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

Rotenone-induced oxidative stress and mitochondrial dysfunction in an SH-SY5Y neuronal cell model of neurodegeneration

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Introduction

Oxidative stress (OS) has been implicated as one of the main contributors to the development and progression of neurodegeneration (Fig 7) [1]. The central nervous system (CNS) is a susceptible target for OS-induced damage, favouring processes that lead to neuronal injury and death [2]. Mitochondria are essential for efficient neuronal function to maintain neuronal integrity and survival, however these organelles are major sources of reactive oxygen species (ROS) and are therefore vulnerable targets for OS-induced damage. Rotenone is a mitochondrial respiratory chain (MRC) complex I inhibitor resulting in inhibition of electron transfer from the iron-sulphur (Fe-S) clusters, located in complex I, to ubiquinone, causing increased ROS and neuronal dysfunction (Fig 1) [4]. These pathological features are frequently observed in neurodegenerative disorders [1], making rotenone a good model to assess OS and mitochondrial dysfunction in neurodegeneration.

The main aims of this study were;

- To determine evidence of toxicity and mitochondrial dysfunction determined in an SH-SY5Y neuronal cell model of neurodegeneration.
- To assess cell viability and oxidative stress (intracellular ROS production and mitochondrial superoxide production) in an SH-SY5Y neuronal cell model of neurodegeneration.

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 37ºC in an incubator containing 5% CO_2. SH-SY5Y cells were grown to 70-80% confluency before being cultured into well plates for analysis.

In vitro model of neurodegeneration

SH-SY5Y cells were treated with increasing concentrations of rotenone (0.25 - 2 µM). Treated SY-SY5Y cells were incubated for 24 hours 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.

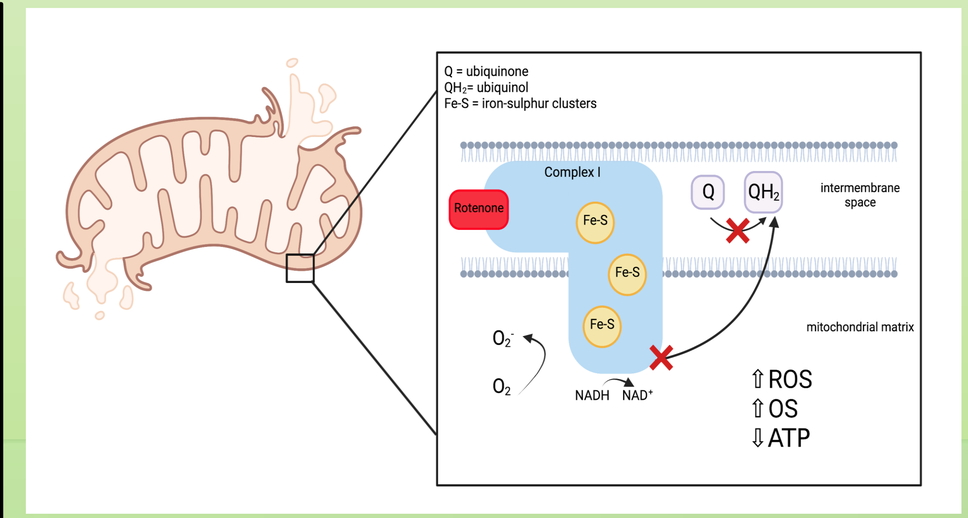

Figure 1. A schematic diagram to show how rotenone, a mitochondrial respiratory chain complex I inhibitor, promotes ROS production resulting in mitochondrial dysfunction and oxidative stress.

Results and Discussion

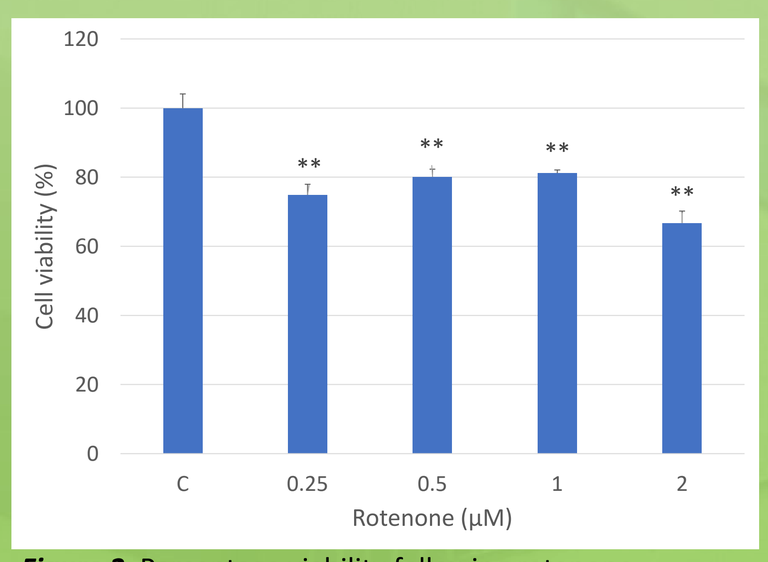

Figure 2. Percentage viability following rotenone exposure in SH-SY5Y cells incubated for 24 hours. * P < 0.05, ** P < 0.01

