MPP+ induced dopaminergic neuron death through necroptosis and autophagy

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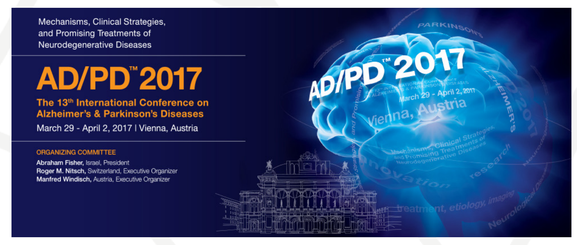


1 Introduction

Parkinson's disease (PD) is characterized by loss of dopaminergic neurons in the substantia nigra pars compacta and intraneuronal protein aggregates (Lewy bodies (LB)). Mitochondrial involvement has been postulated based on observations with mitochondrial toxins, which cause Parkinson's like syndromes. There exist several molecules used to induced Parkinsonism *in vivo*. Among them, MPP+ (1-methyl-4-phenylpyridinium) is the most popular. In this study, we attempted to unravel the mechanisms associated to dopaminergic neuron death induced by MPP+. We carefully dissected the mechanisms involved in the cell death (necrosis versus apoptosis vs necroptosis) and their kinetic of appearance. Additionally, we showed that mitochondrial disturbances induced a moderate aggregation of α-synuclein protein (α-syn) into neurons.

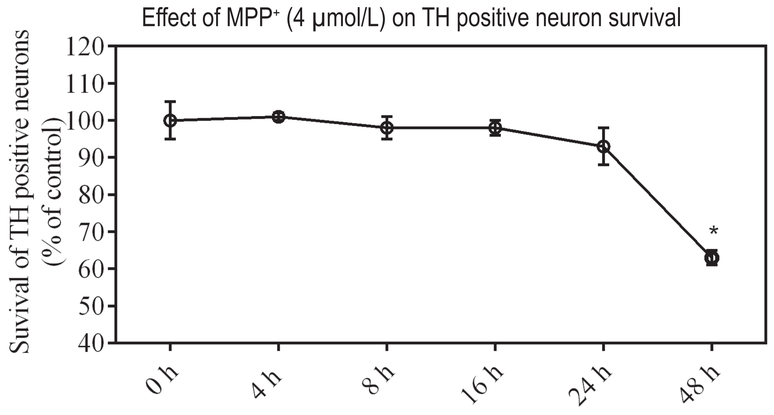
2 Methods

Culture of rat mesencephalic dopaminergic neurons: Neurons were cultured as described by Visanji *et al.*, 2008. Briefly, the midbrains obtained from 15-day old rat embryos were dissected. The primary cells were seeded at a density of 40,000 cells per well in 96-well plates (immunostaining) and 223,000 cells per well in 24-well plates (WB) precoated with poly-L-lysine. Six wells /conditions.
Pharmacological treatments: On day 6, fresh medium was added, without or with 1-méthyl-4-phényl pyridinium (MPP+).
Immunostaining: After toxin application, cells were fixed by a solution of 4 % paraformaldehyde. The cells were incubated with a) monoclonal Anti-Tyrosine Hydroxylase (TH) Ab, b) polyclonal anti-Cytochrome C (CytC) Ab, c) polyclonal anti-apoptosis-inducing factor (AIF) Ab, d) polyclonal anti-α-syn Ab. Abs were revealed with Alexa Fluor 488 goat anti-mouse IgG and Alexa Fluor 568 goat anti-rabbit IgG. The immuno-labelled cultures were examined with ImageXpress (Molecular Devices, USA) at X10 magnification.
Oxidative stress evaluation: After intoxication, the live cells were incubated with cellROX green reagent for 30 min at 37 °C then cells were fixed by a solution of 4 % paraformaldehyde.
Apoptosis and necrosis evaluation: 4, 8, 24 and 48 h after toxin application, apoptosis or necrosis were assessed using Glomax system and Apotoxglo triplex AC Kit (Promega, USA).
LC3II evaluation (Western-blotting): After toxin treatment, cells were lysed with cellytic and immediately frozen at -80 °C. All reagents were prepared and used according to manufacturer's recommendations (Simon™ Protein Simple - www.proteinsimple.com). Anti- LC3-II, primary antibody were used for WB analysis.

