Testing the Ability of Novel Drugs to Modulate TNF-α Secretion in BV-2 Microglial Cells

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Introduction

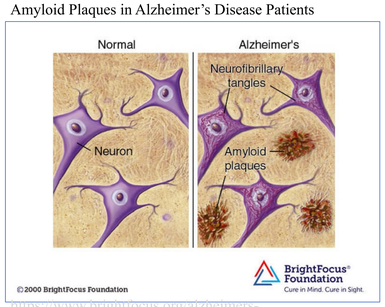
Alzheimer's Disease is one of the most prevalent neurodegenerative disease in the United States, which the projected patient population expected to be 14 million by 2060[1]. Previous research has indicated that the presence of β-amyloid plaques and tau-hyperphosphorylated neurofibrillary tangles (fig.1) are the molecular hallmarks of the disease. The resident immune cells of the brain, microglial cells, typically respond to waste buildup by removing it. Cytokines such as TNF-α recruit microglia to the buildup site for clearance. The inability of the microglia to remove waste leads to chronic inflammation.

The NF-κB pathway (fig.2) is activated when lipopolysaccharide (LPS) from bacteria binds to toll-like receptor 4 (TLR4) or TNF-α binds to the TNF-α receptor (TNFR). This activation results in the translocation of NF-κB into the nucleus, which is a transcription factor that promotes the expression of inflammatory genes, such as the *TNF-α* gene. TNF-α is then translated and secreted out of the microglial cell. Our collaborators at P2D Bioscience and the lab of Dr. Kayla Green at TCU have designed anti-inflammatory drugs that have the potential to attenuate this response.

In this project, LPS is used to stimulate the NF-κB inflammatory pathway. The cytotoxicity of each drug is first assessed using an MTT assay to identify suitable testing concentrations. Plated microglia cells are then treated with varying concentrations of the drugs followed by LPS, with appropriate incubation periods between and after. The cell supernatants are then harvested, and the TNF-α concentration is measured using an enzyme-linked immunosorbent assay (ELISA). Once a reduction is confirmed, different techniques can be employed to elucidate the drug's mechanism of action. Immunofluorescent experiments using antibody probes targeting NF-κB is one such approach. HeLa cells are used here for clear visualization of NF-kB in the cytoplasm versus in the nucleus.

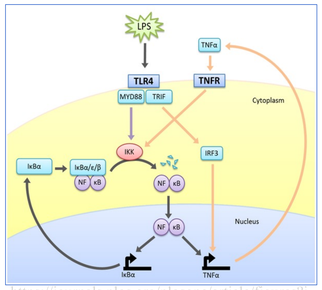
If the drugs are modulating LPS-induced inflammation through the NF-κB pathway, then we should see a reduction in TNF-α secreted.

Figure 1: Accumulation of Neurofibrillary Tangles and Amyloid Plaques in Alzheimer's Disease Patients



https://www.brightfocus.org/alzheimers-disease/infographic/amyloid-plaques-and-neurofibrillary-tangles

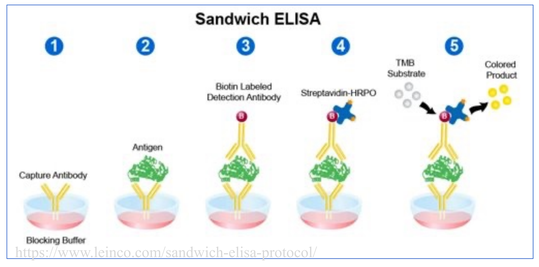
Figure 2: The NF-kB Inflammatory Pathway



https://journals.plos.org/plosone/article/figures?id=10.1371/journal.pone.0070180

Methods

- Microglial and HeLa cell culture
- MTT assay
- Drug treatment followed by LPS or TNF-α stimulant
- Enzyme-linked immunosorbent assay (ELISA, fig.3)
- Immunofluorescence and confocal imaging

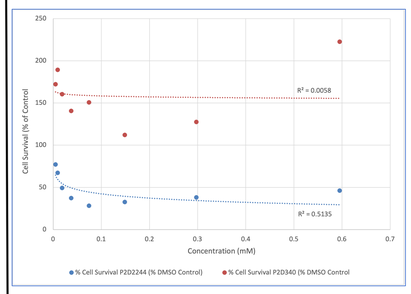


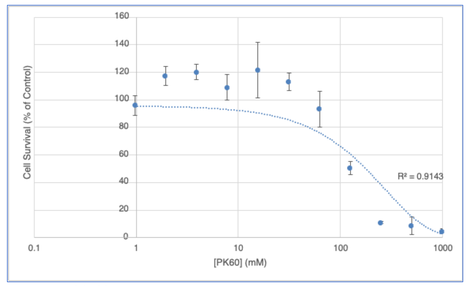
https://www.leinco.com/sandwich-elisa-protocol/

