Abstract

Parkinson's Disease (PD) is the second most common neurodegenerative disorder at older ages and is characterised by the loss of dopaminergic neuronal cells and the deposition of protein inclusions called Lewy bodies that are mainly composed of the protein alpha-synuclein (αS) and other molecules such as lipids. αS has been proposed to interact with lipid membranes as part of its functional role but this interaction was also found to initiate the formation of amyloid fibrils that resemble those found in the brain of patients. Moreover, mutations in the *GBA* gene, that encodes the enzyme Glucocerebrosidase (GCase), is the most important risk factor for PD and can lead to alterations in lipid membrane composition and increased levels of aggregated αS in cell cultures and *in vivo*.

The overall aim of the research described in this project was to test the effects of introduction of PD risk factors such as GCase deficiencies and oxidative stress (OS) in a human neuroblastoma cell line, SH-SY5Y, in order to gain knowledge regarding PD disease progression. The approach for introducing PD risk factors was to knock down the expression of GCase enzyme with shRNA *GBA* silencing (*GBA* KD), inhibition of the GCase enzyme with conduritol β epoxide (CBE) and induction of OS in the cells by treating them with two widely used chemicals for such purposes: 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenylpyridinium (MPP+). The cellular effects of introduction of PD risk factors were characterised by measuring GCase expression levels and activity, αS expression, lipid profile determined with lipidomics and by incubating cell extracted lipids with αS and Thioflavin T (ThT) to study αS aggregation propensity.

In order to test the hypothesis that αS accumulate and that altered lipid profile, caused by introduction of PD risk factors, affect αS aggregation, a valid cell model had to be developed. A protocol for reproducibly generating differentiated SH-SY5Y cells, consistently showing dopaminergic and neuronal phenotype, was developed. *GBA* KD in differentiated SH-SY5Y cells led 50% reduction of GCase protein and activity level compared to wild-type (WT) and scramble control (SCR) cells. *GBA* KD also led to increased C-34:C-36 hexosylceramide (HexCer) acyl chain length ratio, which was reverted when GCase activity was restored to same level as WT and SCR with Ambroxol. A significant inverse correlation between C-34:C-36 HexCer ratio and GCase activity was proposed. CBE treatment of differentiated SH-SY5Y cells led to 95% reduction of GCase activity, accumulation of αS and glucosylceramide (GluCer), and lipids from these cells proved to accelerate αS aggregation compared to dimethyl sylfoxide (DMSO) control both for whole cells and isolated lysosomes. Finally, induction of OS with 6-OHDA led to increased levels of GCase enzyme, GluCer, reduced levels of C-36 HexCer and accelerated αS aggregation for differentiated and 6-OHDA treated *GBA* KD cells compared to WT and *GBA* KD untreated controls. Similar trend was observed for MPP+ treated *GBA* KD cells, where OS led to reduced respiratory function, accumulation of αS, GluCer and sphingolipids and accelerated αS aggregation compared to all control conditions.

The research presented in this dissertation contribute to a better understanding of the mechanisms leading to αS aggregation in PD.