3 Results

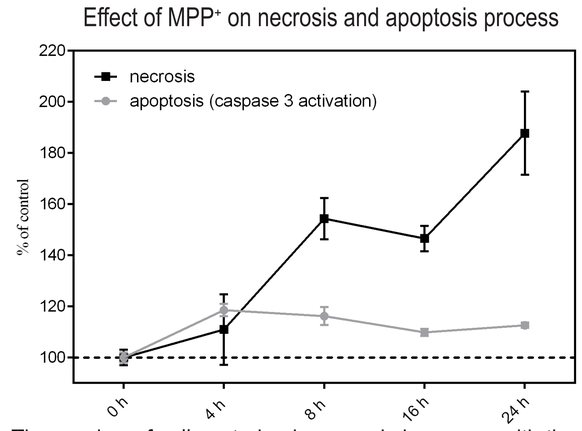
MPP+ induced primary TH neuron cell death (40 %) after 48 h. The cell death induced by MPP+ is mainly caused by a necrosis process

Effect of MPP+ (4 µmol/L) on TH positive neuron survival



Effect of MPP+ at 4 µmol/L on survival of TH-expressing neurons were assessed after 4, 8, 16, 24 and 48 h of intoxication.

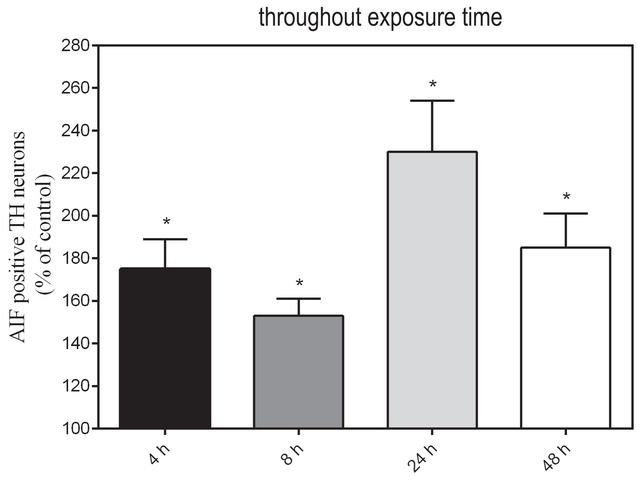
Effect of MPP+ on necrosis and apoptosis process



The number of cells entering in necrosis increases with the time of contact with the MPP+. A moderate apoptosis was observed.

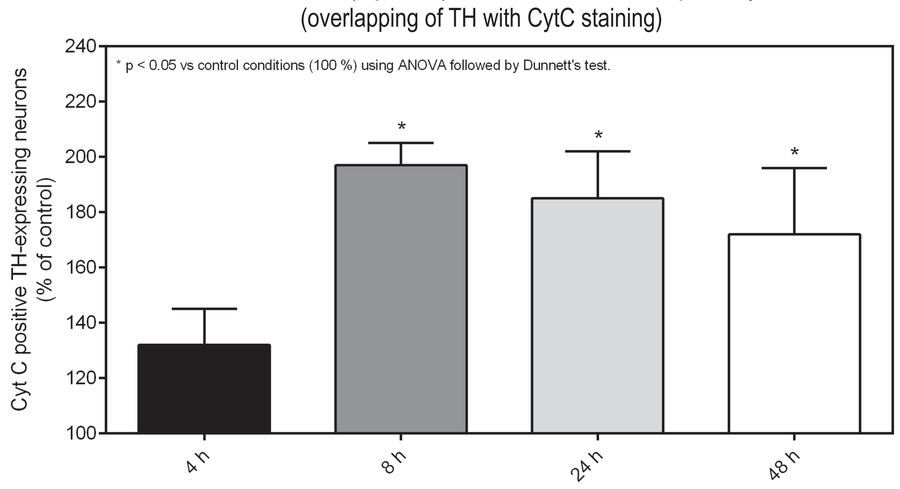
1. MPP+ induced a significant and large increase of AIF levels in the first 4h into TH positive neuron cytoplasm