Figure 3: Schematic of an Enzyme-Linked Immunosorbent Assay (ELISA) Experiment

Results

Figure 4: Cytotoxicity of P2D340, P2D2244, and PK60





Data Provided by Cameron Bowers

Figure 6: PK60 Demonstrates a Dose-Dependent Decrease in LPS-induced TNF-α Secretion BV-2 Microglial Cells

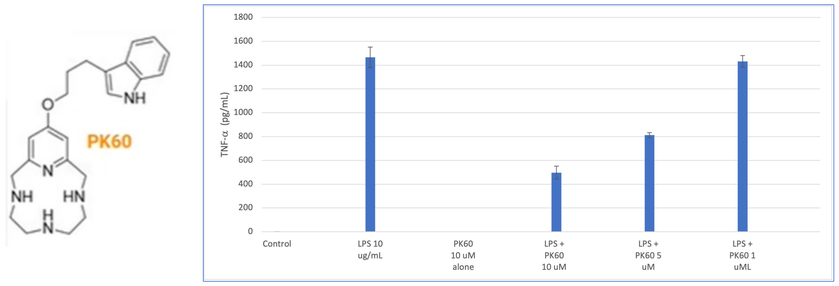


Figure 7: Fluorescently Marked NF-κB in PK60 and TNF-α-Treated HeLa Cells

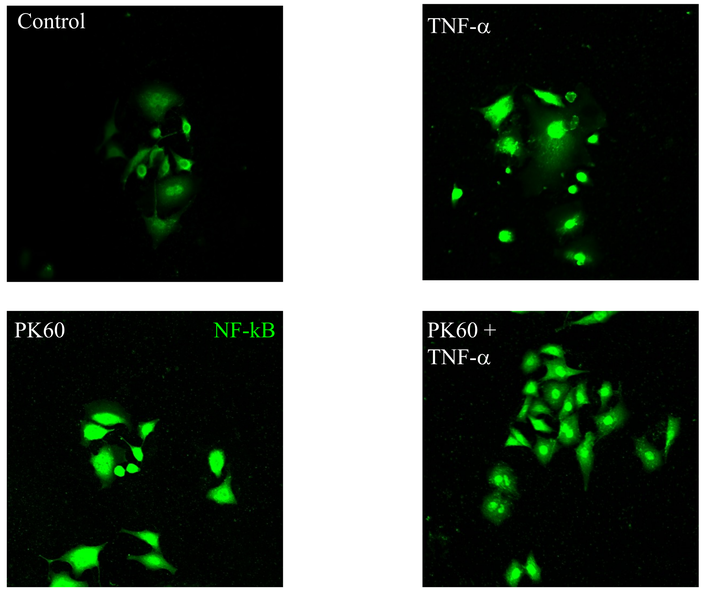
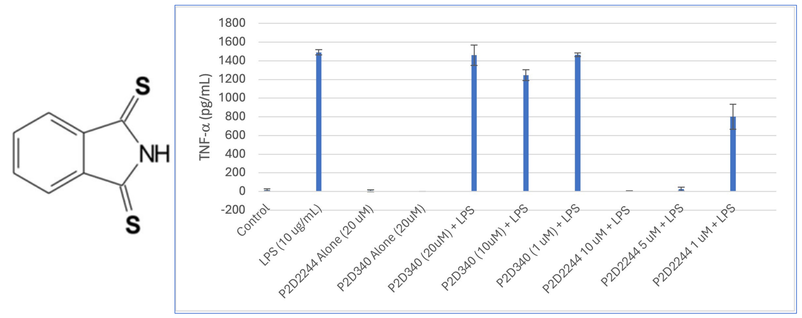


Figure 5: Effect of P2D340 and 2244 on LPS-Induced Secretion of TNF-α



Conclusions

- P2D340, P2D2244, and PK60 are relatively not cytotoxic to the cell
- P2D340 did not significantly reduce the production of TNF-α in microglial cells
- Observed profound reductions in TNF-α production in microglia treated with P2D2244
- PK60 did not prevent translocation of NF-κB into the nucleus of HeLa cells

Future Directions

- Repeat experiment using P2D2244 treatment to affirm replicability
- Assess the ability of other P2D drugs to reduce LPS-induced inflammation
- Perform immunofluorescence experiment using antibodies against NF-κB with P2D2244 to determine its mechanism of action

References

1. Division of Population Health, National Center for Chronic Disease Prevention and Health Promotion. About Alzheimer's Disease. https://www.cdc.gov/aging/alzheimers-disease-dementia/about-alzheimers.html. April 5th, 2024.

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