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

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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 the pro-inflammatory cytokines IL-1β and IL-18 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. Cannabidiol (CBD) is a phytocannabinoid with a number of reported therapeutic benefits including anti-inflammatory, antioxidant and immunomodulatory activities (Atalay et al., Antioxidants. 2020; 9(1):21). 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 (Chang et al., Journal of Natural Products 2020 83 (6), 2025-2029). This suggests that CBD can inhibit inflammasome activation in human immune cells. Here, we established a human whole blood model of inflammasome activation and investigated the NLRP3-inhibitory potential of CBD.

Methods: Human whole blood was obtained from healthy donors (n=6) and stimulated *ex vivo* for 4 hours with 2ng/ml of the TLR4 agonist lipopolysaccharide (LPS; endotoxin). Whole blood was treated with increasing concentrations of CBD (1nM, 10nM, 50nM, 100nM, 1μM, 10 μM) 3 hours after LPS treatment for 30 minutes, followed by stimulation with ATP at 5mM for 30 minutes. The specific NLRP3 inhibitor MCC950 (5μM) was used as a positive control for inflammasome inhibition. Analysis of the cytokines IL-1β, TNF-α, IL-6 and IL-10 in the whole blood supernatant was performed using a MesoScale Diagnostics assay.

Results: 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: Here, we have established a robust model of NLRP3 inflammasome activation in human whole blood and have identified MCC950 as a specific NLRP3 inhibitor that potently supresses IL-1β production. Using this model, we investigated the use of CBD as a novel NLRP3 inflammasome inhibitor. It can be concluded that CBD had no effect on IL-1β production at the time points and concentrations selected. Future experiments will focus on increasing both the concentration of CBD and treatment time, as well as carrying out a similar experiment on human peripheral blood mononuclear cells to identify the potential impact that

plasma protein binding in the whole blood milieu may have on the efficacy of CBD in this model.