Investigating the Potential Therapeutic Effects of CBD *In Vitro*

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Abstract: Alzheimer's disease (AD), currently the seventh leading cause of death in the United States, is a neurodegenerative disease characterized by amyloid beta (Aβ) plaques and chronic inflammation in the brain. Microglial cells, which act as the immune cells of the central nervous system (CNS), function in response to Aβ by secreting pro-inflammatory cytokines and reactive oxygen species (ROS). Microglial activation is a healthy response in the brain, but chronic activation of these cells and thus chronic secretion of neurotoxic factors creates a cyclic process that leads to neuronal cell death. In order to protect against oxidative stress, cells activate the nuclear factor erythroid 2-related factor (Nrf2) pathway. Nrf2 is a transcription factor that regulates the expression of antioxidant enzymes, which can protect the cell from ROS. Here we focus on the therapeutic potential of cannabidiol (CBD) to mitigate oxidative stress in both microglial and peripheral macrophage cell lines. We show that CBD can activate the Nrf2 pathway and thus increases the expression of several antioxidant proteins such as Heme oxygenase-1 (HO-1). This research is significant because it could provide evidence for the use of CBD as a potential therapy in AD patients.

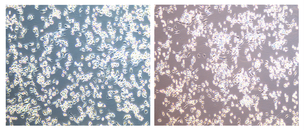
Introduction

- AD is a neurodegenerative disease characterized by progressive memory loss and dementia from neuronal damage/death
- Elements of AD pathology include the deposition of extracellular Aβ plaques and intraneuronal neurofibrillary tangles (NFTs) (Calsolaro et al., 2016)
- Aβ and NFTs exacerbate mitochondrial dysfunction and ROS production (Chen & Zhong, 2014)
- Oxidative stress is the phenomenon caused by an imbalance between production and accumulation of ROS and the ability of a biological system to detoxify these products (Pizzino et al., 2017)
- Oxidative stress plays a role in the progression of many diseases such as cardiovascular disease, diabetes, cancer, and neurodegenerative diseases (Baird & Yamamoto, 2020)
- The Keap1/Nrf2 stress response pathway is a defense system against oxidative stress (see Figure 1) (Baird & Yamamoto, 2020)
- CBD is holding an important therapeutic potential among compounds with null psychoactive properties (Jîtcă et al., 2023)
- There is limited information on the mechanism of action of CBD and its activity on the Nrf2 pathway (Atalay et al., 2022)
- Previous research in our lab showed that treating BV2 cells with CBD led to a decrease in production of pro-inflammatory cytokines (O'Connor, unpublished data)
- Hypothesis: Treating cells with CBD will lead to an increase in Nrf2

Methods

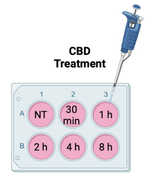
Cell Culture

- BV2, a microglia-like cell line, and RAW 264.7, a macrophage-like cell line, were grown and maintained
- Cells were seeded in a 6-well dish and incubated overnight prior to treatment



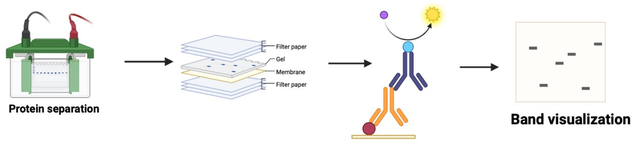
CBD Treatment

- Cells were treated with 1 µM CBD at various time points prior to collection (30 min, 1 h, 2 h, 4 h, 8 h)
- Cells were lysed and the cell lysate samples were collected and stored until needed for analysis



Western Blot Analyses

- Western Blots were performed to semi-quantify levels of Nrf2 in the samples with β-actin as the loading control
- Band pixel densities for Nrf2 and β-actin were computed using ImageJ software, and Nrf2/β-actin ratios were calculated and graphed



Discussion

- Nrf2 protein levels increase with treatment of CBD, which supports our hypothesis
- CBD may be acting as an antioxidant to help neutralize ROS
- Nrf2 increases as treatment length increases, showing duration of action of CBD on the cells is a factor
- Based on these findings and previous research in our lab, there is a strong indication that the mechanism of action of CBD involves both the Nrf2 and NF-κB pathways

