The Influence of LPS-Treated BV2 Supernatants on Glutamate-Induced Cell Death in HT22 Cells.

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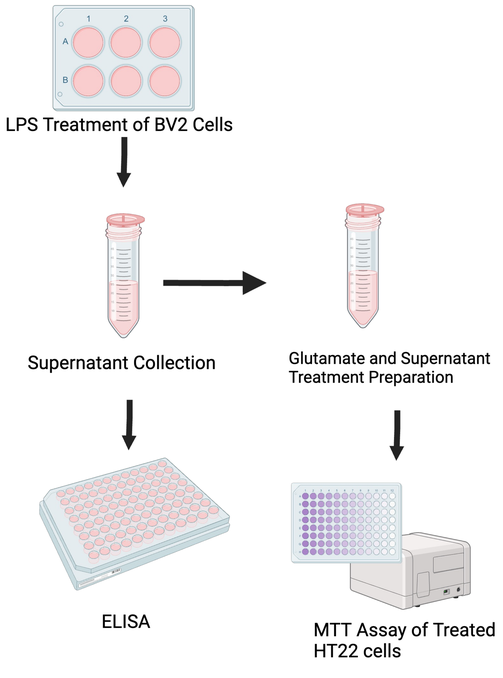
Alzheimer's disease (AD) was the fifth leading cause of death in people over 65 in 2021, and an estimated 13 million Americans will have AD by 2050. AD is a neurodegenerative disease characterized by memory loss and cognitive decline due to neuronal cell death. While the exact causes are still being studied, neuroinflammation, or inflammation in the brain, is known to play a role, with microglial cells—immune cells of the brain—being a key contributor. When overactivated, microglia release excessive inflammatory molecules, which may contribute to AD progression. To investigate this, we used HT22 cells, a mouse neuronal cell, and BV2 cells, a mouse microglial cell that produces inflammatory molecules in their "conditioned" media. We treated HT22 cells with glutamate to induce cell death and exposed them to BV2-conditioned media, then measured cell survival to determine if inflammatory molecules contribute to neuronal death. Unexpectedly, we found that BV2-conditioned media reduced the toxic effects of glutamate and promoted neuron survival. These findings suggest that microglial cells may have protective as well as harmful effects in AD, highlighting the complexity of neuroinflammation in disease progression.

Introduction

- Alzheimer's disease (AD) effects 6.5 million Americans.
- Clinically characterized by memory and cognition loss.
- Inflammation and Oxidative stress key hallmarks of AD disease and its progression.
- Glutamate model for oxidative stress on HT-22 mouse neuron cells
- LPS elicits immune system response
- LPS as a model for Microglial cell (BV2 cells) pro-inflammation signaling
- Develop a coculture system to study the interaction between microglia and neurons in AD pathology.

Methods



Results

TNF-alpha Production in LPS Treated BV2 Cells

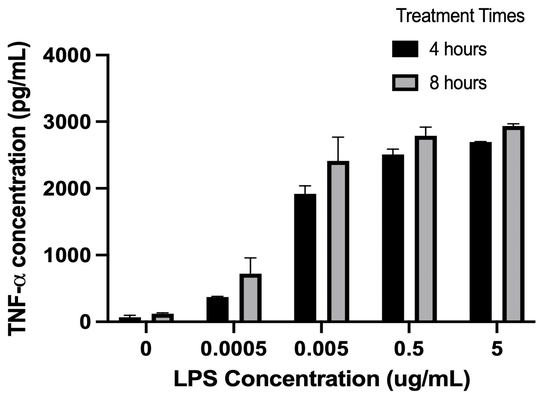


Figure 1.Peripheral cytokine production (pg/mL) in serum of BV2 cells. BV2 cells were treated with increasing concentrations of LPS for either 4 or 8 hours. Supernatants were collected, and TNF-α was measured using an ELISA. Error bars represent standard deviation of the mean.

Glutamate Induced Neurotoxicity in HT22 Cells

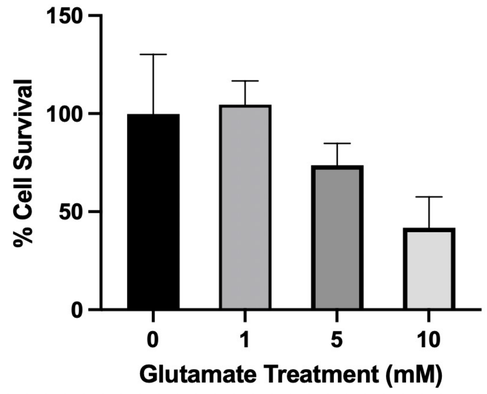


Figure 2. Cell survival following 24-hour Glutamate treatment. HT22 cells were treated with increasing concentrations of glutamate, and cell viability was measured using an MTT assay. Error bars represent standard deviation of the mean.

BV2 conditioned media (CM) may enhance HT22 cell survival despite glutamate treatment.

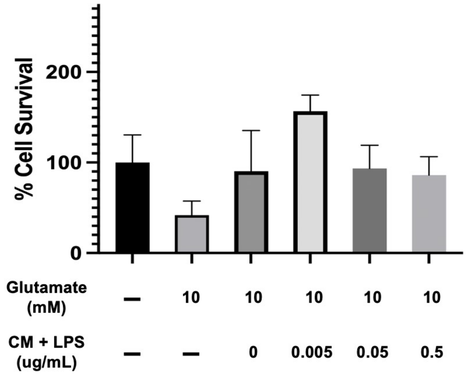


Figure 3. Cell survival following a 24-hour Glutamate and BV2 conditioned media cotreatment. HT22 cells were treated with 10mM glutamate and CM collected from BV2 cells that were treated with various concentrations of LPS. Cell viability was measured in an MTT assay. Error bars represent standard deviation of the mean.

BV2-conditioned media may induce proliferation of HT22 cells.

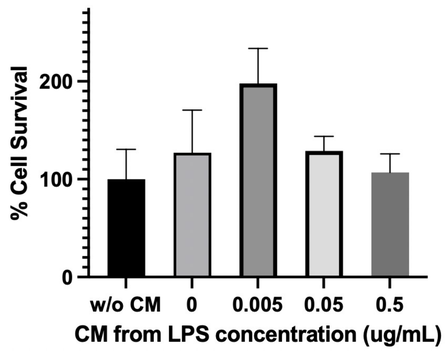


Figure 4. Cell survival following a 24-hour BV2 conditioned media treatment. HT22 cells were treated with CM collected from BV2 cells that were treated with various concentrations of LPS. Cell viability was measured in an MTT assay. Error bars represent standard deviation of the mean.

Conclusions

- Increased concentration of LPS treatment on BV2 leads to increased production of pro-inflammatory cytokines.
- The cytotoxic effect of glutamate on HT22 cells may be ameliorated when combined with BV2 conditioned media.
- BV2 conditioned media may be most effective at enhancing cell proliferation when treated with low concentrations of LPS.

Future Directions

- Investigate effects of coculturing BV2 cells with HT22 cells.
- Determine the component of BV2-conditioned media is causative agent of enhanced cell proliferation.
- Confirm chronic stress is being induced in BV2 cells.

References

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