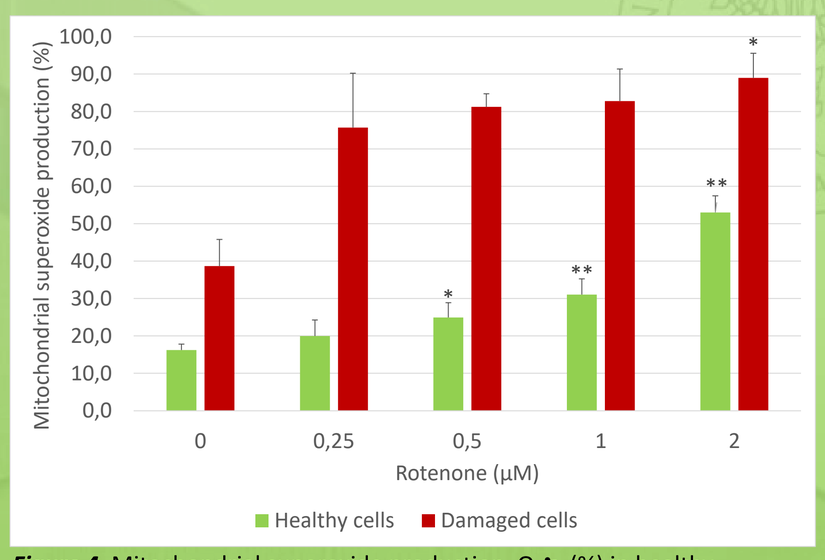

Figure 4. Mitochondrial superoxide production, $O_2^{\bullet-}$, (%) in healthy vs damaged SH-SY5Y cells stained with MitoSOX (5 µM) probe following exposure to increasing concentrations of rotenone for 24 hours. The green bars represent cells producing high levels of $O_2^{\bullet-}$ in healthy cells and the red bars represent cells producing high levels of $O_2^{\bullet-}$ in damaged cells in response to rotenone exposure. * P < 0.05, ** P < 0.01

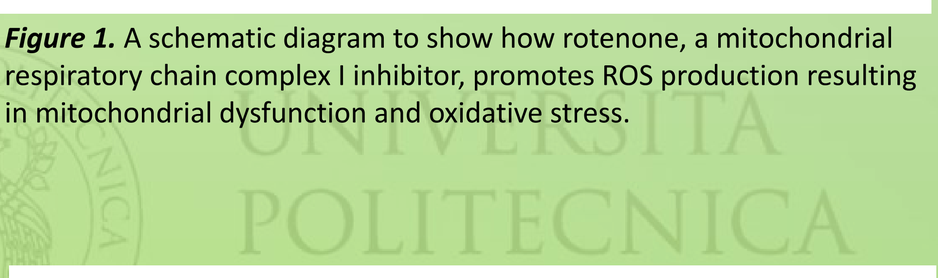

Figure 6. Evaluation of intracellular ROS by $DCFH_2$-DA assay in the SH-SY5Y human neuroblastoma cell line following rotenone treatment (1 µM) for 24 hours. Images taken with Lionheart Automated Microscope.

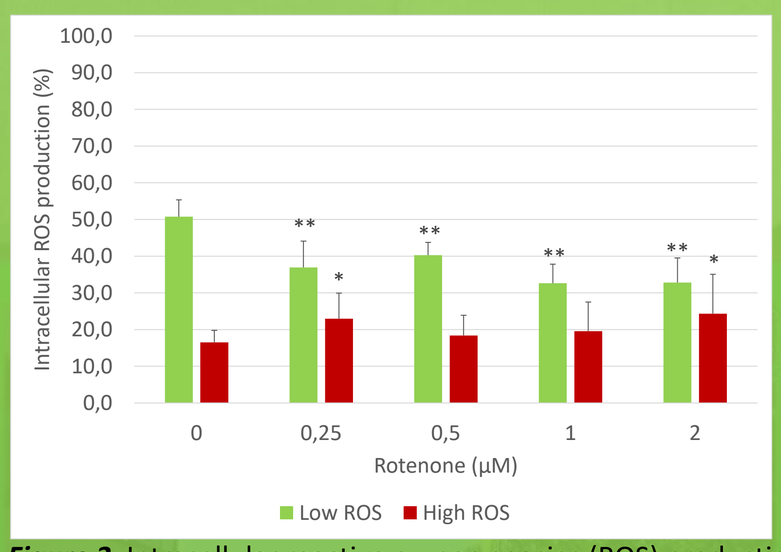

Figure 3. Intracellular reactive oxygen species (ROS) production (%) in SH-SY5Y cells stained with $DCFH_2$-DA (10 µM) probe following exposure to increasing concentrations of rotenone for 24 hours. The green bars represent cells producing a low level of ROS and the red bars represent cells producing a high level of ROS in response to rotenone exposure. * P < 0.05, ** P < 0.01