Future Directions

- Confirm that CBD stabilizes Nrf2 with more Western blots and statistical analysis
- Shift analyses to a more quantitative measure like RT-PCR
- Determine whether CBD increases levels of Nrf2's antioxidant proteins such as HO-1, SOD, etc.
- Investigate CBD's mechanism of action on the Nrf2 pathway by analyzing transcript or protein levels of other known Nrf2 inhibitors such as Keap1 and GSK-3 or activators such as p62 and p21
- Confirm that CBD inhibits the NF-κB pathway
- Investigate CBD's role on the Nrf2/NF-κB cross-talk

Target Pathway

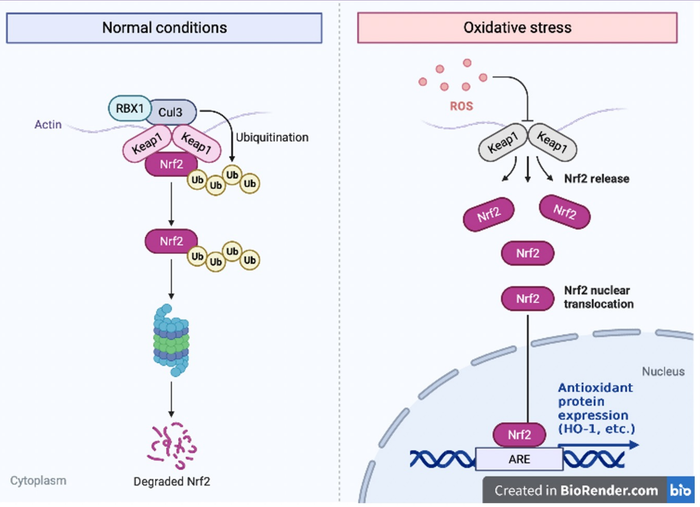


Figure 1. Schematic of the Keap1/Nrf2 pathway. Keap1 protein is the main regulator of Nrf2, a transcription factor for antioxidant proteins. Under normal conditions, Keap1 binds Nrf2 in the cytoplasm and promotes ubiquitination and proteasomal degradation of Nrf2. Under oxidative stress, Keap1 is inhibited, allowing Nrf2 to translocate into the nucleus to initiate the transcription of cytoprotective genes.

Results

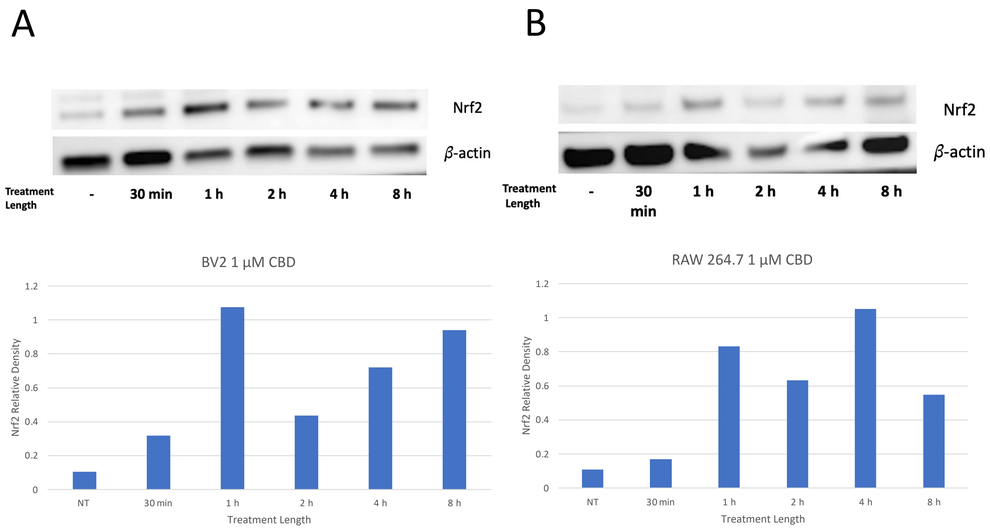


Figure 2. CBD treatment affects levels of Nrf2. A) BV2 and **B)** RAW264.7 cells were incubated with CBD (1 µM) for different periods of time as indicated or with serum-free media (-). Cells were lysed and levels of Nrf2 and β-actin were analyzed by Western blot. Upper panels are Western blots and lower panels show the semi-quantification of Nrf2 protein levels relative to β-actin (loading control) protein levels.

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