Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVI

Evidence of mitochondrial dysfunction and oxidative stress in a neuronal cell model of Parkinson's Disease

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Introduction

Parkinson's Disease (PD) is the second most prevalent neurodegenerative disorder affecting 1.8% of the ageing population [1]. PD is characterised by progressive motor dysfunction and degeneration of dopaminergic neurons. Oxidative stress (OS) and mitochondrial dysfunction are now understood to play critical roles in the development and progression of PD [2]. Mitochondria are the main generators of reactive oxygen species (ROS) making these organelles very susceptible to ROS-induced damage. Mitochondria are vital for efficient neuronal function in order to maintain neuronal integrity and survival, therefore mitochondrial dysfunction can lead to several deleterious consequences, including increased lipid peroxidation (LPO), reduced ATP production, overwhelmed cellular antioxidant system (decreased reduced glutathione (GSH) levels) and mitochondrial respiratory chain (MRC) impairments, particularly deficient complex I (NADH-ubiquinone oxidoreductase) activity [2,4]. These mitochondrial abnormalities and the effects of OS result in progressive neuronal loss in PD [3].

In a rotenone-induced SH-SY5Y neuronal cell model of PD, the main aims of this study were;

- To assess evidence of oxidative stress by assessing lipid peroxidation and reduced glutathione (GSH) content.
- To assess mitochondrial functionality by measuring the activity of MRC complex I and complexes II-III, citrate synthase (CS) activity and cellular ATP content.

Methods

Cell culture

SH-SY5Y neuroblastoma cells, derived from SK-N-SH, were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin-streptomycin and L-glutamine. SH-SY5Y cells were maintained at 37 ºC in an incubator containing 5% CO_2. SH-SY5Y cells were grown to 70-80% confluency before being cultured for analysis.

In vitro model of Parkinson's Disease

SH-SY5Y cells were treated with two concentrations of rotenone (0.5 µM and 1 µM). Treated SH-SY5Y cells were incubated for 24h at 37 ºC in 5% CO_2. After incubation, end-point analyses were carried out and the effects on mitochondrial functionality and oxidative stress were assessed.

Assessment of LPO and cellular GSH and ATP content

SH-SY5Y cells were counted and plated in clear 96 well plates and were then treated with two concentrations of rotenone (0.5 µM and 1 µM) for 24h. The measurement of LPO and cellular GSH content was then measured using flow cytometry and were assessed using the fluorescent probes, BODIPY and Thiol Green Dye respectively. For the assessment of cellular ATP content, SH-SY5Y cells were treated with rotenone as described previously. Cells were counted and plated in white 96 well plates and was assessed using CellTiter-Glo Biolumienscence assay (Promega) and was measured using a plate reader luminometer.

Assessment of MRC complexes and CS activity

SH-SY5Y cells were treated with rotenone as previously described. The MRC enzyme complex kinetics and CS activity were measured spectrophotometrically at 30 ºC. Complex I (NADH-ubiquinone oxidoreductase) activity is measured by a decrease in NADH and a decrease in the absorbance at λ=340 nm. Complex II-III (succinate dehydrogenase cytochrome *c* reductase) activity is measured by cytochrome *c* reduction and an increase in absorbance at λ=550 nm. CS activity is measured spectrophotometrically at λ=412 nm and is a biomarker of mitochondrial content.

Statistical analysis

Data is reported as mean ± standard deviation (SD). The n value represents the number of independently performed experiments. Each experiment was measured in triplicate. The significant difference between the data sets was analysed using the Student's t-test. In all experiments, $p < 0.05$ was considered to be significant.

