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 imparairs 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 °C 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, conduritol B epoxide (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 αsyn injury, cells were treated with an αsyn solution at 250 nM containing oliomers 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 αsyn 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 ImageXpress® (Molecular Devices) at 10x magnification. From images, analysis were 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 exacerbate the toxicity of alpha-synuclein on primary dopaminergic neurons

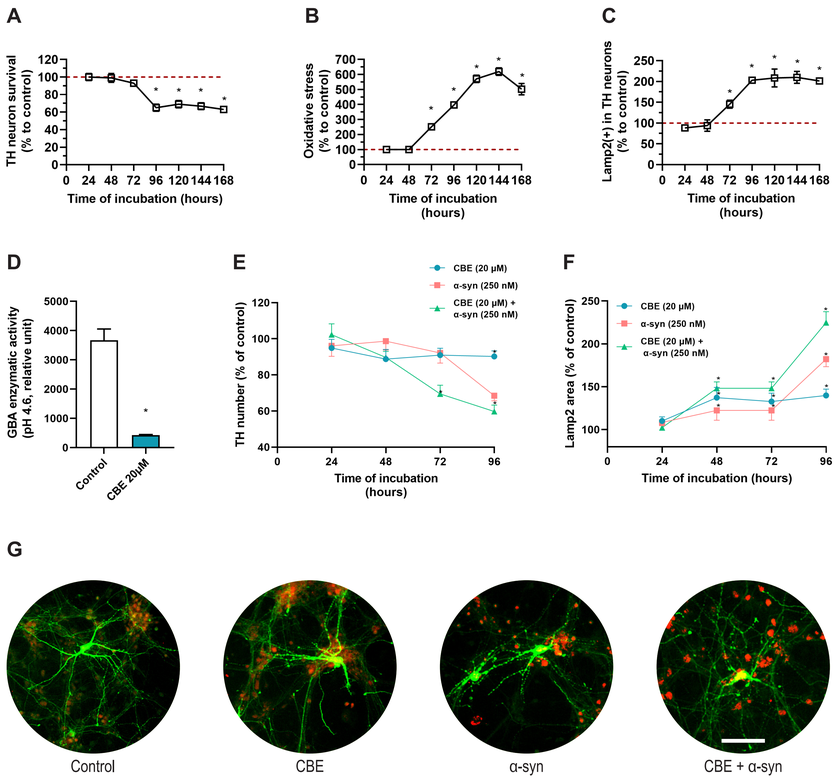

Figure 1: Kinetic of toxicity after injury with CBE, α-syn or α-syn + CBE
Toxic effect of α-syn on survival of primary dopaminergic neurons (A), on mitochondrial dysfunction and oxidative stress (B) and on lysosomal stress (C). (D) Enzymatic GBA activity in mesencephalic cells following CBE application, (E) survival of dopaminergic neurons, (F) lysosomal burden after injury with α-syn, CBE or a combined application of α-syn and CBE and (G) representative pictures of TH neurons in control, α-syn, CBE and α-syn + CBE conditions (72 h of application), TH in green and Lamp2 in red, scale bar: 100 µm.

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.

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

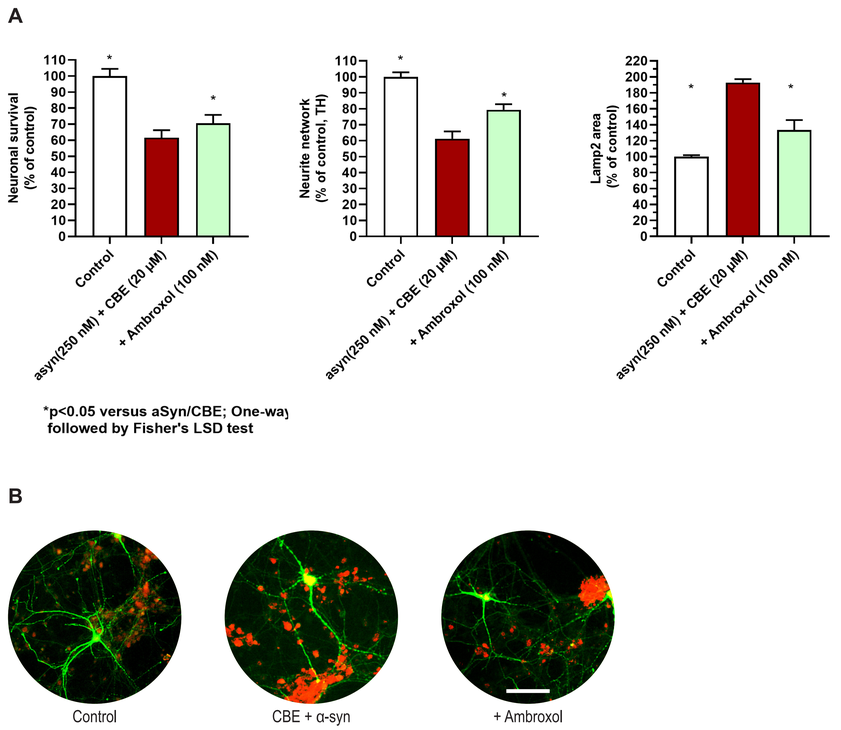

Figure 2: Effect of Ambroxol, a lysosomal chaperon of GBA, on neuronal survival, neurite integrity and lysosomal accumulation
(A) Survival of TH, neurite network and lysosomal accumulation after Ambroxol treatment on cells injured with CBE + α-syn for 96 hours and (B) representative pictures of TH neurons in control, a-syn + CBE conditions with or without Ambroxol treatment, TH in green and Lamp2 in red, scale bar: 100 µm.