Chapter 2

Hypothesis & Objectives

2.1. Major Objectives

Two broad objectives of the current study are:

2.1.1. Target validation for GCase in non-genetic PD Model

2.1.2. Investigate the potential of some drugs as GCase activators for possible disease-modifying effects in PD

Unilateral intrastriatal injection of 6-OHDA in rats is an established and well-validated experimental model of PD (Kirik et al., 1998). Being structurally similar to DA, 6-OHDA binds to DA transporters and transported into DA neurons. Auto-oxidation of 6-OHDA is responsible for generation of highly toxic hydrogen peroxide (H_2O_2), paraquinone, and ROS leading to oxidative stress which is toxic to mitochondria. Oxidative stress is observed with nuclear translocation of Nrf2 (Imaizumi et al., 2012), which regulates antioxidant system including GSH, SOD and CAT (Joshi and A Johnson, 2012). Nrf2 not only leads to α-synuclein degradation through the ubiquitin-proteasome system (UPS) (Lastres-Becker et al., 2012), but also takes part in mitochondrial biogenesis (Tufekci et al., 2011). Besides, 6-OHDA directly inhibits the mitochondrial complex-I. This is followed by the impairment in mitochondrial complex enzyme activities and respiration. Ultimately, there is impairment in mitochondrial bioenergetics and function (Blum et al., 2001). During normal physiology, autophagy is responsible for the lysosome-mediated degradation of damaged proteins and organelles as an essential process to maintain cellular homeostasis and function (Jing and Lim, 2012). Due to 6-OHDA toxicity, the number of dysfunctional mitochondria is increased considerably (Kupsch et al., 2014), which overwhelms the capacity of lysosomal autophagy system to meet the

demand (Marin and Aguilar, 2011). Therefore, it may cause burden on the lysosomal autophagy system and compromised its capacity. GCase, being lysosomal enzyme may also get affected. It is reported that loss of mitochondrial function results into GCase deficiency (Gegg et al., 2012). The relationship is bidirectional as GCase deficiency in lysosomes is reported to directly inhibit autophagy, causing the impairment in degradation of damaged mitochondria, which leads to accumulation of dysfunctional mitochondria (Marin and Aguilar, 2011; Osellame et al., 2013).

GCase deficiency also causes accumulation of α-synuclein toxic oligomers in lysosomes by decreasing its lysosomal autophagy-dependent degradation (Cleeter et al., 2013; Mazzulli et al., 2011). α-synuclein aggregates may further impair the trafficking of wild-type GCase to the lysosome (Mazzulli et al., 2011; Yap et al., 2011). α-synuclein is also reported to inhibit mitochondrial protein import in PD, causing impairment in mitochondrial function (Di Maio et al., 2016). Mitochondrial dysfunction leads to the release of cytochrome-C and activation of caspase-9 and caspase-3, causing apoptosis (Blum et al., 2001). It is accompanied by nigral TH deficiency, as well as striatal DA and DAT reduction, which leads to motor deficits in PD (Chotibut et al., 2012). By following positive feed-back loop, this vicious cycle continues and leads to dopaminergic cell death as shown in **Figure 2.1.** The role of 6-OHDA, ambroxol and rebamipide are observed and discussed separately.

