Loss of GBA activity exacerbates the toxicity of alpha-synuclein oligomers and protofibrils in an in vitro model of Parkinson's disease.

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Introduction

Parkinson’s disease is usually diagnosed at 60 years of age and symptoms include resting tremor and bradykinesia, caused by the degeneration of the nigrostriatal dopaminergic pathways. We know that accumulation of misfolded alpha-synuclein (α-syn), forming lewy-bodies, causes mitochondrial stress and impairs autophagy lysosomal pathway in Parkinson’s disease. Furthermore, mutations on the gene coding for the lysosomal protein GBA is a strong risk factor for Parkinson’s disease. Preclinical models of Parkinson’s disease often rely on one insult despite the multifactorial pathophysiology of this disease. Here, we developed and characterized a novel in vitro model of Parkinson’s disease, based on α-syn toxicity and GBA-linked lysosomal dysfunctions.

Methods

All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and followed current European Union regulations (Directive 2010/63/EU). Agreement number: B1301310.

Primary culture of mesencephalic neurons:

Rat dopaminergic neurons were cultured as described by Visanji et al., 2008 and Callizot et al., 2019. Briefly, the midbrains obtained from 15-day-old rat embryos (Janvier, France) were dissected under a microscope. The ventral portion of the mesencephalic flexure, a region of the developing brain rich in dopaminergic neurons, was used for the cell preparations. The midbrains were dissociated by trypsinization for 20 min at 37 ºC and were seeded at a density of 40,000 cells/well in 96 well-plates (pre-coated with poly-L-lysine) and maintained in a humidified incubator at 37 ºC in 5 % CO2/95 % air atmosphere. Half of the medium was changed every 2 days with fresh medium.

Pharmacological treatment:

On day 6 of culture, culturitB Epoxido (CBE, 20 µM) a covalent inhibitor of glucocerebrosidase enzyme (GBA) was added to the culture for 1 h before the α-syn injury. For the α-syn injury, cells were treated with an α-syn solution at 250 nM containing oligomers and protofibrils from 24 hours to 96 hours. The injury was renewed on day 8 for an additional 48 hours for the cells treated for 96 hours. Ambroxol was applied to the cells at 100 nM 1 hour before the CBE injury and 2 hours before the α-syn injury.

Staining of dopaminergic neurons and automatic microscopic analysis:

After injury, neurons were fixed with PFA (4 % solution). The cells were incubated with a) monoclonal anti-Tyrosine Hydroxylase (TH) antibody produced in mouse b) polyclonal anti-Lamp2 antibody produced in rabbit. For each condition 20 pictures were automatically taken using MetaXpress® (Molecular Devices) at 10x magnification. From images, analysis was directly and automatically performed by MetaXpress® (Molecular Devices). Analysis of total number of TH neurons, total neurite network of TH neurons and area of Lamp2 vesicles in TH neurons were performed.

Results

Reduced activity of lysosomal GBA exacerbates the toxicity of alpha-synuclein on primary dopaminergic neurons

Ambroxol hydrochloride, a molecular chaperon for GBA was able to reduce neuronal loss and lysosomal accumulation

Conclusions

- Toxicity after the application of α-syn oligomers was observed after 72 h with lysosomal accumulation. After 96 h of application neuronal death and neurite network loss were showed.
- After a combined application of CBE, a covalent inhibitor of GBA and α-syn oligomers and protofibrils, a strong accumulation of lysosomes in dopaminergic neurons was observed after only 48 h of injury.
- Moreover, loss of dopaminergic neurons was observed after 72 h of combined injuries. These results showed that toxicity of α-syn oligomers and protofibrils on dopaminergic neurons was exacerbated by the inhibition of GBA, suggesting that lysosomal dysfunction increases α-syn toxicity.