Targeting mu-opioid receptor to alleviate dry eye disease-associated chronic corneal allodynia in mice

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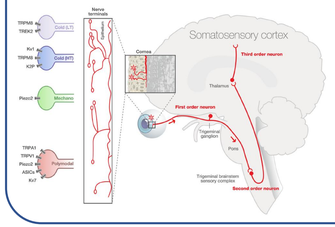
Background

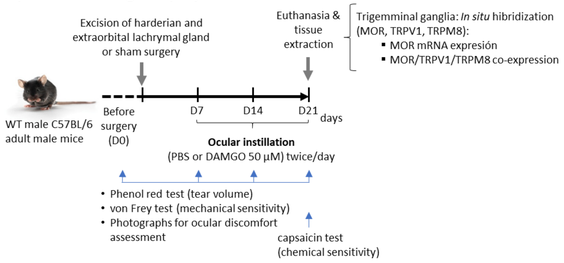
Corneal pain has recently gained recognition due to its increasing prevalence in dry eye disease (DED), a multifactorial pathology of the ocular surface (Belmonte, 2017). Corneal pain remains today as a therapeutic challenge in ophthalmology. The neural pathway implicated in DED-associated pain is initiated in the cornea (**Fig 1**), the most densely innervated epithelium by nociceptors in human body (Marfurt, 2010). Somas of corneal primary sensory neurons are located in the ophthalmic (V1) branch of trigeminal ganglion (TG, **Fig 1**) (Marfurt, 1987; Launay, 2015). Somatic pain can be modulated by exogenous and endogenous opioid peptides, which bind to mu-(MOR), delta- and kappa- opioid receptors. We recently reported that MOR is expressed in corneal nerve fibers and primary sensory neurons (Joubert, 2020). We demonstrated that repeated topical ocular administration of DAMGO ([D-Ala2,N-Me-Phe4,Gly5-ol] enkephalin), a MOR-selective ligand, markedly reduced the mechanical corneal hypersensitivity in an acute inflammatory corneal pain model (Joubert, 2020). Despite the current evidence for the analgesic effects of topical MOR agonist in acute inflammatory ocular pain model, more research is needed to evaluate their use in DED-associated chronic corneal pain.



Physiological corneal pain processing. The corneal nociceptive pathway, from peripheral nociceptors (detecting cold, mechanical or polymodal stimuli) on the corneal surface to the somatosensory cortex integrating the nociceptive input conveyed via the ophthalmic trigeminal branch (V1). Adapted from Guerrero-Moreno, 2020.

Methodology

Preclinical model of DED-associated chronic pain: Extraorbital lacrimal gland (ELG) and harderian gland (HG) excision in mice :

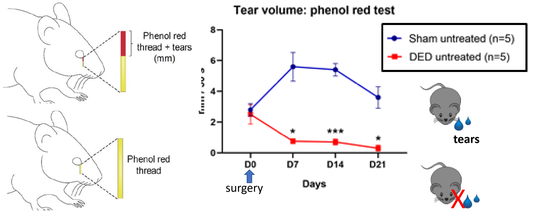


Samples: TG were obtained from sham and DED untreated mice
***In situ* hybridization:** RNAscope® method, MOR, TRPV1 and TRPM8 mRNA probes, https://acdbio.com/.
Image acquisition: Zeiss M1 epifluorescence microscope (Axio ImagerM1; Carl Zeiss).
Image analysis: ImageJ (Fiji) software
Statistical analysis: GraphPad Prism7 software

All animal procedures were performed in strict accordance with institutional guidelines for the care and use of experimental animals.

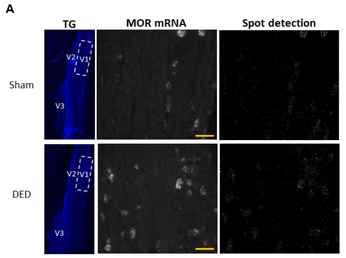
Results

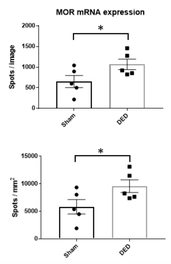
1. ELG and HG excision induces a persistent dry eye



Evaluation of tear volume by phenol red threads in awake mice. Statistical comparations performed by two-way ANOVA. Data represented as mean ± SEM.