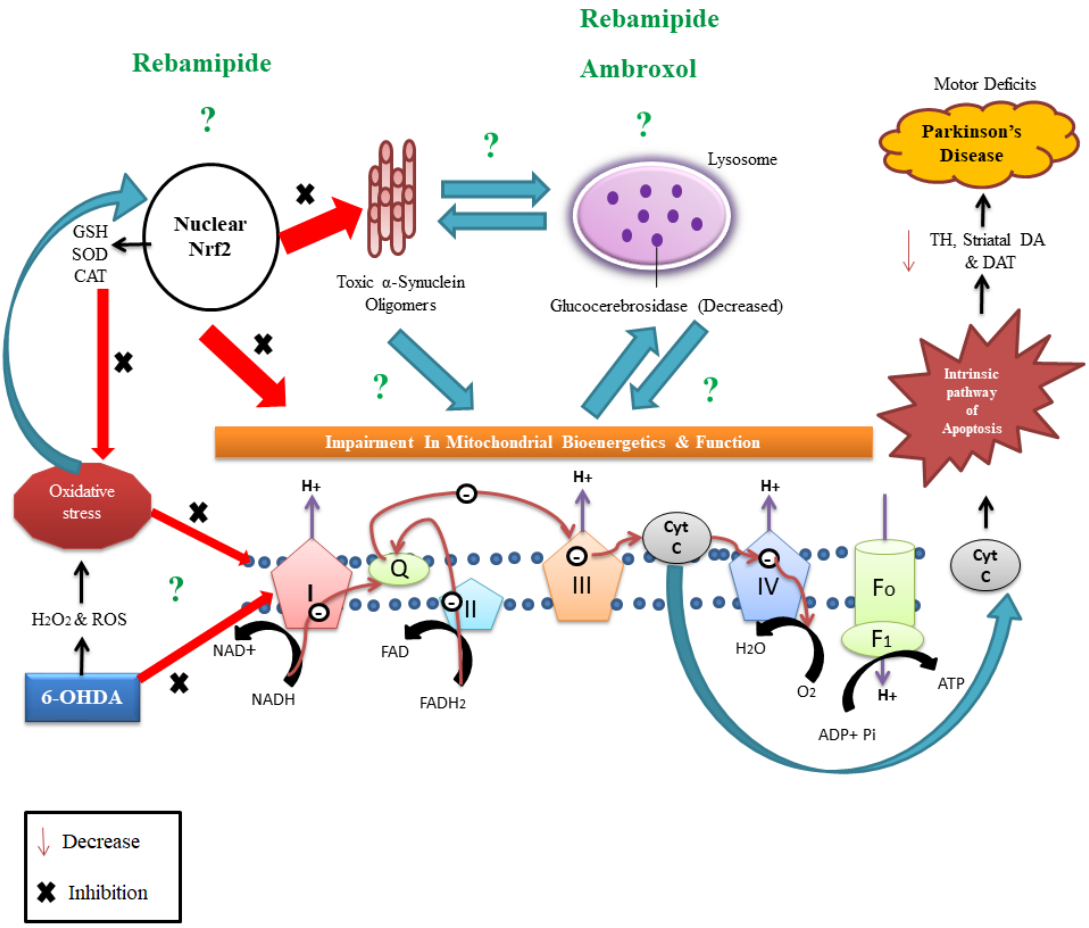

Figure 2.1 The schematic diagram of the hypothesis for the thesis work.
Briefly, 6-OHDA could induce dopaminergic cell death either by direct inhibition of mitochondrial respiratory chain or undergo oxidation process to generate ROS and H_2O_2 leading to oxidative stress. This leads to impair mitochondrial complex enzyme activities and respiration. Oxidative stress also causes nuclear translocation of Nrf2 which regulate antioxidant system including GSH, SOD and CAT. Nrf2 takes part in α-synuclein degradation and regulation of mitochondrial biogenesis. However, mitochondrial dysfunction leads to GCase deficiency which further increases the aggregation of α-synuclein oligomers. Toxic α-synuclein aggregates not only take part in GCase deficiency, but also impair mitochondria function. Additionally, reduction in GCase is one of the reasons for mitochondrial impairment. Mitochondrial dysfunction is responsible for the release of cytochrome C, followed by apoptosis and deficiency of TH, DAT and DA in nigrostriatal pathway. These pathophysiological changes are responsible for behavioral deficits in PD. Effects of 6-OHDA on GCase activity is observed. The role of ambroxol (oral) and rebamipide (oral and novel formulated transdermal patches) is evaluated on 6-OHDA-induced vicious cycle of dopaminergic cell death.

2.2. Specific Objectives

On the basis of **hypothesis (Figure 2.1),** following are the specific objectives of the present study:

2.2.1. Effects of ambroxol and rebamipide on GCase activity *in vitro*

2.2.2. (a) Validation of GCase as a target in 6-OHDA-induced model of PD in rats

(b) Assessing the effects of sub-acute administration of ambroxol in 6-OHDA-induced model of PD in rats

2.2.3. Evaluation of rebamipide in sub-acute doses for its action against 6-OHDA-induced toxicity in rats

2.2.4. To observe the role of nuclear factor erythroid 2-related factor 2 (Nrf2) activity in rebamipide-mediated changes against 6-OHDA toxicity in rats

2.2.5. To assess the sub-chronic dose of ambroxol for neurorestorative effects against 6-OHDA-induced model of PD in rats

2.2.6. Evaluation of sub-chronic administration of rebamipide for disease-modifying effects against 6-OHDA-induced model of PD in rats

2.2.7. Design, characterization and evaluation of transdermal patches of rebamipide in rodent model of PD