Impairment of lysosomal function contributes to synucleinopathy and neurodegeneration in *in vitro* models of Parkinson's disease

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INTRODUCTION

Motor symptoms in Parkinson's disease are caused by the degeneration of the dopaminergic signal in the substantia nigra. The loss of dopaminergic neurons is due to mitochondrial impairments and alpha-synuclein (aSyn) aggregation, which are pathological hallmarks in Parkinson's disease.
Multiple lines of evidence suggest that lysosomes are key for the degradation of alpha-synuclein in dopaminergic neurons. Mutations on the gene coding for the lysosomal protein GBA1 is a strong risk factor for Parkinson's disease.
Here, we have investigated lysosomal function in *in vitro* models of Parkinson's disease (induced with MPP+, a mitochondrial toxin; or with aSyn oligomers). In a second step, we addressed whether an impairment of the lysosomal activity (with conduritol B epoxide, inhibitor of GBA1) can directly lead to the loss of dopaminergic neurons.

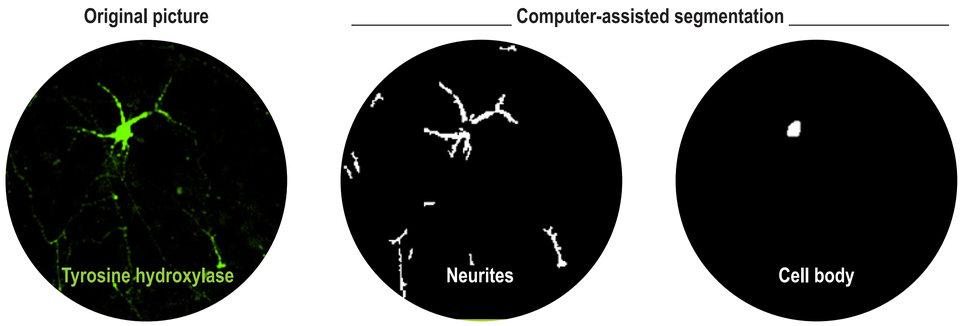
METHODS

Primary culture: Mesencephalic neurons (including dopaminergic neurons) were cultured as described by Visanji *et al.*, 2008 with modifications. Cells were seeded at 40,000 (E15) cells/well in pre-coated PLL 96-well plates maintained at 37 °C.

Injuries: On day 6, mesencephalic neurons were injured with the mitochondrial toxin MPP+ (4 µM, for 48 h) with the lysosomal toxin CBE (50 to 400 µM, for 48 h) or with aSyn oligomers (250 nM, for 96 h, Neuro-Sys' internal procedure).

Immunostaining: After injury, neurons were fixed with a solution of 4 % PFA. The cells were incubated with antibodies anti-tyrosine hydroxylase (TH), anti-aSyn, anti-Lamp1, or anti-Lamp2. Primary antibodies were revealed with Alexa Fluor 488 and Alexa Fluor 596, Pictures (20x) were acquired on an automated microscope with MetaExpress software and automatically analyzed with Custom Module Editor (Molecular Devices).

Statistics: Results are given as a percentage of control (mean +/- SEM, 100 % = control). Statistical analyses were done with GraphPad Prism. *p<0.05 (one-way ANOVA followed by Fisher's LSD test or Student t-test).



RESULTS

Pathological activation of lysosomal pathways in TH neurons after mitochondrial stress or after application of toxic aSyn oligomers

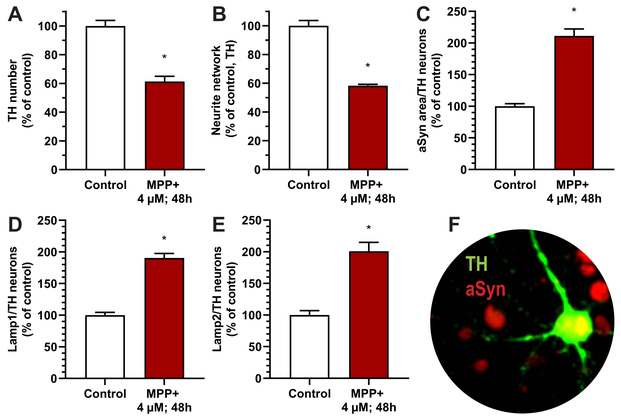


Figure 1: Neuronal stress and pathological activation of lysosomal pathways after mitochondrial stress in dopaminergic neurons. (A) Survival of dopaminergic neurons and (B) integrity of the neurite network after injury with MPP+. (C) Accumulation of alpha-synuclein in dopaminergic neurons after injury with MPP+. Accumulation of lysosomal vesicles positives for Lamp1 (D) and Lamp2 (E). (F) Representative pictures of aSyn accumulation * p<0,05; Student t-test.

