# DEVELOPMENT OF AN *IN VITRO* FERROPTOSIS MODEL FOR EVALUATING NEUROPROTECTIVE AGENTS

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INTRODUCTION: The increase in global life expectancy is a significant achievement, yet it is accompanied by a higher incidence of age-related diseases such as Alzheimer's and Parkinson's. Among the mechanisms implicated in these conditions, ferroptosis—a form of regulated cell death dependent on iron and oxidative stress—has gained prominence in the past decade. This pathway has been associated with the pathophysiology of several neurodegenerative disorders. RSL-3, a known inhibitor of glutathione peroxidase 4 (GPX4), is commonly used to induce ferroptosis in cell-based models. OBJECTIVE: This study aimed to establish a ferroptosis model using differentiated SH-SY5Y human neuroblastoma cells treated with RSL-3 and to assess the protective potential of deferoxamine, an iron chelator. MATERIALS AND METHODS: SH-SY5Y cells were plated, differentiated, and treated with increasing concentrations of RSL-3 (0.25–25 μM). Deferoxamine was then tested at concentrations of 5 to 100 μM in co-treatment with RSL-3 at 10 and 25 μM. After 24 hours, cell viability was evaluated using the MTT and Neutral Red (NR) assays. Results were expressed as percentages relative to the control group (0.625% DMSO) and analyzed using one-way ANOVA followed by Tukey's post hoc test, with significance set at $p<0.05$. RESULTS: RSL-3 significantly reduced cell viability at 5, 10, and 25 μM in the MTT assay, and at 10 and 25 μM in the NR assay. Deferoxamine conferred protection only at lower concentrations, suggesting that iron accumulation is a key driver of RSL-3-induced ferroptosis, while higher doses of the chelator may have limited efficacy. CONCLUSION: RSL-3 effectively induces ferroptosis in differentiated SH-SY5Y cells, especially at 10 μM. This model may contribute to the study of neurodegenerative disease mechanisms and the development of potential neuroprotective strategies.

Keywords: ferroptosis; RSL-3; SH-SY5Y cells; Alzheimer's disease; Parkinson's disease.