Antioxidant Therapy: A Potential Treatment for Alzheimer's Disease & Chronic Inflammation

Margaret Vo[1,2], Evan Chandlee[1,3], Caroline O'Connor[1,2], Shelby Kay Miller[1,3], Chelsy Mani[1,2], Paige Braden-Kuhle[1,3], Kayla Green[4], Gary Boehm[1,3], and Michael Chumley[1,2]

[1]Neurobiology of Aging Collaborative [2]Department of Biology [3]Department of Psychology and [4]Department of Chemistry at Texas Christian University
Fort Worth, TX 76129

Neurobiology of Aging Collaborative

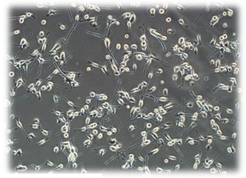
Alzheimer's Disease (AD) is a progressive neurodegenerative disease associated with old age and marked by deficits in memory and cognitive function. AD pathology is characterized by amyloid-beta (Aβ) accumulation, which leads to Aβ plaque formation and ultimately neuronal death. Additionally, Aβ activates microglial cells, which function as immune cells in the brain. Microglial cells secrete proteins that induce inflammation, known as pro-inflammatory cytokines. The chronic activation of microglia engenders oxidative stress in the brain, which further exacerbates AD pathologies. Dr. Kayla Green's lab in the TCU Chemistry Department has successfully created potent small molecules, such as L2 and L4, that act as potent antioxidants. We collaborated with Dr. Green's lab to research the possible, therapeutic effects of L2 and L4 treatment against inflammation in immortalized, BV2 microglial cells. Moreover, the main purpose of the current experiment was to further study the effects of these molecules against key AD pathologies, and to understand L2 and L4's therapeutic potential against inflammation *in vitro*. The overall goal of this research was to demonstrate the capacity of L2 and L4 to minimize the immunological mechanisms that drive AD pathologies. AD is the sixth leading cause of death in America, but the availability of therapies is limited. Our research will contribute to the understanding of the link between the immune system and central nervous system in AD development.

Introduction

- Alzheimer's Aβ plaques disrupts communication between neurons, and ultimately disrupts learning and memory processes (LaFerla et al., 2007).
- Chronic inflammation is a key component of AD.
- Brain microglial cells are immune cells that become activated in response to inflammation and release pro-inflammatory cytokines (Horvath et al., 2008; Stansley et al., 2012).
- Lipopolysaccharide (LPS) isolated from bacteria activates microglia to produce cytokines (Kahn et al., 2012).
- Metal ion dysregulation is also believed to be a contributor to Aβ plaque formation and AD (Fischer & Maier, 2015), and produces excess reactive oxygen species (ROS) (Johnston, et al., 2019).
- The compounds L2 and L4 may be promising therapeutics for several neurodegenerative diseases (Johnston, et al., 2019; Lincoln, et al., 2013).
- L4 treatment is protective against oxidative stress, while L2 and L4 are both effective at rescuing microglia from ROS-induced death (Johnston, et al., 2019; Lincoln, et al., 2013).
- The aim of this project is to examine the potential therapeutic properties of L2 and L4 against LPS-induced inflammation in microglial cells as a possible treatment for Alzheimer's Disease.

Methods

- BV2 microglial cells were maintained in a cell culture incubator at 37 degrees Celsius with 5% CO_2.



- **LPS Treatment**

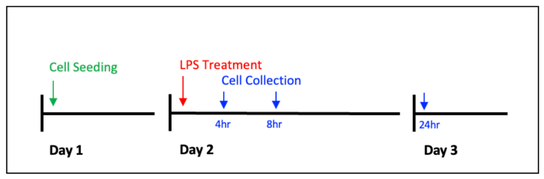


Figure 1. BV2 cells were seeded in culture wells on day one. At the start of day 2, cells were treated with various concentrations of LPS. Cell supernatant and lysates were collected at 4, 8, and 24 hours after LPS treatment.

- **L2 & L4 Pretreatment and LPS Treatment:**

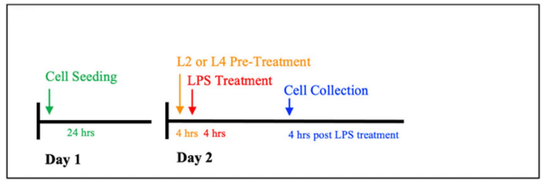


Figure 2. Cells were seeded on day one. At the start of day 2, cells were treated with various concentrations of L2 or L4 and treated with LPS 4 hours later. Cell supernatant and lysates were collected 4 hours after LPS treatment.