Effect of MPP+ (4 µmol/L) on AIF in TH-expressing neurons throughout exposure time



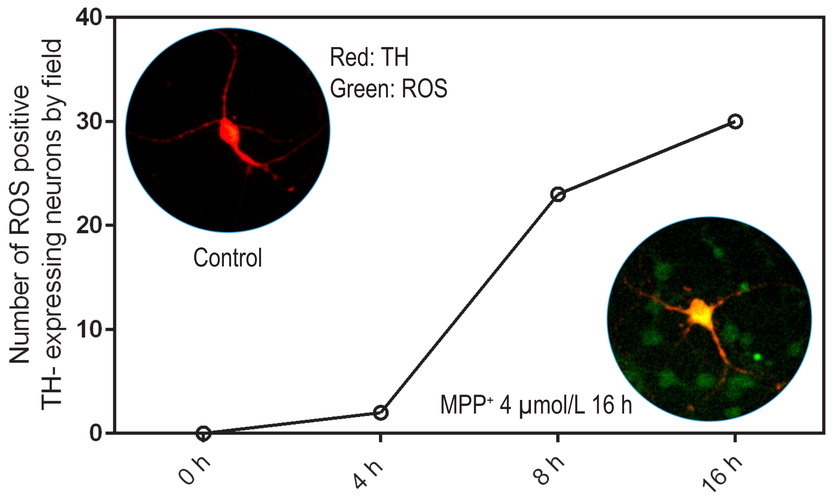
2. MPP+ induced a significant increase of cytoC levels 8 h after application

Effect of MPP+ (4 µmol/L) on mitochondrial death pathway (overlapping of TH with CytC staining)



3. MPP+ induced a significant and increase of ROS levels after 8 h application

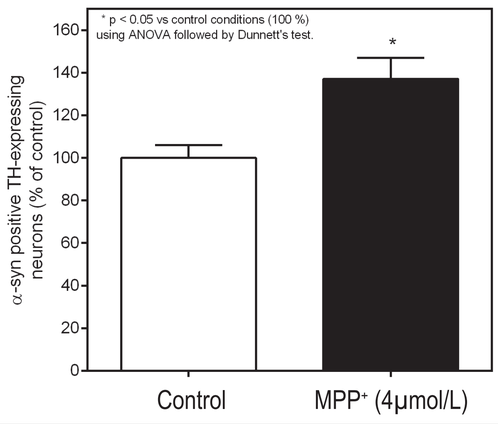
Effect of MPP+ on oxidative stress in TH-expressing neurons throughout exposure time



4. Autophagy pathway: MPP+ induced a large and significant increase of LC3-II

We observed a large and important increase (by 200 %) of LC3-II after MPP+ application. In addition, significant increase of cytoplasmic aggregated a-syn was observed into TH positive neurons 24 h after MPP+ application.

Effect of MPP+ (4 µmol/L, 24 h exposure) on α-syn aggregation in TH-expressing neurons (overlapping of TH and α-syn staining)



4 Conclusions

These results showed that :
- Lethal effects of MPP+ on TH positive neurons, slowly developed with time (48 h).
- MPP+ induced expression of AIF in the first 4 h after application. AIF is required for the maintenance of the mitochondrial respiratory complex 1.
- MPP+ induced early oxidative stress and release of Cytochrome C (without major increase of caspase3).
- MPP+ induced a large increase of LC3-II associated with aggregation of α-syn.

This finding may support the role of the autophagy at the early stage of MPP+ toxicity, to remove the protein aggregates. All together these results suggest that at early stage, MPP+ induced autophagy, this process being not able to remove the overwhelming amount of aggregated protein; the neuron therefore, died via necroptosis.

References: Visanji *et al.*, 2008 Faseb J 2008 Jul;22(7):2488-97