The Effects of Novel Antioxidant Treatment on Microglial Cell Function in BV2 Cells

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Alzheimer's disease (AD) is a progressive neurodegenerative disease that is projected to affect almost 14 million American adults by the year 2050. While the prevalence of this detrimental disease is rapidly increasing in the United States, researchers have established the key pathologies connected to AD, including the development of extracellular, amyloid beta (Aβ) plaques, and intracellular, hyperphosphorylated, neurofibrillary tau tangles. Overall, AD engenders general atrophy of the brain and damage to key brain regions including the cerebral cortex and hippocampus, which are both involved in the neural mechanisms of learning and memory. AD pathologies develop in these regions, which commonly results in neuronal death. The presence of AD pathologies, such as Aβ, activates microglial cells in the brain. Glial cells are the most common brain cells that provide support to neurons. Microglia specifically serve as the resident immune cell in the brain because they clear cellular debris, such as dead neurons. Therefore, microglia play a key role in the progression of several neurodegenerative diseases. When microglia are activated, they release effector proteins known as pro-inflammatory cytokines. Cytokines are released when inflammatory agents, such as Aβ, are present in the brain. Microglial cells commonly produce pro-inflammatory cytokines, such as TNF-alpha. Although microglial activation is advantageous at first, continual activation of microglial cells results in a constant inflammatory state. Chronic inflammation can lead to detrimental tissue damage that plays a vital role in neurodegeneration. Another key AD pathology, oxidative stress, is connected to chronic inflammation. Oxidative stress develops when the antioxidant system is unbalanced, resulting in the accumulation of reactive oxygen species (ROS). The presence of inflammatory agents and ROS have the potential to activate microglial cells. Accordingly, our lab utilizes microglial cells to study the harmful effects of inflammation on the brain. Dr. Kayla Green's lab in the TCU Chemistry Department has successfully created potent small molecules, such as L4, that act as potent antioxidants. We collaborate with Dr. Green's lab to research the possible, therapeutic effects of L4 treatment against inflammation in immortalized, BV2 microglial cells. In our lab's previous research, we have demonstrated that L4 has the capacity to rescue BV2 cells and increase cell survival during oxidative stress. Moreover, the main purpose of the current experiment is to further study the effects of this molecule against key AD pathologies, to understand L4's therapeutic potential against inflammation *in vitro*. In the current experiments, we utilized lipopolysaccharide (LPS), an element from the cell wall of gram-negative bacteria, to induce an inflammatory response in BV2 cells. First, we determined several timepoints and concentrations in which LPS treatment successfully induced the secretion of TNF-alpha. Next, we pre-treated cells with the molecule, L4, for one hour prior to LPS treatment, to study the possible rescue effects of the drug against pro-inflammatory cytokine production. We are currently working on cytokine assays to determine what concentration of L4 is the most protective against pro-inflammatory cytokine production in BV2 cells. Therefore, we aim to further explore how antioxidant treatment could possibly mitigate AD pathologies, such as inflammation, as this could be a key therapeutic strategy for AD.

Introduction

- Macrophages of the brain, known as microglial cells, rapidly respond to injury and infection by modulating inflammation in the central nervous system (Saijo & Glass, 2011; Wyss-Coray & Rogers, 2012).
- Inflammatory triggers, such as Aβ, continually activate microglial cells and engender the release of pro-inflammatory mediators and neurotoxic factors, such as pro-inflammatory cytokines and reactive oxygen species (ROS). Chronic microglial cell activation promotes oxidative stress and neuronal damage (Block et al., 2007; Fischer & Maier, 2015). See Figure 1.
- Chronic inflammation and oxidative stress are both connected to other AD pathologies. Dysfunction of the antioxidant system leads to the production of ROS, therefore increasing levels of pro-inflammatory cytokines generated by microglia (McGarry, et al., 2018).
- Additionally, accumulation of metal ions is believed to be a contributor to Aβ plaque formation (Fischer & Maier, 2015).
- Unregulated redox-active metals react with molecular oxygen and produce excess ROS (Johnston, et al., 2019). Past research has shown that metal ion capture can promote metal ion equilibrium and attenuate Aβ accumulation (Lannfelt, et al., 2008).
- The compound L4, a N-heterocyclic amine, may be a promising therapeutic for several neurodegenerative diseases.
- Prior studies have demonstrated that L4 treatment is protective against oxidative stress (Johnston, et al., 2019; Lincoln, et al., 2013).
- This current experiment aimed to further examine the potential therapeutic properties of L4 against the inflammation in BV2 microglial cells, following LPS stimulation.

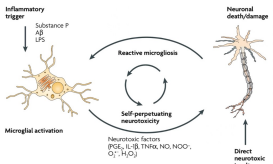

Figure 1. Activated microglial cells play a key role in the neuropathology of AD (Block et al., 2007).

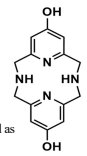

Figure 2. L4 is an N-heterocyclic amine used as a promising antioxidant and therapeutic drug against reactive oxygen species.