Results

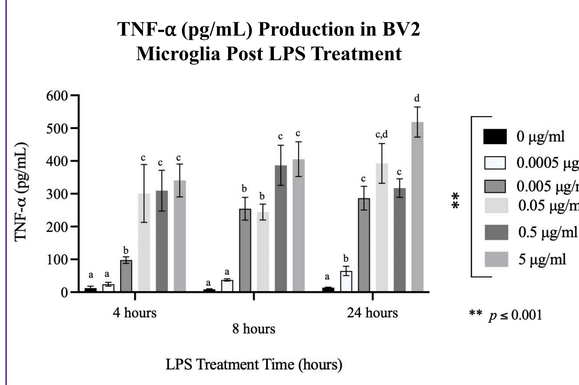


Figure 3. LPS Treatment Induces TNF-alpha Production in BV2 Cells. TNF-alpha ELISAs were conducted with BV2 cell supernatant following LPS treatment. ANOVA revealed a significant main effect of LPS treatment (μg/ml) on TNF-alpha production at 4, 8, and 24 hours of LPS treatment, $p \leq 0.001$. Different letters (a,b,c,d) represent significant differences at $p \leq 0.05$. Bars represent mean ± SEM. N's = 6 – 8.

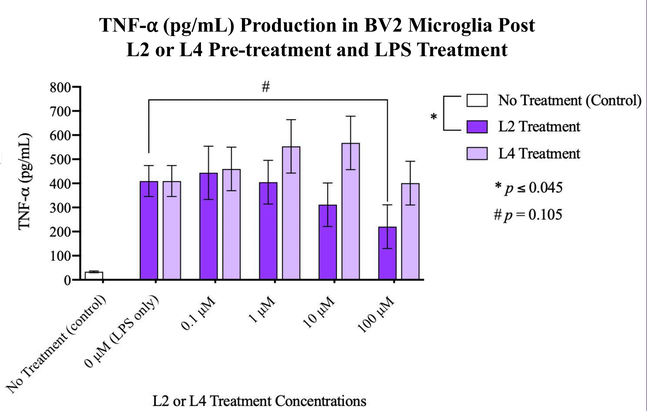


Figure 4. Drug Pre-Treatment Attenuates TNF-alpha Production. TNF-alpha ELISAs were conducted with BV2 cell supernatant following L2 or L4 pre-treatment and LPS treatment. ANOVA revealed a significant main effect of drug treatment, such that L2 attenuated TNF-alpha (pg/mL) production more so than L4, ($p \leq 0.045$). Post hoc tests did not reveal any significant differences between drug treatment concentrations for L2 or L4. However, the difference between 0 μM (LPS only) and 100 μM of L2 treatment was approaching significance, ($p = 0.105$). Bars represent mean ± SEM. Ns = 2– 6.

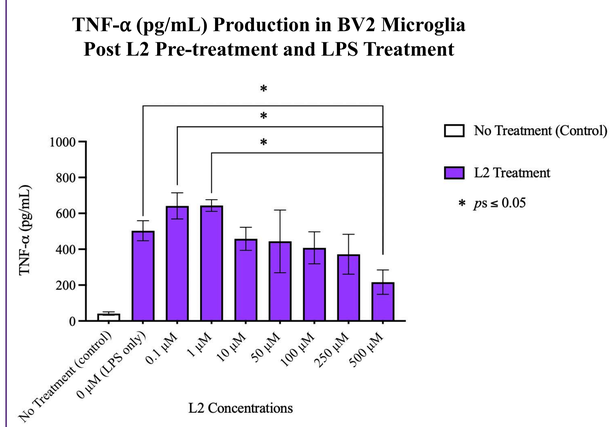


Figure 5. L2 Pre-Treatment Moderately Reduces LPS-Induced TNF-alpha Production. ANOVA revealed a significant main effect of L2 treatment was approaching significance, ($p = 0.053$). Post hoc tests revealed significant differences in TNF-alpha (pg/mL) production between BV2 cells treated with 0 μM and 500 μM of L2 ($p = 0.026$), 0.1 μM and 500 μM of L2 ($p = 0.005$), and 1 μM and 500 μM of L2 ($p = 0.002$). Bars represent mean ± SEM. Ns = 2 – 3.

