CB2 Receptor Activation is Anti-Inflammatory in an Endotoxin-Induced Uveitis Model

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Uveitis, an inflammatory disease of the uvea, can cause vision loss. Experimental endotoxin-induced uveitis (EIU), generated by intravitreal injection (IVT) of lipopolysaccharide (LPS), induces leukocyte-endothelial interactions and the release of proinflammatory mediators that cause inflammation and can lead to tissue damage[1]. The endocannabinoid system (ECS) modulates immune cell activity, and cannabinoid drugs that interact with cannabinoid 2 receptors (CB2R) have immunomodulatory effects[2]. This study used intravital microscopy (IVM) to investigate: 1) The effects of CB2R activation on leukocyte-endothelial interactions in the iridal microcirculation. 2) The efficacy of CB2R agonists in comparison to current topical ocular anti-inflammatory treatments.

Lewis rats were divided into 11 groups: control (saline, IVT), EIU (100 ng LPS,IVT), EIU + vehicle (Tocrisolve®), EIU + vehicle (DMSO), EIU + vehicle (DMSO) + vehicle (Tocrisolve®), EIU + CB2R agonist, HU308 (1.5 µg/µL eye drop), EIU + CB2R antagonist, AM630 (2.5 mg/kg, i.v.), EIU + CB2R agonist, HU308 + CB2R antagonist AM630, EIU + Maxidex® (0.1% dexamethasone), EIU + Pred Forte® (1% prednisolone), EIU + Nevanac® (0.1% nepafenac). Drug treatments were given 15 min after EIU. IVM of the iridal microcirculation was performed in 4 locations hourly,, for 6 hrs post-LPS administration. Leukocyte adhesion was measured offline in a blinded manner. Statistical analysis was conducted by a two-way ANOVA with a Bonferroni post hoc test.

Iridial leukocyte adhesion was significantly increased at 4, 5 and 6 hr (146 ± 68, 182 ± 101, 232 ± 176, respectively, $n = 15$, $p<0.05$) after LPS in EIU animals compared with the control group at the same time-points (37 ± 23, 65 ± 51, 69 ± 33, $n = 11$, respectively). Vehicle treatment did not change leukocyte-endothelial adhesion ($p>0.05$) in animals with EIU. However, administration of HU308, significantly attenuated leukocyte adhesion at 4, 5 and 6 hr (39 ± 12, 58 ± 7, 89 ± 72, respectively, $n = 12$, $p<0.05$). No significant difference was found in leukocyte-endothelial adhesion at 6 hr in EIU animals treated with AM630 + HU308 (225 ± 102, $n = 7$, $p>0.05$). In contrast, application of AM630 alone to EIU significantly increased leukocyte adhesion at 4, 5 and 6 hr (418 ± 330, 576 ± 309, 539 ± 338, respectively, $n = 8$, $p<0.05$). The clinical drugs, dexamethasone ($n = 9$), prednisolone ($n = 8$), and nepafenac ($n = 6$) did not significantly reduce leukocyte adhesion at 6 hr of EIU (247 ± 105, 214 ± 99, 134 ± 30, respectively).

This data demonstrates that activation of CB2R by the agonist, HU308, significantly attenuates leukocyte adhesion in the iridal microvasculature in EIU. The anti-inflammatory actions of HU308 were superior to treatments of dexamethasone, prednisolone and nepafenac, which failed to significantly mitigate leukocyte adhesion. These results are consistent with the immunosuppressive action of CB2R agonists, and indicate that future drugs targeting CB2R could aid in the treatment of ocular inflammatory diseases, such as uveitis.

1. Becker MD *et al*, Investigative Ophthalmology and Visual Science 42:2563, 2001.

2. Eisenstein T *et al*, Journal of Neuroimmunology 189:17, 2007.