# CBD Treatment Attenuates Inflammation in Microglial Cells

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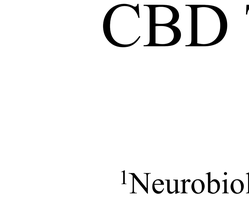
Alzheimer's Disease (AD) is a progressive neurodegenerative disease associated with old age and marked by deficits in memory and learning skills. AD pathology is characterized by amyloid-beta (AB) accumulation, which leads to plaque formation and ultimately neuronal death. Additionally, AB activates microglial cells, which function as an immune cell in the brain. Microglial cells secrete proteins that induce inflammation, known as pro-inflammatory cytokines. The chronic activation of pro-inflammatory cytokines engenders neuroinflammation and oxidative stress, which then further exacerbates AD pathologies. This project aims to study the effectiveness of cannabidiol (CBD) as a potential treatment for AD, due to its known anti-inflammatory properties. The overall goal of the research is to demonstrate the capacity of CBD to minimize the immunological mechanisms that drive AD pathologies. Our research will contribute to the understanding of the link between the immune system and central nervous system in AD development. AD is the sixth leading cause of death in America, but the availability of therapies is limited. CBD represents a natural and possible effective therapy for those suffering from Alzheimer's disease, and our research will contribute to determining its efficacy.

## Introduction

- Brain microglia function release pro-inflammatory cytokines following injury or infection (Horvath et al., 2008; Stansley et al., 2012).
- Chronic secretion of pro-inflammatory cytokines contributes to the onset and progression of AD (Block & Hong, 2005; LaFerla et al., 2007).
- Cannabidiol is a cannabinoid that contains both antioxidant and anti-inflammatory properties (Klein, 2005; Nagarkatti et al., 2009).
- CBD decreases pro-inflammatory cytokine production by microglia (Iuvone et al., 2004; Janefjord et al., 2013; Kozela et al., 2009).
- The current study investigated the ability of CBD to reduce microglial production of pro-inflammatory cytokines in response to bacterial LPS.

## Methods

- BV2 cells from an immortalized microglia cell line were maintained in a cell incubator at 37 degrees Celsius 5% $CO_2$. Cells were grown in complete cell medium. When the cells became 80-90% confluent, they were passaged following our standard protocol.



- **LPS Treatment**

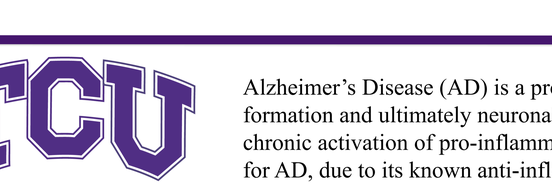



**Figure 1.** Cells were seeded on day one. At the start of day 2, cells were treated with various concentrations of LPS. Cell supernatant and lysates were collected at 4, 8, and 24 hours after LPS treatment.

- **CBD Pre-treatment and LPS Treatment**

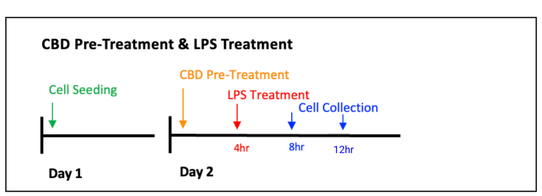



**Figure 2.** Cells were seeded on day one. At the start of day 2, cells were treated with various concentrations of CBD and treated with LPS 4 hours later. Cell supernatant and lysates were collected after 4 or 8 hours of LPS treatment.

## Results

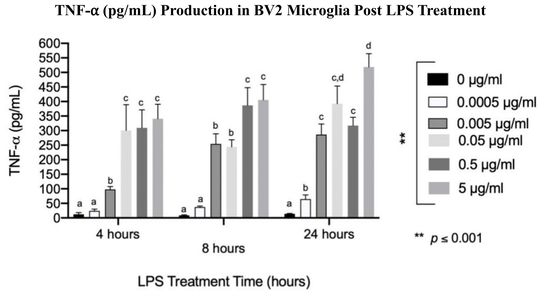



**Figure 3. LPS Treatment Induces TNF-alpha Production in BV2 Cells.** ANOVA revealed a significant main effect of LPS treatment (µg/ml) on TNF-alpha production at 4, 8, and 24 hours of LPS treatment, $p \leq 0.001$. Different letters (a,b,c,d) represent significant differences at $p \leq 0.05$. Bars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