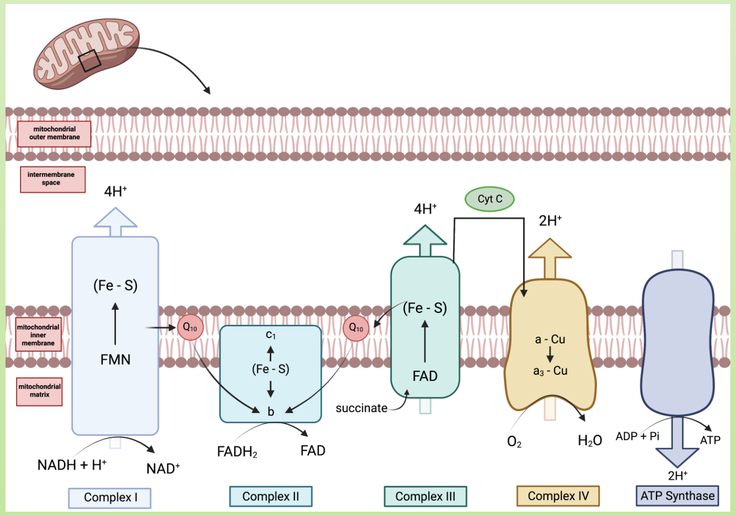


Figure 1. Diagram of the MRC demonstrating the process of oxidative phosphorylation. The complexes (I, II, III, IV and ATP synthase) are located within the inner mitochondrial membrane.

Results

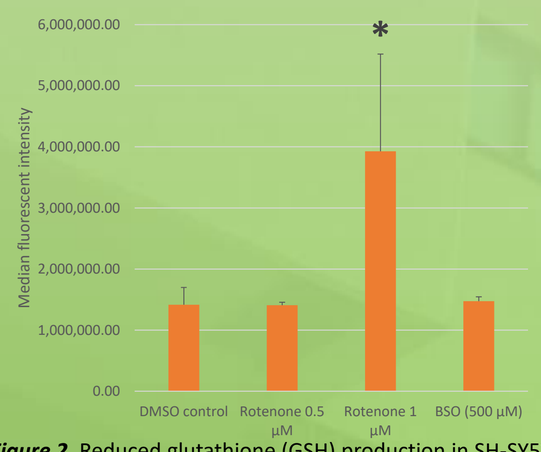


Figure 2. Reduced glutathione (GSH) production in SH-SY5Y cells stained with Thiol Green Dye following treatment with rotenone for 24h. SH-SY5Y cells were incubated with buthionine sulfoximine (BSO) for 48h (500 µM) to deplete GSH levels. The median fluorescent intensity of Thiol Green Dye was measured with a flow cytometer.
* P < 0.05 n = 4

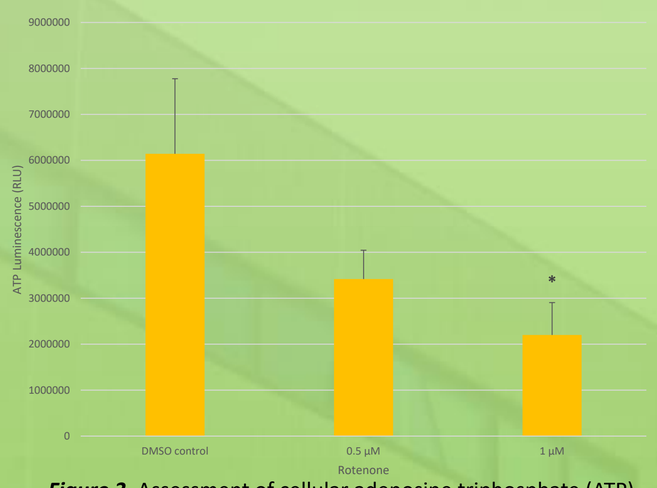


Figure 3. Assessment of cellular adenosine triphosphate (ATP) production in SH-SY5Y cells using CellTiter-Glo Bioluminescence assay (Promega) following treatment with rotenone for 24h. ATP luminescence was measured using a plate reader luminometer.
* P < 0.05 n = 2

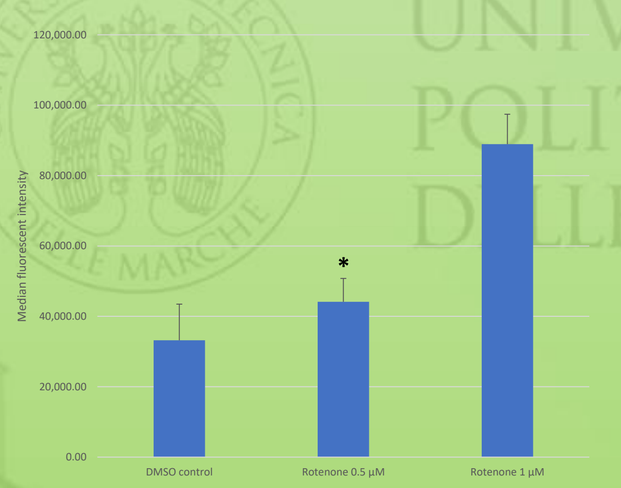


Figure 4. Assessment of lipid peroxidation (LPO) in SH-SY5Y cells stained with BODIPY (10 µM) probe following treatment with rotenone for 24h. The median fluorescent intensity of BODIPY was measured with a flow cytometer.
* P < 0.05 n= 3