Methods

- Immortalized BV2 microglial cells were maintained in a cell incubator at 37 degrees Celsius and 5% CO_2. Cells were grown in complete cell medium. When the cells became 80–90% confluent, they were passaged following our standard protocol.

LPS Treatment

- **Day 1**: BV2 cells were seeded into 6 well plates.
- **Day 2**: After 24 hours of growth, BV2 cells were treated with various concentrations of LPS. Cells were collected 4, 8, 12, 14, and 24 hours following LPS treatment.

L4 Pre-treatment and LPS Treatment

- **Day 1**: BV2 cells were seeded into 6 well plates.
- **Day 2**: After 24 hours of growth, BV2 cells were pre-treated with L4 (1uM, 0.1uM, 0.01uM, 0.001uM, 0.0001uM, 0.0uM) and then treated with LPS (0.5μg/mL and 0.05μg/mL) one hour later. Cells were collected 8 hours following LPS treatment.

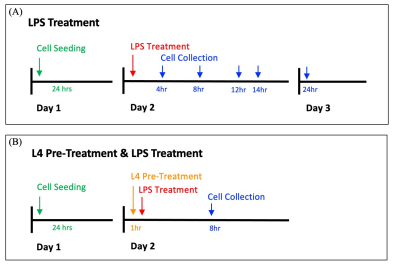

Figure 3. Timeline of experimental procedures. (A) LPS treatment timeline. (B) L4 pre-treatment and LPS treatment timeline.

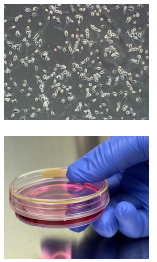

Figure 4. BV2 microglial cells (top) and tissue culture dish (below) .

Results

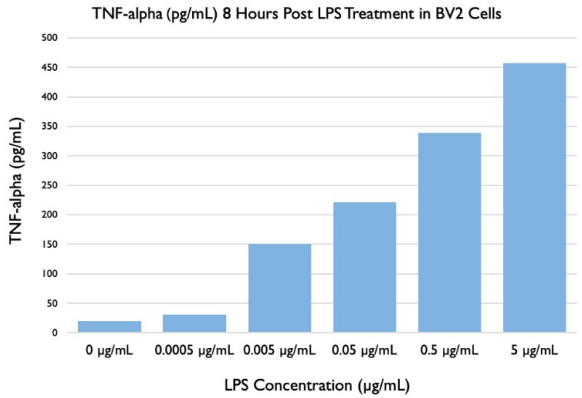

Figure 5. TNF-alpha ELISA Results Following LPS Treatment. The results revealed that 8 hours of LPS treatment induces high levels of TNF-alpha production in BV2 microglial cells. Additionally, 8 hours of LPS (0.05 μg/mL and 0.5 μg/mL) treatment were selected for use in future experiments with combined treatment of LPS and L4.

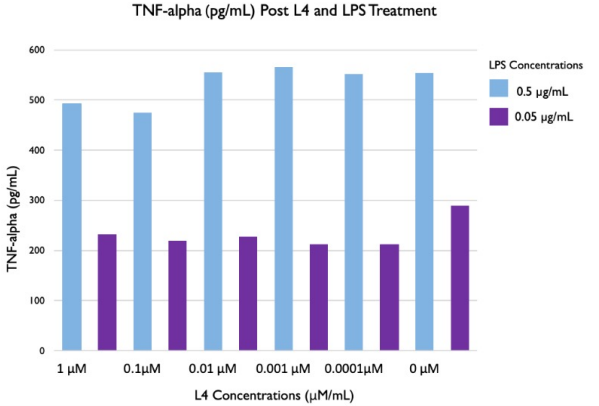

Figure 6. TNF-alpha ELISA Results Following L4 and LPS Treatment. BV2 cells were pre-treated with L4 molecule (1uM, 0.1uM, 0.01uM, 0.001uM, 0.0001uM, 0.0uM) for 1 hour prior to LPS (0.5μg/mL and 0.05μg/mL) stimulation for 8 hours. Preliminary data reveal that these concentrations of L4 pre-treatment were likely not high enough to block the pro-inflammatory response induced by LPS.

Conclusions

- LPS induced an inflammatory response via TNF-alpha production in BV2 cells.
- Preliminary data demonstrated that the L4 concentrations utilized in the current study did not protect BV2 cells from inflammation following LPS stimulation.
- Previous studies have shown that L4 has high antioxidant properties and rescues BV2 microglial cells from oxidative stress (Johnston et al., 2019).
- This research aimed to explore the potential therapeutic properties of L4 against the inflammatory response.
- Future research needs to assess L4's anti-inflammatory capacity.

Future Direction

- Determine the appropriate L4 concentrations and pre-treatment time necessary to provide protection against inflammation.
  - Measure other pro-inflammatory markers, like TNF-α, IL-1β, or IL-6.
  - Measure anti-inflammatory markers, like IL-10, Nrf2, or HO-1.
- Replicate this study in HT-22 neuronal cells and co-cultures with BV2 cells and HT-22 cells.

Funding

This project was made possible through a grant provided by the John V. Roach Honors College.

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