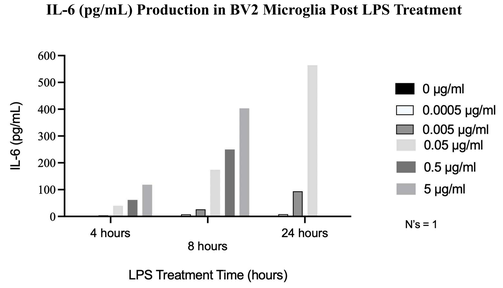



**Figure 4. LPS Treatment Induces IL-6 Production in BV2 Cells.** N's =1.

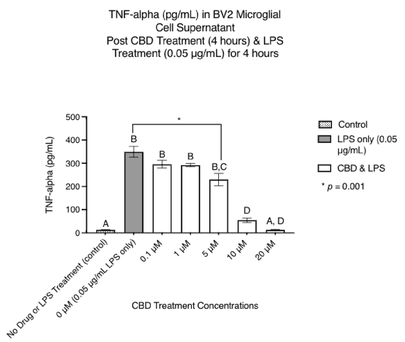



**Figure 5**. CBD Pre-treatment attenuates LPS-induced TNF-alpha production following 4 hours of LPS treatment (0.05 µg/mL of LPS).

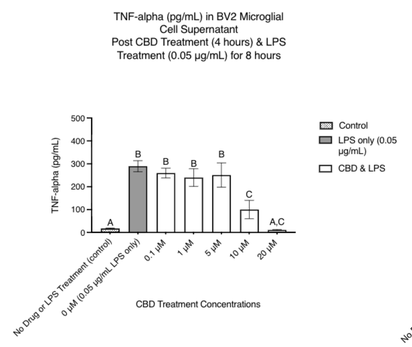



**Figure 7.** CBD Pre-treatment attenuates LPS-induced TNF-alpha production following 8 hours of LPS treatment (0.05 µg/mL of LPS).

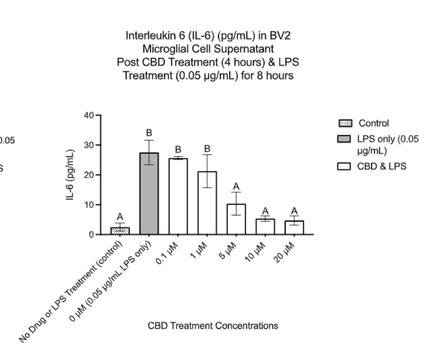



**Figure 9.** CBD pre-treatment attenuates LPS-induced IL-6 production following 8 hours of LPS treatment (0.05 µg/mL of LPS).

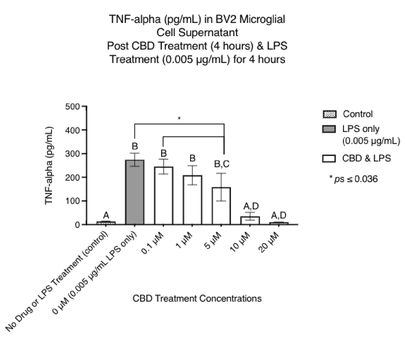



**Figure 6**. CBD Pre-treatment attenuates LPS-induced TNF-alpha production following 4 hours of LPS treatment (0.005 µg/mL of LPS).

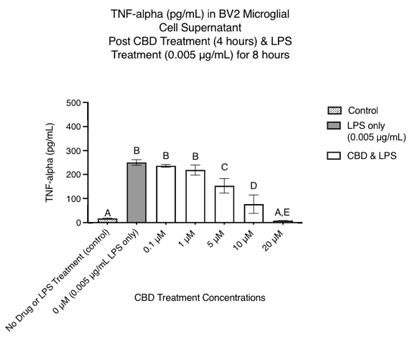



**Figure 8.** CBD Pre-treatment attenuates LPS-induced TNF-alpha production following 8 hours of LPS treatment (0.005 µg/mL of LPS).

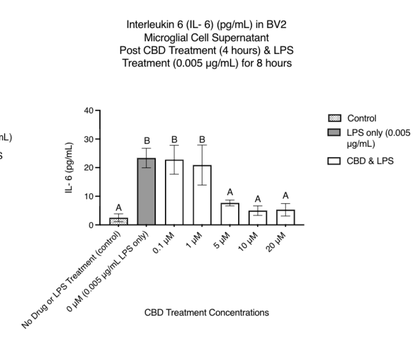



**Figure 10.** CBD pre-treatment attenuates LPS-induced IL-6 production following 8 hours of LPS treatment (0.005 µg/mL of LPS).

## Conclusions

- LPS induced an inflammatory response in BV2 microglial cells.
- CBD pre-treatment effectively reduced LPS-induced TNF-a and IL-6 production in a dose-dependent manner.
- These data suggest that CBD could potentially be used as a natural, plant-based therapeutic to reduce inflammation.
- Further studies are needed to examine the potential, therapeutic properties of CBD against chronic inflammation and AD.

**Future Studies:**

- Determine if CBD activates the Nrf2 cellular antioxidant pathway.
- Determine if CBD inhibits the signaling pathway that leads to pro-inflammatory cytokine production.
- Determine if CBD induces the production of anti-inflammatory cytokines, such as IL-4 and IL-10.

## References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