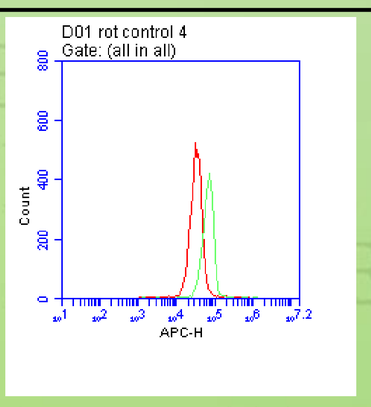


Figure 5. BODIPY staining for lipid peroxidation in control cells vs H_2O_2 (50 mM) treated cells. The red line represents the control cells, and the green line represents the H_2O_2 treated cells.

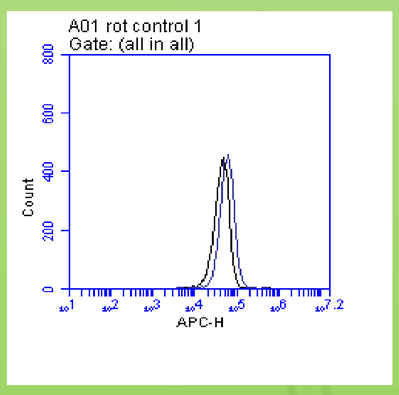


Figure 6. BODIPY staining for lipid peroxidation in control cells vs rotenone (1 µM) treated cells. The black line represents the control cells, and the blue line represents the rotenone treated cells.

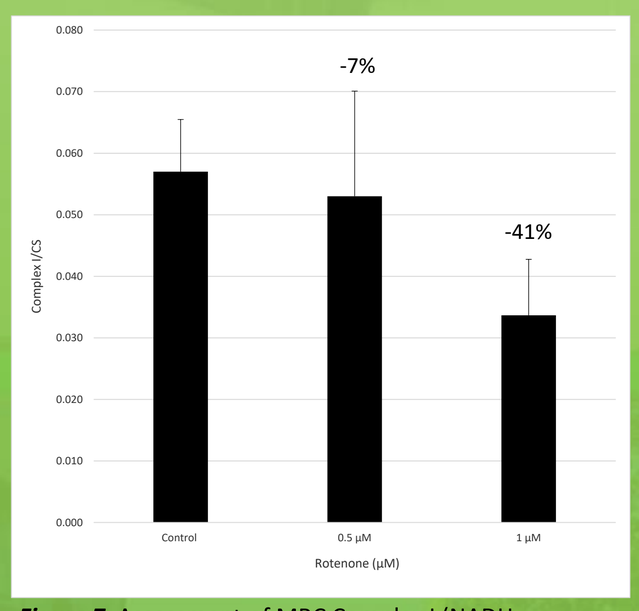


Figure 7. Assessment of MRC Complex I (NADH-ubiquinone oxidoreductase) activity in SH-SY5Y cells treated with rotenone for 24h. The % represents the % reduction in the activity of complex I following rotenone treatment. n = 3

