Studying the Mode of Action of Novel Anti-Inflammatory Drugs

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Project Overview: Alzheimer's disease (AD) is ranked as the seventh leading cause of death in the US with over 6 million Americans currently diagnosed, and that number is projected to reach about 13 million by 2050. AD is currently believed to be caused by numerous factors ranging from genetics, lifestyle, and environmental conditions. The exact pathogenesis of AD remains uncertain, but the pathology of the disease includes the presence of amyloid beta (Aβ) plaques and neurofibrillary tangles composed of the protein tau in the brain. These two proteins are normally found in the brains of healthy individuals, but Aβ peptides are often degraded under normal conditions, while tau plays a role in stabilizing our cell's cytoskeletal structures. In Alzheimer's however, these proteins are misfolded and accumulate, causing disruptions in cell signaling and neuronal death, therefore worsening the disease. Aβ plaques also activate microglial cells, which produce cytokines and induce inflammation. Cytokines are signaling molecules produced by immune cells that mediate inflammatory signaling. Activation of an inflammasome complex found in mic.oglial cells, NLRP3, leads to the production of the cytokine IL-1β which has been implicated in Alzheimer's due to its ability to induce and maintain this chronic cycle of inflammation, and possibly results in more amyloid-beta deposition. Our research looks into the mode of action of novel anti-inflammatory drugs and their potential to reduce inflammation at the level of the NLRP3 inflammasome as a mechanism to slow down the progression of AD.

Introduction

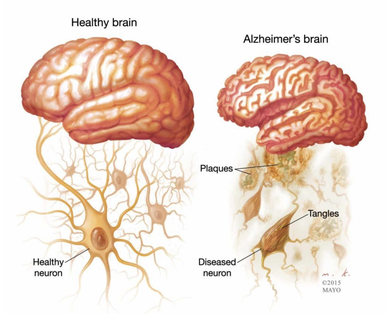

Figure 1: Alzheimer's Pathology[1]

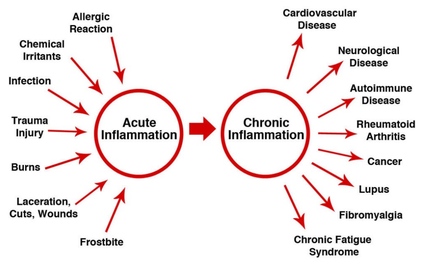

Figure 2: Effects of Chronic Inflammation[2]

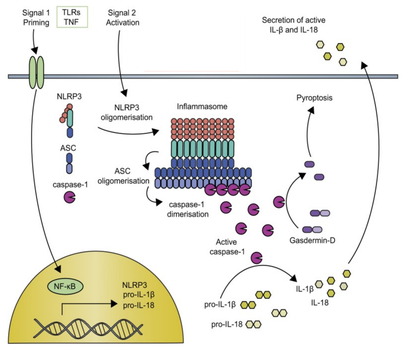

Figure 3: NLRP3 Inflammasome Pathway[3]

