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EXPRESSION AND FUNCTIONAL ROLE OF ENDOCANNABINOID RECEPTORS IN DRY EYE DISEASE

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INTRODUCTION

- Dry Eye Disease (DED) is a multifactorial disease with its pathogenesis forms a vicious circle[1]
- The endocannabinoid system (ECS) and its receptors (CBRs) are present in many organs and are involved in many physiological processes (CB1R: nervous system, CB2R: immune cells)
- Stimulating CBR was reported to modulate inflammation, wound healing and pain, which are also core mechanisms of DED[4, 5]
- Are CBR ligands a potential multiple-target therapy for DED?

HYPOTHESES

- CB1R and CB2R are present at the ocular surface and its related structures and their expression varies following DED induction
- Topical application of tetrahydrocannabinol (THC), a non-selective agonist, improves experimental DED conditions

METHODS

- Expression of CB1R and CB2R: RT-qPCR and in-situ hybridization
- Experimental DED: mouse desiccating stress model[2,3] (14 days of desiccating stress, followed by a recovery phase in standard housing conditions)
- Lipophilic THC (0.5 %) was formulated in semifluorinated alkanes (F4H5) for topical use. THC was applied from day 1 of experimental DED
- Phenotypes: tear production, corneal sensitivity, corneal fluorescein staining
- Corneal nerves: beta-III-tubulin staining in corneal whole mounts
- Inflammatory cells: flow cytometry analysis of draining lymph nodes

RESULTS

1. CB1R and CB2R are present at the ocular surface and their expression alters during experimental DED

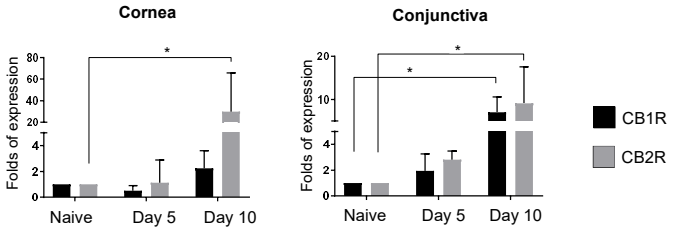

Figure 1: RT-qPCR analysis of CB1R and CB2R expression. CB1R and CB2R are present in cornea and conjunctiva. Expression increases during experimental DED.

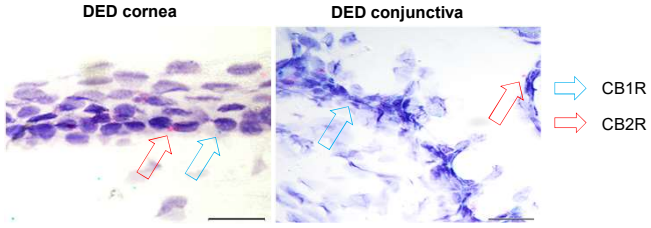

Figure 2: In-situ hybridization of cornea and conjunctiva: CB1R and CB2R are present mainly in the epithelial layer. Scale bar: 20 µm

2. Application of THC has therapeutic effects on experimental DED

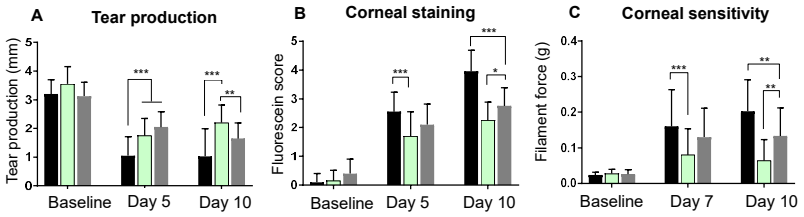

Figure 3: Ocular phenotypes following desiccating stress in untreated, F4H5 and THC/F4H5 treated mice (n=10 each). THC/F4H5 group showed a higher tear production **(A)**, lower fluorescein score **(B)** and maintained corneal sensitivity after 10 days of experimental DED **(C)**.

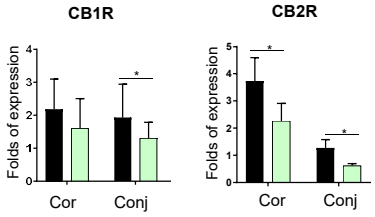

Figure 4: RT-qPCR analysis of CB1R and CB2R expression on day 10 of experimental DED. Application of THC reduced CB1R and CB2R expression compared to the untreated group (Cor: Cornea, Conj: Conjunctiva)

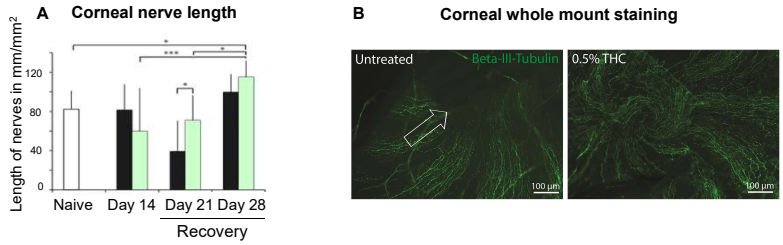

Figure 5: Corneal nerve assessment. A) Nerve length analysis in central cornea. B) beta-III tubulin staining of corneal whole mounts on day 21. THC/F4H5 showed a protective effect in the recovery phase (day 21).

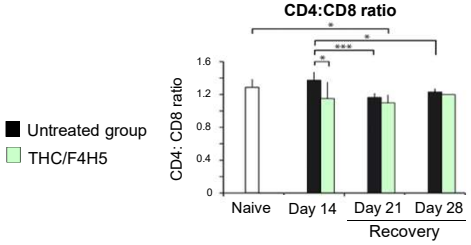

Figure 6: Flow cytometry analysis of CD4+, CD8+ T-cells in regional lymph nodes in untreated and THC/F4H5 treated mice. CD4:CD8 ratio is lower in the THC treated group than the untreated group at day 14 in experimental DED.

CONCLUSION

- CB1R and CB2R are present at the ocular surface and their expression altered after DED induction suggesting an important role of ECS in DED pathogenesis
- Topical application of THC (an agonist) has therapeutic effects on tear production, corneal damage and neurosensory-abnormalities
- CB1R and CB2R ligands are promising to become a multiple-target therapy for DED treatment

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Ref:
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