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Efficacy of topical application of cannabinoid receptor ligands in experimental dry eye disease

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PURPOSE

- Cannabinoid receptors (CBRs), as part of the endocannabinoid system, were previously reported to be expressed at the ocular surface.
- Applying CBR ligands (agonist or antagonists) can stimulate CBR functions on neuro-sensation, inflammation, and wound healing, which are core pathomechanisms of dry-eye disease (DED)[1,2].
- This study investigates CBRs functions during DED-induction and effects of CBR ligands, that are applied topically in an experimental DED mouse model.

METHODS

- DED was induced in C57/Bl6 female mice by a modified desiccating-stress (DS) protocol[3,4].
- CBR ligands were tetrahydrocannabinol (THC, agonist), SR141716A (Anta CB1, selective CBR1 antagonist), and SR144528 (Anta CB2, selective CBR2 antagonist). Drug formulations and corresponding aqueous carriers were topically applied 3 times/day from day 1 of DS.
- Clinical readouts included corneal fluorescence staining (FL) and corneal sensitivity. Data are representative of two sets of independent experiments.
- At the end of each experiment, tissues were collected and qPCR was performed to analyze the expression of CBRs and IL-1β in the corneas.
- Corneal nerve morphology were observed and semi-automatically quantified from β3-tubulin staining images.

RESULTS

1. CBR 1 and 2 expression in the cornea increases during DED induction

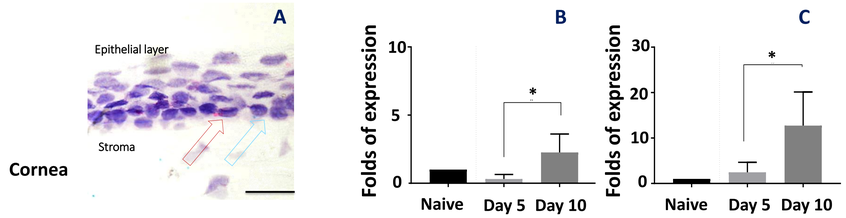

Figure 1: **A, B:** In-situ hybridization confirmed the expression of CBRs in the cornea epithelium (scale bar 20µm).
B, C: RT-qPCR results showed CBRs expressions increasing in cornea during 10 days of DS (n=5) (*: $p < 0.05$)

2. Topical application of CBR ligands reduces CBR expression

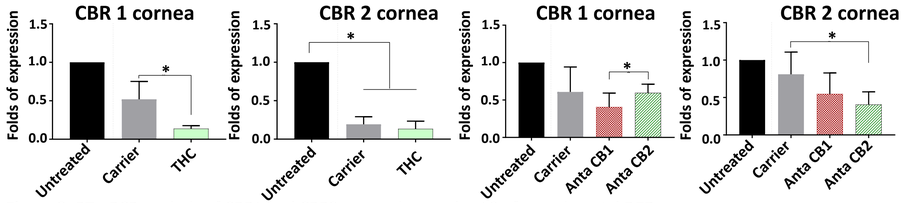

Figure 2: RT-qPCR analysis of CBR1 and CBR2 expression on day 10 of experimental DED.
Applying THC and 2 antagonists reduced CBRs expression compared to untreated DED mice (n =5).

3. Topical application of CBR ligands reduces cornea epithelial damage and inflammation

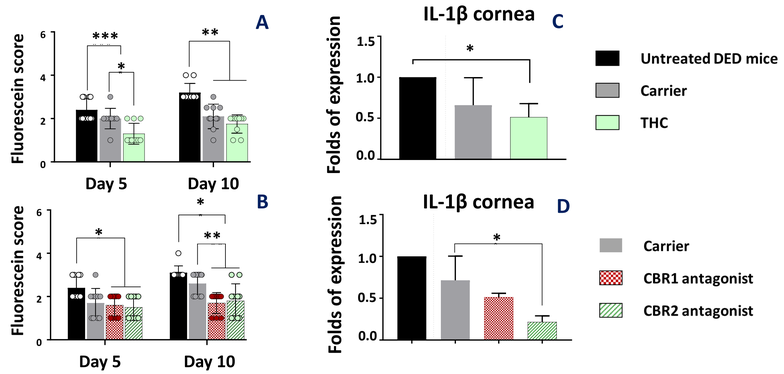

Figure 2: **A,B:** Applying CBRs ligands reduced fluorescein score (FL) of DED mice during 10 days of DS (n=5). **C, D:** RT-qPCR results of the IL-1β in the cornea at day 10 of DS (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

4. THC and Anta CB1 maintain corneal sensitivity, THC preserves corneal nerve morphology

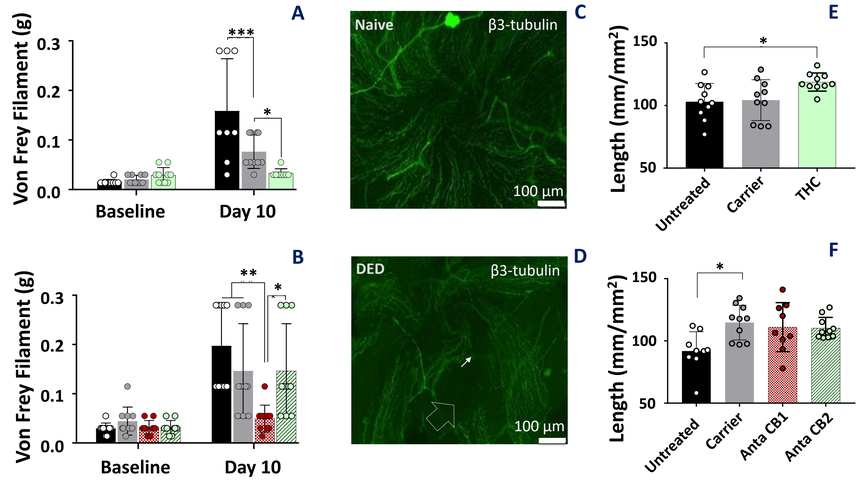

Figure 3: **A, B:** Applying THC (A) and CBR1 antagonist (B) maintained corneal sensitivity (von-Frey test).
C, D: Representative immunohistology images showed that DS reduced corneal nerve morphology (green: β3-tubulin, bar=100µm), white arrows indicate the reduction in the nerve morphology.
E, F: Effects of CBR ligands on nerve-length (in mm/mm2): only THC maintained nerve length significantly compared to untreated DED mice (n=10) (*: $p < 0.05$, **: $p < 0.01$)

CONCLUSIONS

- CBR1 and 2 seem to be involved in DED pathogenesis. CBRs increase during DED-induction and topical application of cannabinoid ligands reduces their expressions.
- THC (agonist) and selective antagonists reduced corneal epithelial damage and inflammation in the cornea after DED induction.
- Activating CBRs by THC protected nerve density, thus maintained corneal sensitivity. Meanwhile, the selective CBR1 antagonist maintained cornea sensitivity without changing nerve density, which indicated a role of CBR1 in corneal neurotransmission.
- This study adds evidence to the development progress of a new DED therapy using cannabinoids.

Literature:

1.Bron et al., TFOS DEWS II 2017
2.Toguri et al., Front. Pharm. 2016
3.Dursun et al., IOVS 2002
4.Gehlsen et al., Graefes Arch. Clinic. 2017

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