Examining oxidative stress models in mouse neuron HT-22 cells to explore neuroprotective features of anti-oxidant compounds.

Caleb Pryor[1], Raleigh Robinson[1], Maddie Perkins[1], Logan Gunerson[2], Morgan Bertrand[1], Gary Boehm[2], and Michael Chumley[1]

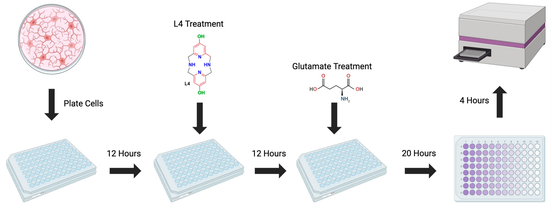
[1] Department of Biology and [2] Department of Psychology, Texas Christian University, Fort Worth, TX 76129

Alzheimer's disease (AD) affects millions worldwide and has shown increasing prevalence. Over the last few years, it has become increasingly apparent that oxidative stress from reactive oxygen species (ROS) resulting in cell death and chronic inflammation plays a crucial role in AD disease progression along with other neurodegenerative diseases. To investigate oxidative stress and potential neurotherapeutics, the HT-22 mouse neuronal cell line was developed. Glutamate treatment on HT-22 cells has been demonstrated to cause oxidative stress and cell death. By utilizing this model system, we can investigate the efficacy of novel compounds in their ability to block oxidative stress and neuronal cell death.

Introduction

- Alzheimer's disease (AD) effects 6.5 million Americans.
- Oxidative stress is a key hallmark of AD disease and its progression.
- Glutamate model for oxidative stress on HT-22 mouse neuron cells
- Antioxidant L4 Created by Dr. Green's research lab shown to promote cell survival following H202 treatment.
- Examine HT-22 cell survival in presence of glutamate following pre-treatment of L4.

Methods

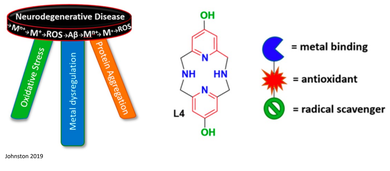


MTT Assay

- 10,000 cells/well
- L4 Pre-treatment 12 hours before Glutamate
- 24 Hour Glutamate Treatment
- Add MTT and Analyze

Follow Up Experiment

- Add H2O2 treatment to look for effective cell protection following L4 treatment.



Results

HT-22 Cell Viability with Glutamate Treatment

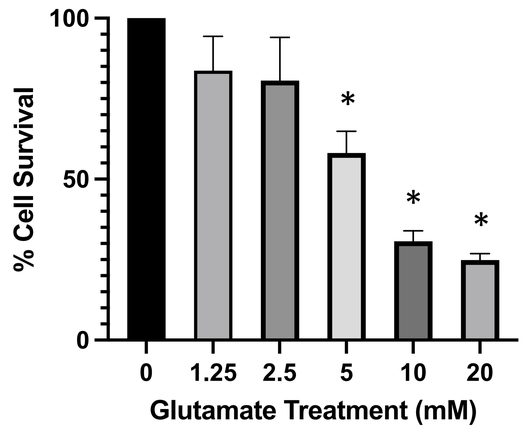


Figure 1.Cell survival following 24-hour Glutamate treatment. An independent samples 2-tailed t-test found a significant difference in cell viability at and above 5 mM treatment ($p \leq 0.05$). N=4 experiments, each treatment in triplicates.