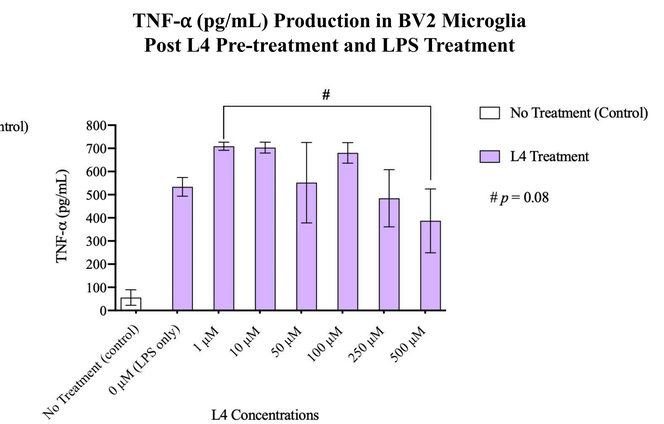


Figure 6. L4 Pre-Treatment Does Not Protect Against LPS-Induced Inflammation. ANOVA revealed that the main effect of L4 treatment was not significant, such that BV2 cells pre-treated with L4 were not protected from LPS-induced inflammation. Post hoc tests did not reveal any significant differences between treatment conditions. However, post hoc tests did reveal that the difference between 1 μM and 500 μM of L4 treatment was approaching significance, ($p = 0.08$). Bars represent mean ± SEM. Ns = 2 – 3.

Conclusions

- LPS induced an inflammatory response in BV2 microglial cells following 4, 8, and 24 hours of treatment.
- L2 is more effective than L4 at reducing LPS-induced TNF-α production.
- Higher concentrations of L2 were more effective at mitigating TNF-α production, but these concentrations were likely toxic to the cells.
- Ongoing studies are investigating if lower levels of L2 applied for extended periods will attenuate TNF-α production without toxicity to the microglial cells.

Future Studies:

- Determine if L2 & L4 activate the Nrf2 pathway antioxidant pathway in microglia.
- Determine if L2 & L4 inhibit proteins in the microglial cell signaling pathway that leads to transcription of TNF-α.
- Determine if L2 & L4 induce production of anti-inflammatory cytokines, such as IL-4 and IL-10.

References

Fischer, R., & Maier, O. (2015). Interrelation of Oxidative Stress and Inflammation in Neurodegenerative Disease: Role of TNF- *α. Oxidative Medicine and Cellular Longevity*,1–18.

Horvath, R. J., Nutile-McMenemy, N., Alkaitis, M. S., & Deleo, J. A. (2008). Differential migration, LPS-induced cytokine, chemokine, and NO expression in immortalized BV-2 and HAPI cell lines and primary microglial cultures. *Journal of Neurochemistry*, *107*(2), 557–569.

Johnston, H. M., Pota, K., Barnett, M. M., Kinsinger, O., Braden, P., Schwartz, T. M., ... Green, K. N. (2019). Enhancement of the Antioxidant Activity and Neurotherapeutic Features through Pyridol Addition to Tetraazamacrocyclic Molecules. *Inorganic Chemistry*, *58*(24), 16771–16784. Kahn, M., Krunjac, D., Alonzo, C., Haase, J., Cedillos, R., McLinden, K., Boehm, G.W.,Chumley, M.J. (2012). Prolonged elevation in hippocampal Aβ and cognitive deficits following repeated endotoxin exposure in the mouse. *Behavioral Brain Research*, *229*(1), 176–84.

LaFerla, F. M., Green, K. N., & Oddo, S. (2007). Intracellular amyloid-β in Alzheimer's disease. *Nature Reviews Neuroscience*, *8*(7), 499–509.

Lannfelt L, Blennow K, Zetterberg H, Batsman S, Ames D, Harrison J. (2008). Safety, efficacy, and biomarker findings of PBT2 in targeting Abeta as a modifying therapy for Alzheimer's disease: a phase IIa, double-blind, randomized, placebo-controlled trial. *Lancet Neurol*,7:779–786.

Lincoln, K. M., Gonzalez, P., Richardson, T. E., Julovich, D. A., Saunders, R., Simpkins, J. W., & Green, K. N. (2013). A potent antioxidant small molecule aimed at targeting metal-based oxidative stress in neurodegenerative disorders. *Chemical Communications*, *49*(26), 2712.

Stansley, B., Post, J., & Hensley, K. (2012). A comparative review of cell culture systems for the study of microglial biology in Alzheimer's disease. *Journal of Neuroinflammation*, *9*, 115.

Funding

JOHN V. ROACH HONORS COLLEGE

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