IL-6/ATP Dependent Microglial Disfunction is Potentially Diminished by Treatment with Cannabidiol

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Introduction: Autism spectrum disorder (ASD) is a developmental disorder with few predictable causes nor broadly accepted treatments for core symptoms. Despite the heterogeneity of ASD, there are conserved features across affected individuals namely in differences in synaptic connectivity and immunological landscape compared to typically developing (TD) individuals; with the ASD cohort demonstrating hyperconnectivity of synapses, chronic neuroinflammation, and disrupted proteostasis.[1,2] These pathological features implicate microglia, the native immune cell to the central nervous system who responds to inflammation and guides synaptic development, in having a central role in the pathogenesis of ASD.[3,4] Treatments targeting cytokine interleukin 6 (IL-6) and purinergic signals such as adenosine triphosphate (ATP) have shown efficacy *in vivo* in reducing key symptoms of ASD,[5,6] implicating these signals as potentially critical components of ASD-specific immune dysregulation. Cannabidiol (CBD) is a non-psychotropic phytocannabinoid that has been demonstrated as an effective immunomodulator and regulator of proteostasis,[7,8] and additionally has shown some promise as an effective treatment for core symptoms of ASD.[9] The goal of this present work is to 1) demonstrate the ability of combinatorial IL-6/ATP activation of a human microglial cell line, HMC3, to recapitulate key microglial deficits observed in ASD; as well as 2) explore the capacity to which CBD abolishes these deficits.

Materials and Methods: *Cell Culture:* Human embryonic microglial cell line HMC3s were subcultured in EMEM media supplemented with 10% heat inactivated FBS. For experiments HMC3s were plated in 24 well plates at a density of 2.63×10^4 cells/cm^2. Following, they were activated with IL-6 (5 ng/mL) for 24 hours and ATP (1mM) for 30 minutes. Following this, cells were treated with CBD (1 nM, 100 nM, and 1 µM) and dexamethasone (DEX; 10 µM) for 6 hours prior to collection of media and lysates. *Endpoint Analysis*: Western blot analysis was performed for intracellular protein targets. Results were normalized to total DNA content assessed using Quant-iT^{TM} PicoGreen dsDNA Assay (Invitrogen).

Results and Discussion: Activation of HMC-3 microglia with IL-6/ATP increased levels of p-mTOR (Ser2448) as well as NOS2. p-mTOR (Ser2448) is strongly associated with mTORC1 activation, a primary governor of proteostasis.[10] This increase suggests a suppression of autophagy in favor of upregulated synthetic activity. Similarly, NOS2, a major contributor of nitric oxide species, was upregulated by IL-6/ATP. Treatment with

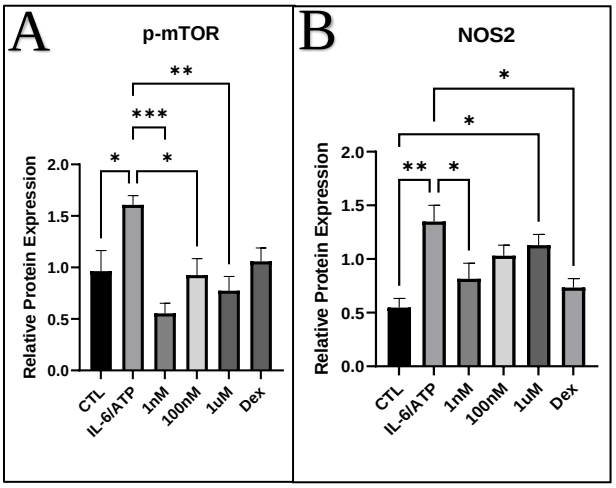

Figure 1. Relative protein expression levels of intracellular protein targets**: A** p-mTOR and **B** NOS2. Results are normalized to DNA (n = 4). *denotes significant differences between indicated groups, (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) as assessed by Tukey *post hoc* test. Error bars indicate the standard error of the mean.

CBD significantly improved IL-6/ATP driven deficits restoring levels of p-mTOR and NOS2 to those of the unactivated control (CTL). Interestingly increasing concentrations of CBD seemed to have decreasingly desirable effects, while lower concentrations of CBD seem more effectual at improving IL-6/ATP induced microglial deficits.

Conclusions: These preliminary data demonstrate the ability of CBD to alter the IL-6/ATP based activation state of HMC3 microglia as well as provides initial insight into possible mechanisms by which these effects are conferred. Future work resolving the effects of CBD on microglial ROS/nitric oxide species balance as well as impact on autophagy and phagocytosis will be necessary to understand how the cannabinoid alters microglial behavior as well as to further develop cannabinoid based therapeutic strategies towards treatment of ASD.

References: [1] DL Vargas, *Ann Neurol.* 2005. [2] RS Louros, *J Neurochem.* 2016. [3] H Kettenmann, *Neuron.* 2012. [4] T Takano, *Dev Neurosci.* 2015. [5] TB Kirsten, *PLoS One*. 2018. [6] RK Naviaux, *PLoS One.* 2013. [7] DJ Yeisley, *Cannabis and Cannabinoid Res.* 2021. [8] LC Koay, *Am J Physiol – Gastrointest Liver Physiol.* 2014. [9] S Poleg, *Prog Neuro-Psychopharmacology Biol Psychiatry*. 2018. [10] M. Rosner. *Amino Acids*. 2010.