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Advancing Parkinson's disease biopathology and drug discovery by dual cellular modelling

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ABSTRACT

Parkinson's disease (PD) is the fastest growing neurological disorder in the world. Its pathologic hallmarks are dopaminergic neuronal loss in the substantia nigra and alpha-synuclein accumulation in neurons. However, the patho-biologic mechanisms are largely unknown. Current drugs cannot slow or halt disease progression while clinical trials are mostly unsuccessful. Hence better cellular models are needed for pathological and drug discovery studies prior to *in vivo* validation. PC12 cells are commonly used for neurotoxicity studies but the Neuroscreen-1 (NS-1) variant has a faster doubling time and higher basal rate of neurite growth. We developed a NS-1 PD model with the neurotoxin 6-hydroxydopamine (6-OHDA) and MTT cell viability assay as readout. We optimized 6-OHDA concentration to a uniquely low 10 μM for a closer approximation to *in vivo* neurotoxicity. NS-1 cells treated with 6-OHDA displayed hallmark dopamine loss and apoptotic cell death. We used the model to screen a series of xanthones – polyphenolic compounds found in many medicinal plants. We report a novel activity of thwaitesixanthone in the PD model. The model was validated using alpha-mangostin (a neuroprotectant in *in vivo* and *in vitro* PD models) which was the most active in restoring cell viability. Alpha-synuclein is now a therapeutic target for stopping PD progression. Human HEK293 cells have neuronal attributes and reported to express pathologic alpha-synuclein. We hypothesized the transfection-efficient HEK293T cells is an optimal cell line for monitoring human alpha-synuclein levels. We make the first report that 6-OHDA treatment increased pathologic alpha-synuclein expression in HEK293T cells. This alpha-synucleinopathy model was validated using alpha-mangostin which attenuated 6-OHDA-induced pathologic alpha-synuclein to baseline levels. Thus we developed a novel NS-1 PD model more representative of *in vivo* neurotoxicity complemented by a human HEK293T cell-based alpha-synucleinopathy model for tracking pathologic alpha-synuclein levels. We present these dual models for producing *in vitro* findings with increased likelihood of clinical translation.

1. Introduction

Parkinson's disease (PD) is the fastest-growing neurological disorder in the world. Current PD drugs only treat the symptoms, without slowing down or stopping the underlying neurodegenerative processes (Stoker & Barker, 2020). The main pathological hallmark of PD is the loss of dopaminergic neurons in the substantia nigra pars compacta of the midbrain (Dauer & Przedborski, 2003). PD is also referred to as an α-synucleinopathy- a neurodegenerative disorder characterized by aggregated alpha-synuclein (α-syn) in neurons forming inclusions termed Lewy bodies (Koga et al., 2021). Currently α-syn is a therapeutic target and potential biomarker (Visanji et al., 2021). The classical PD motor symptoms such as resting tremor, bradykinesia and rigidity can be preceded by non-motor signs which have been attributed to synucleinopathy outside of the nigro-striatal pathway (Adler & Beach, 2016). Such dysfunction of other brain areas without a dopaminergic basis points to PD as a complex multisystem disorder.

Although postmortem brain analysis can shed light on end-stage PD, it is not possible to access the human brain or neurons to study the early stages. Hence it is necessary to develop experimental PD models that possess features of the dopaminergic system and reflect the disease phenotype (Lopes et al., 2017). The first PD animal model used rats injected with the neurotoxin 6-hydroxydopamine (6-OHDA), a hydroxylated metabolite of dopamine (DA) (Ungerstedt, 1968). Still widely

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