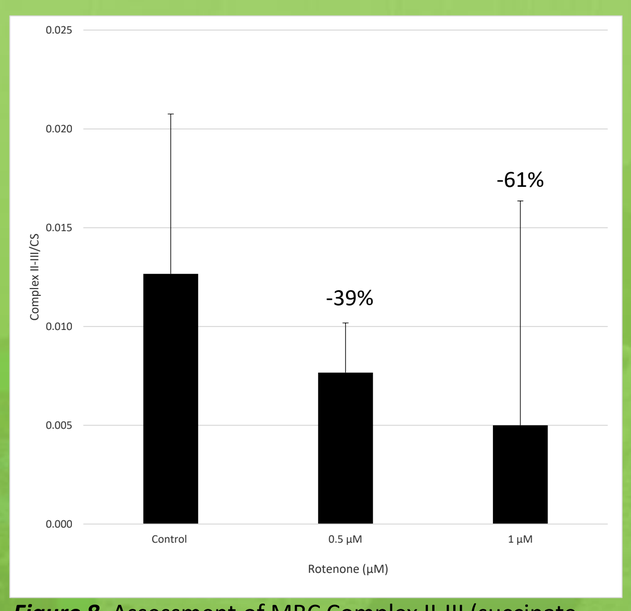


Figure 8. Assessment of MRC Complex II-III (succinate dehydrogenase cytochrome *c* reductase) activity in SH-SY5Y cells treated with rotenone for 24h. The % represents the % reduction in the activity of complex II-III following rotenone treatment. n = 3

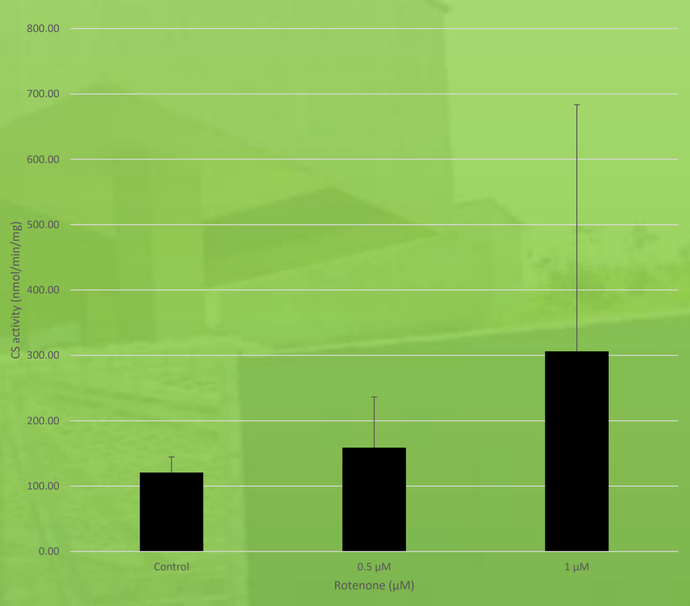
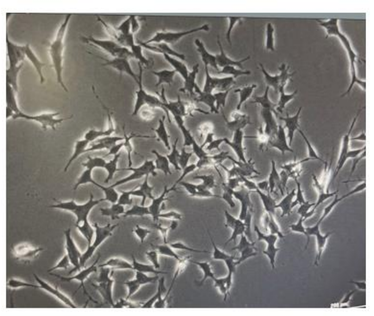


Figure 9. Citrate synthase (CS) activity (nmol/min/mg) assessed in SH-SY5Y cells treated with rotenone for 24h.
n = 3



Undifferentiated SH-SY5Y cells

Discussion

The data from this study demonstrates that there is evidence of OS and mitochondrial dysfunction in a neuronal cell model of PD. Evidence of OS can be demonstrated by redox dysfunction including increased LPO (Fig 4-6) and decreased GSH production (Fig 2). However, increases in GSH have also been observed demonstrating that GSH is involved in the cell's response to OS, therefore preventing OS-induced damage leading to neuronal dysfunction and death (Fig 2).

The data demonstrates that there is a decrease in the activity of both complex I and complex II-III of the MRC (Fig 1), therefore leading to mitochondrial dysfunction and increased OS, which as a result contributes to neuronal dysfunction and death (Fig 7-8). Compromised mitochondrial functionality can also be assessed via a reduction in cellular ATP production (Fig 3), leading to the dysfunction of energy metabolism and neuronal cell death [5].

This study also demonstrates that there is an increase in CS activity, suggesting upregulation of mitochondrial biogenesis, which is believed to be an adaptive response to the perturbation in cellular oxidative phosphorylation, leading to neuronal cell death (Fig 9) [6].

The data from this study will be used further to investigate several therapeutic strategies that aim to prevent and/or reduce mitochondrial dysfunction and oxidative damage in a neuronal cell model of PD.

References

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