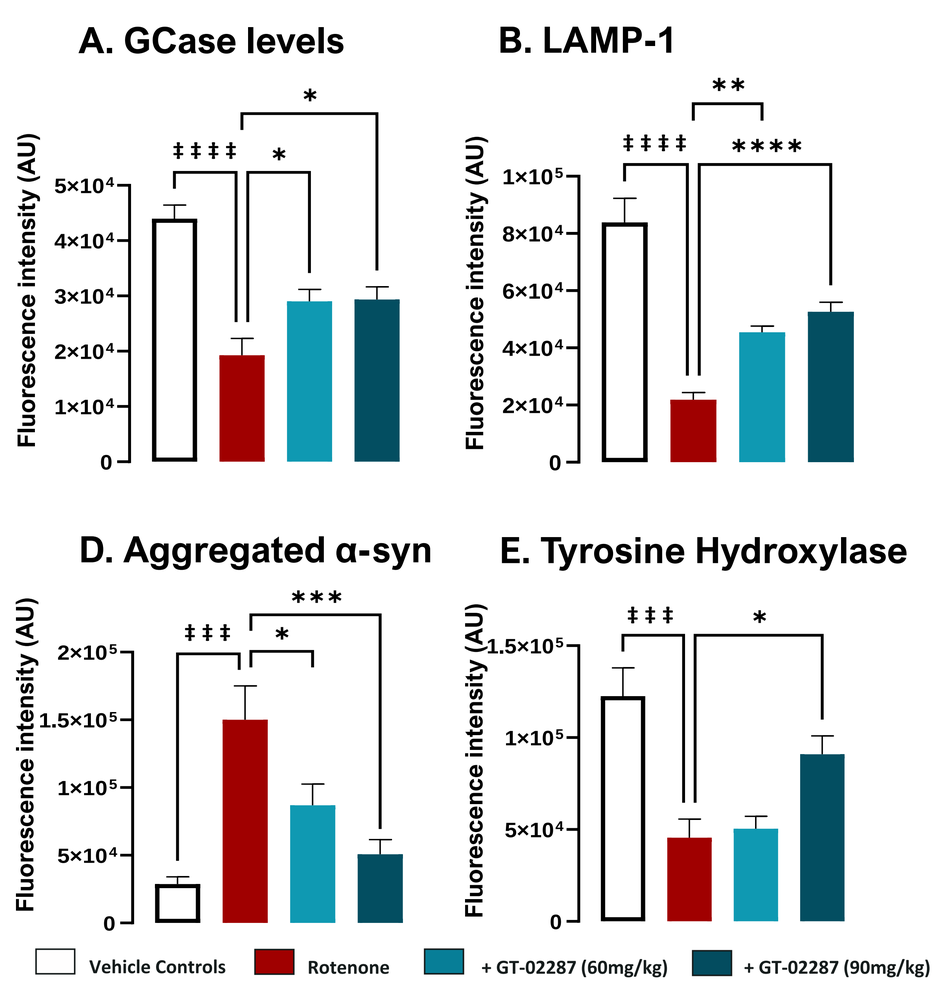


Fig.2 GCase protein (A), LAMP-1 (B), TNF-α (C), aggregated α-synuclein (D) and tyrosine hydroxylase (E) levels in the substantia nigra of vehicle controls (white bars), rotenone-injected rats (red bars) and rotenone-injected rats orally treated with GT-02287 at 60 mg/kg Q.D. (light blue bars) and 90 mg/kg Q.D. (dark blue bars) for 10 days. Data is shown as mean ± S.E.M. (n=6-12), One-way ANOVA followed by Dunnett's Multiple Comparison Test. Significant difference as compared to rotenone group. ‡Significant difference as compared to vehicle control. ‡*P < 0.05, ‡‡**P < 0.01, ‡‡‡***P < 0.001, ‡‡‡‡****P < 0.0001.

Conclusions

Augmentation of GCase function by GT-02287 is reflected in decreased GlcCer and GlcSph levels in the CSF, which leads to protection against key pathophysiological hallmarks of PD, including α-synuclein and lysosomal pathology as well as neuroinflammation, which ultimately leads to the increase of dopaminergic neuronal survival. GT-02287 emerges as a potential disease-modifying orally bioavailable therapy for PD.

References

Rocha EM, De Miranda BR, Castro S, Drolet R, Hatcher NG, Yao L, Smith SM, Keeney MT, Di Maio R, Kofler J, Hastings TG, Greenamyre JT. LRRK2 inhibition prevents endolysosomal deficits seen in human Parkinson's disease. Neurobiol Dis. 2020 Feb;134:104626. doi: 10.1016/j.nbd.2019.104626. Epub 2019 Oct 13. PMID: 31618685; PMCID: PMC7345850.