ESTABLISHMENT OF A HUMAN WHOLE BLOOD NLRP3 INFLAMMASOME ACTIVATION ASSAY FOR EVALUATING NOVEL INHIBITORS: ASSESSMENT OF CANNABIDIOL

Davison L.R.[1], Prenderville J.A.[1]

[1]*Transpharmation Ireland Ltd., Trinity College Institute of Neuroscience, Dublin, Ireland*

Introduction

Inflammation is the process by which the immune system responds to pathogen and damage-associated stimuli, and one of the main biological processes through which this is achieved is activation of the inflammasome. Inflammasomes are multi-protein complexes that regulate the production of pro-inflammatory cytokines in response to a wide range of stimuli. One of the best studied of these inflammasomes is the nucleotide-binding and oligomerization domain and leucine-rich repeat-containing pyrin domain containing 3 (NLRP3) inflammasome, which is classically activated by a two-step process involving sequential inflammatory stimuli, leading to the activation of caspase-1 and production of IL-1β and IL-18. Cannabidiol (CBD) is a phytocannabinoid with a number of reported therapeutic benefits including anti-inflammatory, antioxidant and immunomodulatory activities[1]. Recently, it has been reported that CBD is capable of inhibiting the NLRP3 inflammasome in human THP-1 monocytes through modulation of the P2X7 receptor[2]. The NLRP3 inflammasome has been shown to be activated in a number of inflammatory disorders, thus, inhibition of NLRP3 constitutes a useful therapeutic target for the attenuation of inflammation in diseases such as Parkinson's and Alzheimer's disease. Here, we established a human whole blood model of inflammasome activation and investigated the NLRP3-inhibitory potential of CBD.

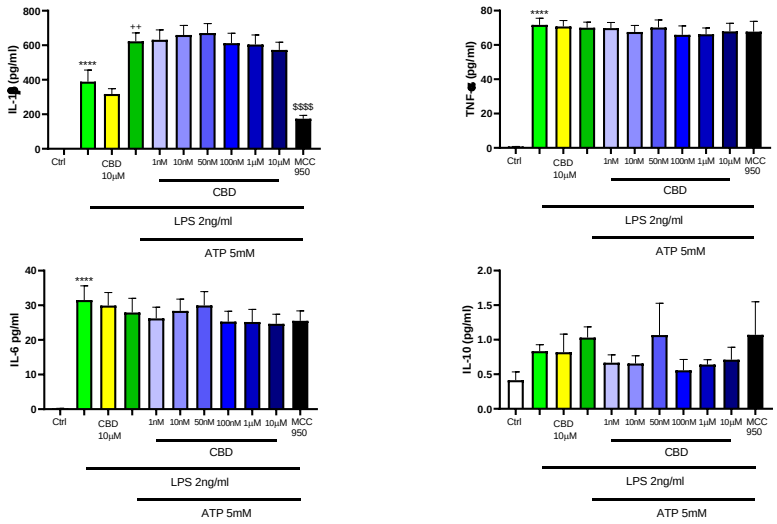
Methods

- Whole blood obtained from n=6 healthy donors
- 3 hours LPS 2ng/ml stimulation
- 30 minutes of vehicle, MCC950 NLRP3 inhibitor or CBD treatment
- 30 minutes of vehicle/5mM ATP treatment
- Whole blood supernatant levels of IL-1β, TNF-α, IL-6 and IL-10 measured using Meso Scale Diagnostics assay

Results

- ❑ Significant increase in IL-1β, TNF-α and IL-6 observed following LPS alone
- ❑ IL-1β further significantly increased with ATP treatment, which is potently reduced by MCC950
- ❑ No significant effect of CBD at any concentration for any of the cytokines measured

Results



The effect of cannabidiol on LPS and ATP-mediated NLRP3 inflammasome activation

A significant increase in the expression of IL-1β, TNF-α and IL-6 was observed following LPS stimulation alone ($P<0.0001$). IL-1β was further increased by ATP treatment ($P<0.01$). MCC950 was found to significantly reduce IL-1β release following LPS and ATP treatment ($P<0.0001$). There was no significant effect of CBD treatment at any concentration for any of the cytokines measured. Data was analysed using One-Way ANOVA and Fisher's LSD post-hoc test, data presented as mean ± SEM

Conclusions

- LPS/ATP co-stimulation of human whole blood is a robust model of NLRP3 inflammasome activation that is potently suppressed by the specific NLRP3 inflammasome inhibitor MCC950
- CBD had no significant effect on LPS/ATP-induced NLRP3 activation and subsequent IL-1β release at the time points and concentrations selected
- Future experiments will focus on increasing CBD concentration and assessing the efficacy of CBD in isolated immune cell populations such as PBMCs to assess the potential effect of plasma-protein binding of CBD in whole blood on CBD anti-inflammatory efficacy

References

[1]Atalay S, Jarocka-Karpowicz I, Skrzydlewska E. Antioxidative and Anti-Inflammatory Properties of Cannabidiol. *Antioxidants (Basel)*. 2019;9(1):21. Published 2019 Dec 25. doi:10.3390/antiox9010021

[2] Liu C, Ma H, Slitt AL, Seeram NP. Inhibitory Effect of Cannabidiol on the Activation of NLRP3 Inflammasome Is Associated with Its Modulation of the P2X7 Receptor in Human Monocytes. J Nat Prod. 2020 Jun 26;83(6):2025-2029. doi: 10.1021/acs.jnatprod.0c00138. Epub 2020 May 6. PMID: 32374168.