**INTRODUCTION**

Parkinson’s disease (PD) is the second most common age-related neurodegenerative disorder leading to severe motor symptoms. Major pathological features of PD are the loss of dopaminergic neurons in the substantia nigra (SN) and the presence of intraneuronal proteosomatic cytoplasmic inclusions (termed Lewy bodies), (Yamada, 1992). Numerous hypotheses have been developed to explain the pathogenesis of PD such as oxidative stress and mitochondrial dysfunction, aspects that have been widely studied especially by 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenylpyridinium (MPP+) in vitro cellular models which NeuroExperts already developed.

Here we investigated the role of α-synuclein (α-syn), a pre-synaptic protein implicated in dopamine trafficking and membrane dynamics, predominantly found in Lewy bodies (Yamada, 1992). Indeed, a large body of evidence suggests that aggregation of α-synuclein plays a crucial role in the pathogenesis because of its neurotoxic activity and its ability to trigger inflammatory process. To study the mechanisms by which aggregated α-synuclein acts, we developed a relevant in vitro cellular model where dopaminergic neurons are reproducibly intoxicated by α-synuclein. This improved and reproducible cellular model displays a prerequisite to further experiments on α-synuclein involvement in Parkinson disease and drug discovery through in vitro high content screening.

**MATERIALS AND METHODS**

**Dopaminergic neuron culture**

Rat dopaminergic neurons were cultured as described by Schnelli et al., 1988. Briefly, midbrain from 15 day old Wistar rat fetuses were removed and placed in ice-cold medium of Lautus. The ventral portions of the mesencephalic flexure were dissociated by trypsinisation for 20 min at 37°C and reaction was stopped by addition of Dulbecco’s modified Eagle’s medium and 10% of foetal calf serum. Cells were mechanically dissociated and centrifuged at 180 x g for 10 min at 4°C on a layer of 3.5% BSA. Pellet was re-suspended in Neurobasal supplemented with 2% B27, 0.2 mM L-glutamine and 1% of PS. Cells were seeded in 96 well plates pre-coated with poly-L-lysine and cultured at 37°C in a humidified air (95%/CO2 (5%) atmosphere.

α-synuclein toxicity was increased in dopaminergic neurons after a MPP+ injury and its own toxicity depends on its form, concentration and time of incubation.

**α-synuclein preparation and neuron treatments**: α-syn peptide was reconstituted in define culture medium at 4µM and was immediately or slowly shaken at 37°C for 3 days in dark. These α-syn peptide preparations were used on primary mesencephalic neurons (after 7 days of culture) at the desired final concentrations by dilution in control medium for 1, 2, 4, 7 or 14 days incubation in order to define the optimal incubation time. Brain-derived neurotrophic factor (BDNF), GDNF, α-synuclein, Nicotine, α-methyltyrosine were incubated in the same time of α-syn (protective effect) or 24 hours after α-synuclein intoxication (restorative effect).

**MPP+ preparation and neuron treatments**: MPP+ was incubated for 48h at 4µM. BDNF and GDNF were incubated in the same time of MPP+.

**Immunolabeling and analyses**

Cells were fixed by a solution of 4% paraformaldehyde, permeabilized and non-specific sites were blocked with phosphate buffered saline containing 0.1% of saponin and 1% fetal calf serum. Dopaminergic neurons were stained by incubating cells for 2h with a monoclonal antibody anti-Tyrosine Hydroxylase (TH) and α-syn positive neurons with a polyclonal antibody anti-phosphorylated α-syn. These antibodies were revealed with an Alexa Fluor 488 goat anti mouse and an Alexa Fluor 568 goat anti-rabbit for 1h. Nuclei were labeled by a Hoechst solution. For each condition, 20 pictures per well were taken using InCell Analyzer TM 2000 (GE Healthcare, x20 or x60). Analysis of TH positive cell bodies and α-syn positive TH cell bodies were performed using Developer software (GE healthcare).

**RESULTS**

A study was performed to establish a standard model of Parkinson disease based on the use of α-synuclein protein. Second to validate the model by using several reference compounds.

We showed (a) the predominantly toxic effect of presumed α-synuclein pre-fibrillar oligomers and investigated its toxicity according to its concentration and time of incubation, (b) the protective and restorative effects of BDNF and two neurotrophic factors belonging to the GDNF Family of Ligands (GFLs); (c) the relevance of our cellular model by employing compounds acting on specific pathways involved in PD like Nicotine known to bind to F2 and limiting oxidative damages (Linet et al., 1999) or like α-methyltyrosine known to inhibits dopamine synthesis that stabilizes α-syn oligomers. In conclusion, the 96 well in vitro model developed in this study is a good and relevant model that can be used for the screening of test compounds on α-synuclein aggregation in Parkinson disease.

**CONCLUSION**

The aims of this study were multiple. First, it was to establish a robust model of Parkinson disease through the use of α-synuclein protein. Second to validate the model by using several reference compounds.

We showed (a) the predominantly toxic effect of presumed α-synuclein pre-fibrillar oligomers and investigated its toxicity according to its concentration and time of incubation, (b) the protective and restorative effects of BDNF and two neurotrophic factors belonging to the GDNF Family of Ligands (GFLs); (c) the relevance of our cellular model by employing compounds acting on specific pathways involved in PD like Nicotine known to bind to F2 and limiting oxidative damages (Linet et al., 1999) or like α-methyltyrosine known to inhibits dopamine synthesis that stabilizes α-syn oligomers. In conclusion, the 96 well in vitro model developed in this study is a good and relevant model that can be used for the screening of test compounds on α-synuclein aggregation in Parkinson disease.

**REFERENCES**


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