HT-22 Cell Viability with Glutamate & L4 Treatment

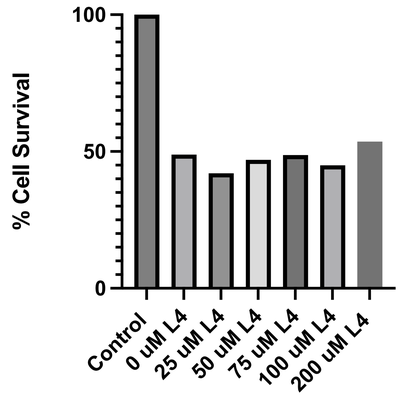


Figure 2. Cell survival following 24-hour Glutamate treatment. Cells were pre-treated for 24 hours with varying concentrations of L4 prior to 5 mM glutamate treatment. No statistics were run from the data, but findings are compelling evidence that there is no significant effect of adding L4 treatment to protect against glutamate-induced cell death.

Cell Survial following H2O2 Treatment

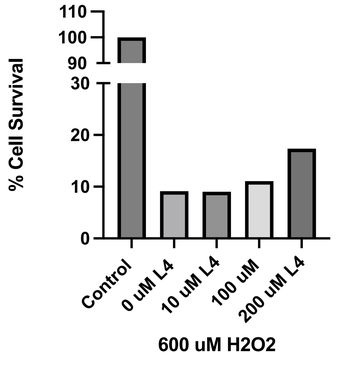


Figure 3. Cell survival following 4-hour Hydrogen Peroxide (H2O2) treatment. To demonstrate previously published findings that L4 treatment of cells protected against H2O2 mediated cell death, we repeated the experiment to ensure the assay was working properly. Cells were pre-treated for 24 hours with varying concentrations of L4 prior to 600 uM H2O2 treatment.

Cell Survival Following H2O2 Treatment

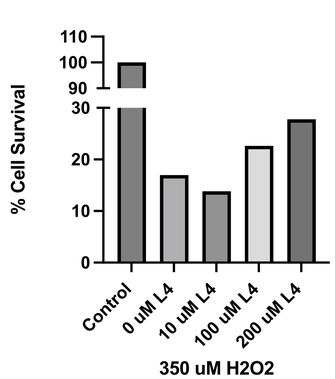


Figure 4. Cell survival following 4-hour Hydrogen Peroxide (H2O2) treatment. After low survival rate of HT-22 cells from 600 uM H2O2 treatment, we lowered H2O2 treatment to 350 uM. Cells were pre-treated for 24 hours with varying concentrations of L4 prior to 350 uM H2O2 treatment.

Conclusions

- Glutamate treatment of HT-22 cells leads to increased cell death.
- L4 pre-treatment has been shown to protect HT-22 cells from H2O2-induced toxicity, however our H2O2 treatment was too toxic to see a beneficial effect of L4.
- L4 pre-treatment likely protects cells from H2O2 induced cell death but does not protect from glutamate induced cell death.

Future Directions

- Confirm that L4 upregulates antioxidant gene expression.
- Confirm that glutamate treatment leads to lipid peroxidation in HT-22 cells.
- Determine if L4 is capable of blocking lipid peroxidation.

References

- Prasansuklab, A., Sukjamnong, S., Theerasri, A. *et al.* Transcriptomic analysis of glutamate-induced HT22 neurotoxicity as a model for screening anti-Alzheimer's drugs. *Sci. Rep.* **13**, 7225 (2023).
- 2022 Alzheimer's disease facts and figures. *Alzheimers Dement.* **18**, 700–789 (2022). Dai, X. J. *et al.* Activation of BV2 microglia by lipopolysaccharide triggers an inflammatory reaction in PC12 cell apoptosis through a toll-like receptor 4-dependent pathway. *Cell Stress Chaperones* **20**, 321–331 (2015).
- Johnston, H. M. *et al.* Enhancement of the antioxidant activity and neurotherapeutic features through pyridol addition to tetraazamacrocyclic molecules. *Inorg. Chem.* **58**, 16771–16784 (2019).
- Tan, S., Sagara, Y., Liu, Y., Maher, P. & Schubert, D. The regulation of reactive oxygen species production during programmed cell death. *J. Cell Biol.* **141**, 1423–1432 (1998)

Funding

reSEaRCh Science and Engineering Research Center
TCU College of Science & Engineering
NIH National Institutes of Health