Results

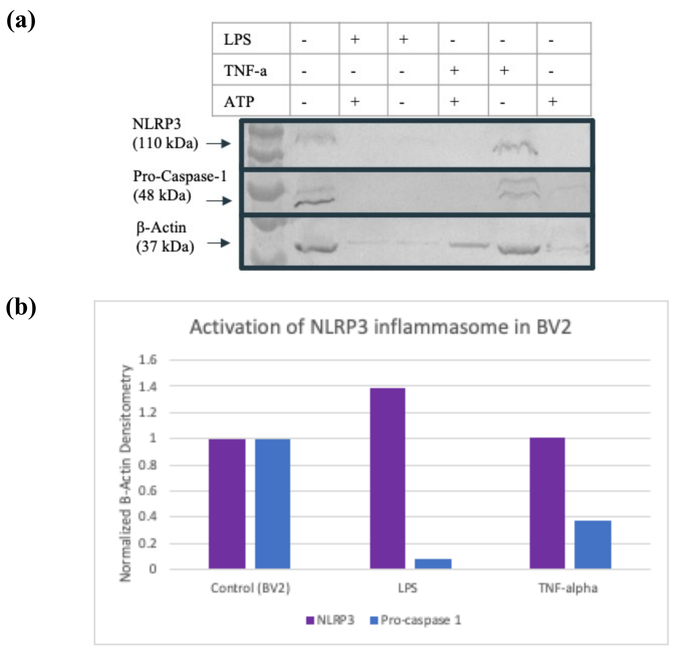

Figure 1: Effect of LPS, TNF-α, and ATP on the activation of the NLRP3 inflammasome in BV2. (a) Western Blot of BV2 treated with LPS and TNF-α (b) Quantification of Western Blot using ImageJ analysis

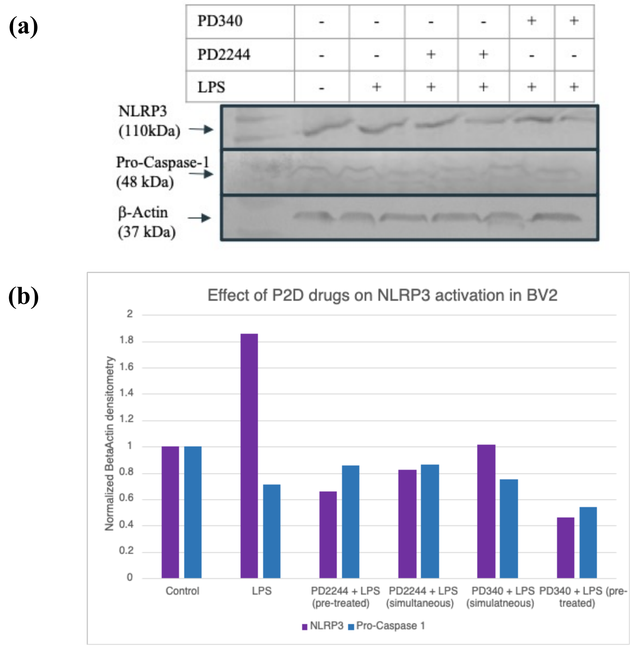

Figure 2: Effect of P2D2244 and P2D340 on the Activation of the NLRP3 inflammasome. (a) Western Blot of BV2 treated with LPS, O2D2244, and P2D340. (b) Quantification of western blot using ImageJ analysis

Methods

- Cell culture: BVS (mice microglial cells) cultured in DMEM media and incubated at 37 ºC with 5% CO_2
- Treatment: BV2 cells were treated with LPS, TNF-α, and/or P2D2244 or P2D340 for 4 hours, followed by 1 hour with ATP.
- Cells were then harvested and treated with a cell lysis buffer to break open cells and extract proteins. Protein samples were then further processed for SDS-PAGE and Western Blot.
- Western blot Images analyzed using ImageJ software.

Conclusions

- Results suggest that P2D2244 may be acting at the level of the NLRP3 inflammasome (or an upstream component of inflammation)

Future Directions

- Repeat previous experiments under identical conditions
- Look at the effect of the drugs used in this study on the activation of the NLRP3 pathway in HT22 (neuronal cells)

Funding

- This research was funded by the undergraduate SERC Research Grant Program.

Resources

(1) Mayo Foundation for Medical Education and Research. (2024b, February 13). *Alzheimer's disease*. Mayo Clinic. https://www.mayoclinic.org/diseases-conditions/alzheimers-disease/symptoms-causes/syc-20350447
(2) Solanki, S. (2022, June 6). *Inflammation: Types, causes, symptoms and properties*. RapidLeaksIndia. https://rapidleaks.com/lifestyle/health/inflammation-types-causes-symptoms-properties/
(3) Coll, Rebecca & O'Neill, LAJ & Schroder, Kate. (2016). Questions and controversies in innate immune research: what is the physiological role of NLRP3?. Cell Death Discovery. 2. 16019. 10.1038/cddiscovery.2016.19.