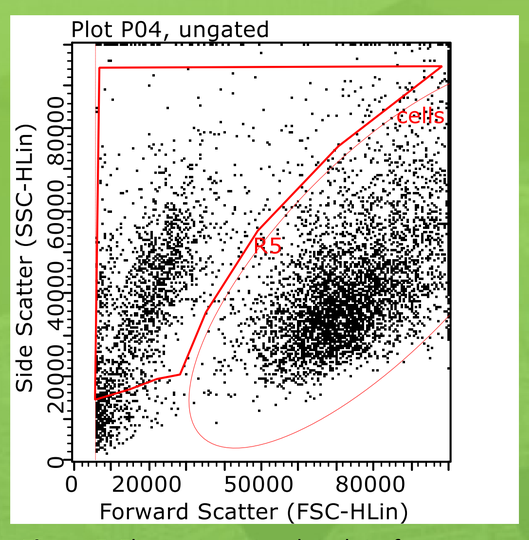

Figure 5. Flow cytometry dot plot of mitochondrial superoxide production analysis in SH-SY5Y cells following rotenone exposure (24h).

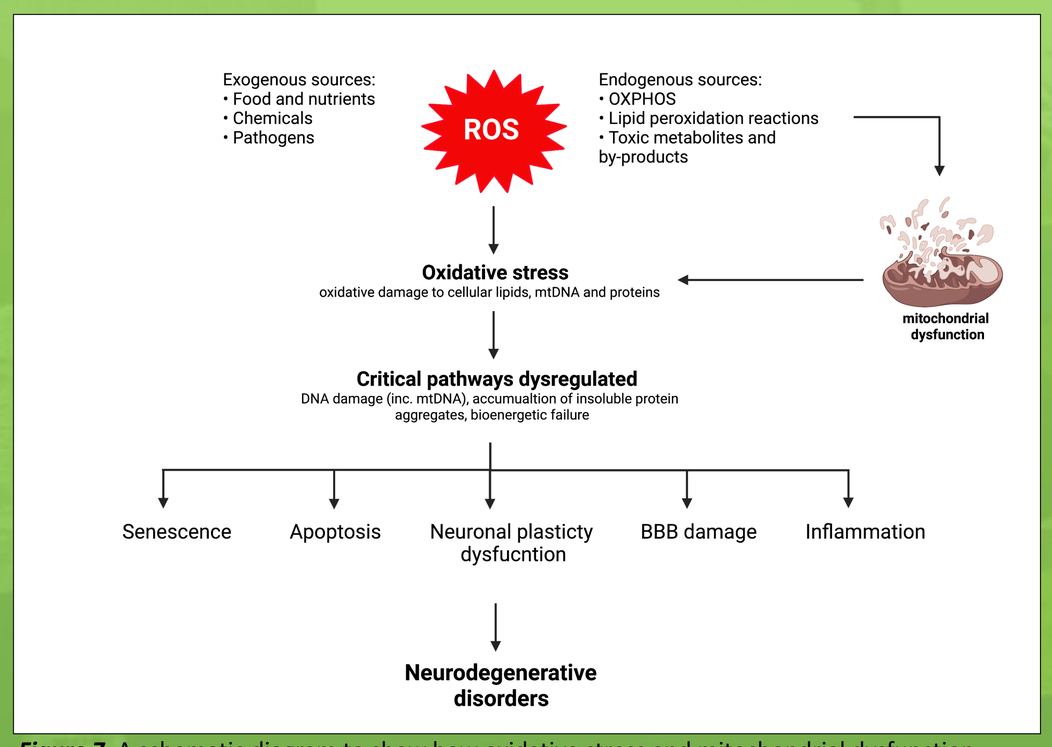

Figure 7. A schematic diagram to show how oxidative stress and mitochondrial dysfunction contribute to the development of neurodege.

The data from this study demonstrates that rotenone contributes to neuronal dysfunction and death as shown by increased intracellular ROS (Fig 3) and mitochondrial superoxide production (Fig 4), in addition to a reduction in SH-SY5Y viability (Fig 2).

The data from this study will be used to further investigate the methods of action of rotenone, in addition to analysis of CoQ_{10} levels and treatments with bioactive compounds that aim to reduce rotenone-induced ROS production, preventing mitochondrial dysfunction and oxidative damage in order to promote neuronal survival.

References

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