# Oxidative Stress as a Target for Alzheimer's Disease Therapeutics

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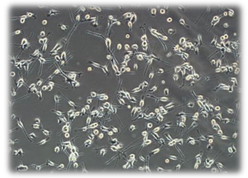
Alzheimer's Disease (AD) is a progressive neurodegenerative disorder that is currently ranked as the sixth leading cause of death in the United States. Those affected by the disease experience symptoms such as decline, memory loss, confusion, difficulty with language, as well as behavioral and mood changes. One of the main hallmarks of AD is inflammation that occurs in the brain and periphery. While inflammation is the natural response to activation of our immune system, neuroinflammation is heavily associated with AD pathology. Two main cellular mediators involved with the inflammatory response are members of the glial cell family, microglial cells and astrocytes. Chronic inflammation can lead to harmful cell and tissue damage. The continuous presence of damaged neurons results in the constant activation of microglia and astrocytes, which promotes neurodegeneration. Linked to inflammation, another process up regulated in the brains of aging individuals is oxidative stress, which plays a major role in age-related neurodegeneration and cognitive decline. This process can occur due to dysfunction of the antioxidant system, causing the accumulation of reactive oxygen species (ROS) in the brain. The presence of ROS can activate macrophages in the presence of specific microglia. This can produce major proinflammatory cytokines such as IL-1β and TNF-α, illustrating the connection between oxidation and inflammation in the brain. One of the primary biological markers of AD is the aggregation of amyloid beta (Aβ). Aβ has been shown to act as a pro-inflammatory agent, promoting the activation of many inflammatory components. For example, Aβ causes microglia to produce higher levels of TNF- α, causing toxicity in neurons. The accumulation of Aβ is thought to be exacerbated by essential bio metal ions, such as zinc and copper. Dr. Kayla Green's lab in the TCU Chemistry Department has successfully created compounds that can simultaneously chelate metal ions and act as powerful antioxidants. They have developed a family of compounds all consisting of N-heterocyclic amines (L2 and L4) that in turn, have the capacity to perform radical scavenging and metal ion capture. For the experimental design, BV2 microglia cells were treated with either $H_2O_2$ or Lipopolysaccharide (LPS) for 16 hours. Following treatment, MTT assays were performed to measure cell viability. After a treatment concentration was found to significantly decrease cell viability, varying concentrations of L2 and L4 were tested with an oxidative stress inducer to measure rescue capacity in overall cell viability.

## Introduction

- Research has shown that Aβ plaques can lead to an increase in oxidative stress and promote cellular degeneration by increasing reactive oxygen species (ROS) (Roman and Olaf, 2015).
- Microglia cells, the resident macrophages of the brain, are one of the main cell mediators involved in neuroinflammation and clearing excess proteins or waste (Wyss-Coray and Rogers, 2012).
- Chronic inflammation is connected to oxidative stress. Dysfunction of the antioxidant system leads to the production of ROS, therefore increasing levels of proinflammatory cytokines generated by microglia (McGarry, et al., 2018).
- Aβ is a proinflammatory agent and can generate ROS within cells. ROS can develop from metal-ion cores in Aβ plaques, most likely produced during inflammatory states (Roman and Olaf, 2016).
- Past research has shown that metal ion capture can promote metal ion equilibrium and attenuate Aβ accumulation (Lannfelt, et al., 2008).
- Compounds L2 and L4, N-heterocyclic amines, were used as promising antioxidant and therapeutic drugs to perform radical scavenging and metal ion capture (Lincoln, et al., 2013).
- In this experiment, BV2 murine microglia cells were used to conduct MTT assays. This colorimetric assay uses a tetrazolium dye to analyze cell viability.

## Methods

- BV2 cells from an immortalized microglia cell line were maintained in a cell incubator at 37 degrees Celsius 5% Co2. Cells were grown in complete cell medium. When the cells became 80-90% confluent, they were passaged following our standard protocol.



- **Experiment 1:**

| Treatment | Concentrations |
|---|---|
| $H_2O_2$ | 1.5 µM - 12 µM |
| Lipopolysaccharide (LPS) | 2.5 µg/mL - 20 µg/ml |

**Figure 1.** MTT Assay assessed cell viability with oxidative stress inducer, $H_2O_2$, or inflammatory inducer, LPS, in BV2 cells. Cells were treated for 16 hours with either $H_2O_2$ (1.5 µM - 12 µM) or LPS (2.5 µg/mL - 20 µg/ml).

- **Experiment 2:**

| Treatment 1 | Concentrations | Treatment 2 | Concentrations |
|---|---|---|---|
| $H_2O_2$ | 1.5 µM - 12 µM | L2 | 0.0000125-12.5 µM |

**Figure 2.** MTT Assay assessed the rescue effect of L2 compound (0.0000125-12.5 µM) in combination with oxidative stress inducer, $H_2O_2$ (3 µM).

