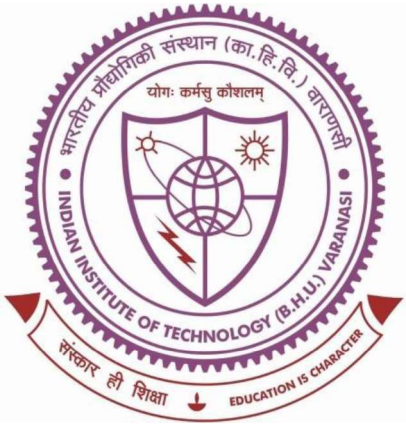
Glucocerebrosidase Chaperone as a Treatment Strategy for Parkinson's disease



Thesis submitted in partial fulfilment for the Award of Degree

Doctor of Philosophy

By

Pratigya Tripathi

DEPARTMENT OF PHARMACEUTICAL ENGINEERING & TECHNOLOGYINDIAN INSTITUTE OF TECHNOLOGY (BANARAS HINDU UNIVERSITY)
VARANASI– 221005
INDIA

Roll No. 17161008 **2023**

Chapter 7

Summary & Scope for Further Work

7 Summary

These studies give the 3D-structural insight of the rGCase and contributed to understanding the interactions between rGCase and chaperone and its mechanism. Swiss models were used to predict 3D models of apo rGCase at neutral and acidic pH. pH-dependent docking and MD simulation studies used to understand the nature of interactions between rGCase and chaperones. These studies revealed that chaperone made a stable complex with rGCase residues TRP 198, TYR 263, GLN 266, TYR 331, GLU 358, and TRP 399 at the ER pH 7.0 than at lysosomal acidic pH 4.5. Consistent with *in silico* studies, *in vitro* studies showed higher inhibitory activity and binding affinity of chaperone at pH 7.0 than pH 4.5. Novel chaperones with possible GCase stabilizing activity have been identified with a built rat 3D model since AMB is used at greater doses in preclinical and clinical research for PD treatment due to issue in the CNS penetration ability. From the *in silico* and associated analyses, we were able to select four compounds (GC466, GC519, GC329, and GC607) with appropriate BBB penetration, drug-likeness properties, and GCase stabilization abilities. Chaperones stabilize GCase due to the made a stable complex with rGCase residues TRP 198, TYR 263, GLN 266, TYR 331, GLU 358, and TRP 399. Only one of them, GC466, has been shown by *in vitro* TDA and enzyme kinetics assay to have better chaperoning ability and binding affinity towards GCase compared with others. It required 6-fold (60 μM) and 15-fold (150 μM) less concentration than those of GC519 and GC329 to stabilize the GCase under *in vitro* TDA, respectively. Similarly, GC466's binding affinity (K_i) for GCase was 6.5 times stronger and required just 0.64 ± 0.2 μM to stabilize the enzyme, compared to the second most active compound GC519 (4.17 ± 3.4 μM) evaluated in the *in vitro* enzyme kinetic assay. MD modeling accurately anticipated this GCase-GC466 complex stability and was

further verified by *in vitro* CD, FT-IR, and Raman studies. All these properties led to the emergence of the GC466 have the potential rGCase stabilizing capacity. GC466 was also found to have neuroprotective action, GCase potentiating activity, and ROS scavenging capabilities in the cell line investigation. In *in-vivo* 6-OHDA-induced PD model, GC466 showed an anti-PD effect by enhancing GCase activity and its related cell protective mechanisms, such as the suppressing ER-stress mediated apoptotic pathways and inhibiting α-syn oligomerization. To monitor the pharmacokinetic parameters and brain penetration ability of GC466, HPLC method was meticulously developed. Through this research endeavor, we sought to assess the plasma pharmacokinetics and the extent to which GC466 traverses the blood-brain barrier after oral administration at a dose of 25 mg/kg. Remarkably, our research stands as the first to demonstrate the successful and efficient traversal of the blood-brain barrier by GCase chaperone, swiftly reaching target regions within the brain, namely, the CSF, ST, and SNp. Overall outcomes of the thesis work have been summarized as given below:

- 3D-model of rat glucocerebrosidase was developed.
- GC466 was identified as the ideal rGCase chaperone.
- Asp146, Phe265, His329, and Tyr331 residues play a vital role in the GC466-rGCase complex stabilization.
- GC466 shows anti-PD activity in SHSY-5Y cell lines because it enhanced GCase and ROS scavenging activities
- LD_{50} of GC466 in acute toxicity study was found to be 500 mg/kg *b.w.*
- Histological analysis showed the absence of any indications of organ toxicity in rats at doses as high as 300 mg/kg.
- GCase dysfunction may triggers PD through ERS-induced apoptotic (Caspases-12/3 and PERK/CHOP) pathways.

- GC466 blocks apoptotic cell death and improves impaired GCase activity in PD rats.
- GC466 required approximately 129 to 144 minutes to cross the brain.
- 50-60% of the drug's exposure in the plasma is also observed in the brain striatum and nigra tissues by GC466, suggesting good brain penetration.

7.1 Scope for Further Work

- ✓ This study would facilitate the screening and designing of chaperones at an early stage of drug discovery to improve the treatment strategies of PD.
- ✓ This can be an effective screening method for the evaluations of chaperones for new drug discovery.
- ✓ Further, sub-acute or sub-chronic toxicity study is to be performed to evaluate the long –term toxicity profile of chaperone.
- ✓ This research endeavor holds tremendous promise in elucidating the intricate molecular mechanisms underlying the pathogenesis of PD associated with GCase.