Synucleinopathy and toxicity in dopaminergic neurons following inhibition of lysosomal function

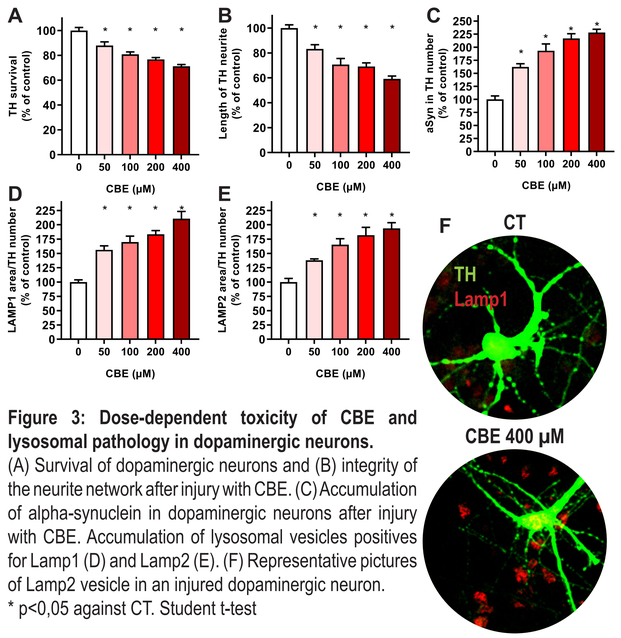


Figure 3: Dose-dependent toxicity of CBE and lysosomal pathology in dopaminergic neurons. (A) Survival of dopaminergic neurons and (B) integrity of the neurite network after injury with CBE. (C) Accumulation of alpha-synuclein in dopaminergic neurons after injury with CBE. Accumulation of lysosomal vesicles positives for Lamp1 (D) and Lamp2 (E). (F) Representative pictures of Lamp2 vesicle in an injured dopaminergic neuron.
* p<0,05 against CT. Student t-test

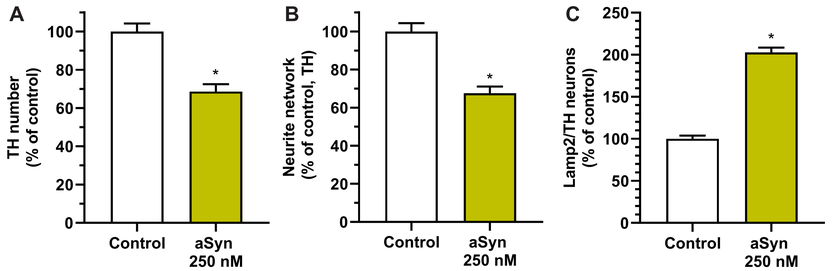
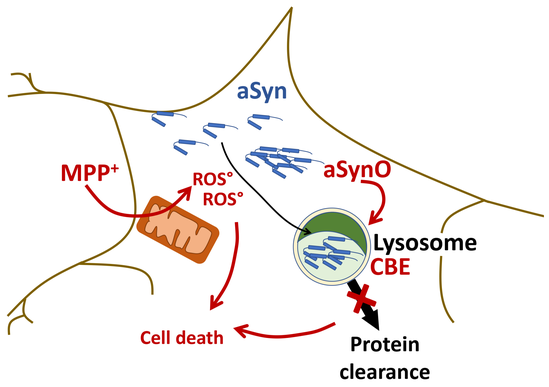


Figure 2: Long application of aSyn oligomers induced a loss of TH neuronal and of their neurite network and pathological overactivation of lysosomal pathways. (A) Survival of dopaminergic neurons and (B) integrity of the neurite network after application of aSyn oligomers. (C) Accumulation of lysosomal vesicles positives for Lamp2.
* p<0,05; Student t-test.

CONCLUSIONS

- **Mitochondrial stress** induced a loss of dopaminergic neurons, associated with an aggregation of aSyn, through the production of ROS, and an activation of lysosomal function. Lysosomal dysfunction might contribute to the direct neurotoxicity of MPP+.
- **Toxic aSyn oligomers** induced a strong activation of lysosomal pathway and a simultaneous loss of dopaminergic neurons. The increased signal in Lamp2 might results from an overactivation of the protein clearance pathway or to a lysosomal disorder.
- **Pharmacological inhibition of lysosomal function** triggered a dose-dependent accumulation of aSyn and lysosomal vesicles, and an acute neurotoxicity. **Lysosomal dysfunction is sufficient to induce a loss of dopaminergic neurons.**

Altogether, our results indicate that lysosomal dysfunction is tightly associated with the alpha-synucleinopathy and with loss of dopaminergic neurons, in three different *in vitro* models of Parkinson's disease.



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