## Results

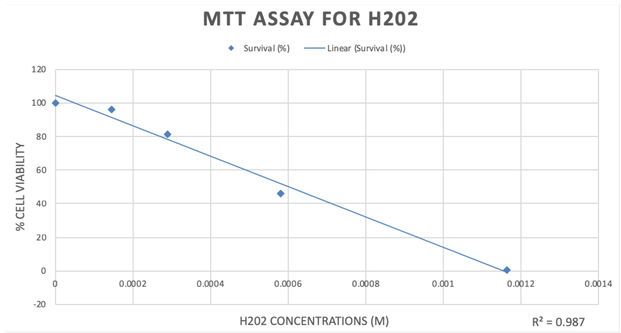



**Figure 3.** BV2 cell survival decreased with increased $H_2O_2$ concentration.

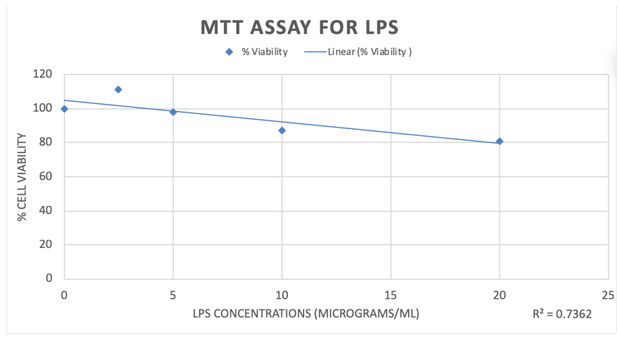



**Figure 4.** LPS had a weak effect on overall BV2 cell viability.

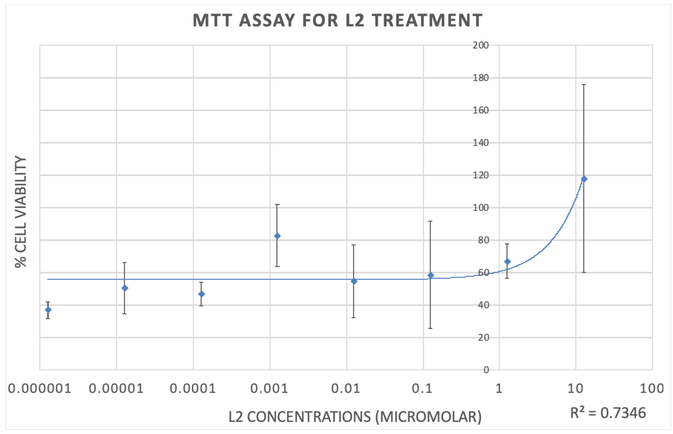



**Figure 5**. L2 treatment enhanced BV2 cells survival following $H_2O_2$, oxidative stress inducer. This demonstrates that L2 could play a possible therapeutic role in protecting microglial cells from oxidative stress.

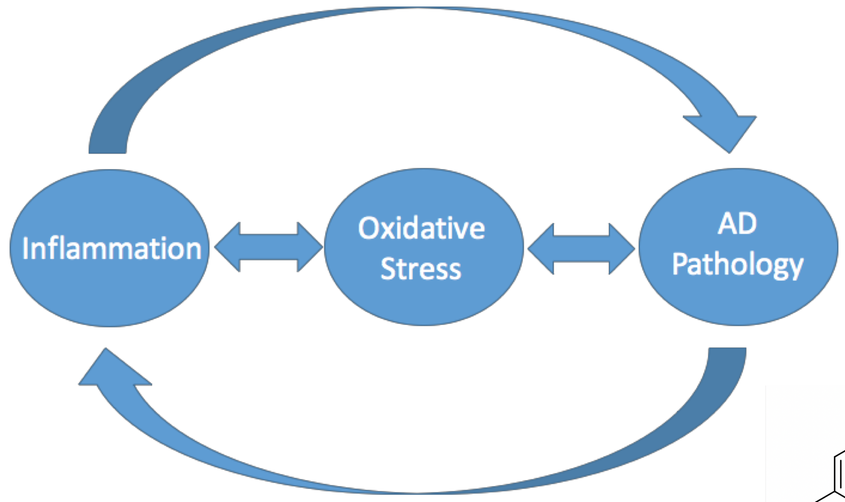



**Figure 6.** There is a direct connection between inflammation, oxidative stress and Alzheimer's disease pathologies. There are many contributing factors to oxidative stress, including the accumulation of reactive oxygen species (ROS), microglial activation and the production of proinflammatory cytokines.

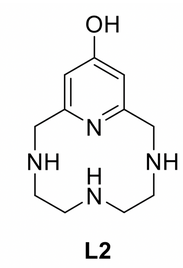



**Figure 7.** L2, a potent antioxidant molecule consisting of N-heterocyclic amines.

## Conclusions

- $H_2O_2$ had a negative dose-dependent effect on BV2 cell viability.
- LPS showed a weak effect on overall BV2 cell viability.
- L2 administration demonstrated an effective rescue capacity for BV2 cells, illustrating a clear rebound effect.
- In conclusion, L2 ameliorates oxidative stress and has a promising future as a potential therapeutic treatment for AD.
- **Future Studies:**
- Further explore LPS effect on cell viability with increased concentrations and assess what stage BV2 cells produce more ROS.
- Utilize ELISA assays to measure pro-inflammatory cytokine levels, such as TNF-alpha, in BV2 cells.

## References

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## Funding

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