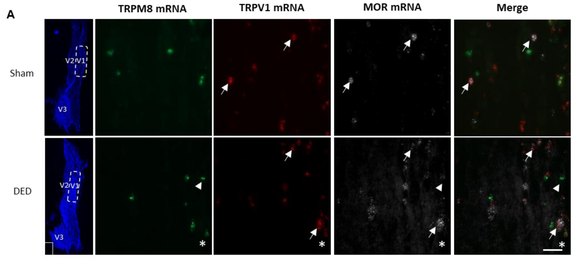
2. MOR mRNA expression increases after chronic DED in V1 branch of TG

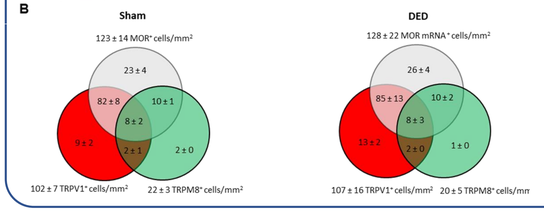




MOR mRNA levels were evaluated by *in situ* hybridization RNAscope technology. (A) dashed-line rectangle: ophthalmic branch (V1) of the TG. Right pannels: automatic detection of MOR⁺ spots by ImageJ (Fiji). Scale bar: 50 µm. (**B**) Histograms showing the quantitative analysis of the spots representing individual MOR mRNA molecules. Data as mean ± SEM. N = 5 mice per group. One-tail unpaired *t* test. *: p<0.05.

3. MOR mRNA is expressed in TRPV1⁺ and TRPM8⁺ primary sensory neurons

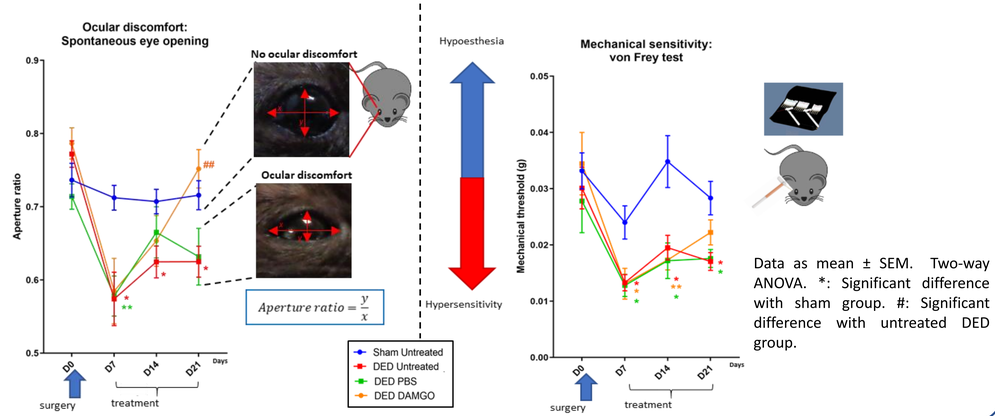




Some cells co-express TRPV1 (red) and MOR mRNA (grey, arrows), TRPM8 (green) and MOR mRNA (grey, arrowheads) or the three markers (TRPM8, TRPV1, MOR mRNA, asterisk). Scale bar: 50 µm.

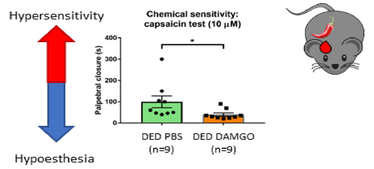
Number of cells/mm² expressing MOR (grey circle), TRPV1 (red circle) and/or TRPM8 (green circle) mRNA. 2-way ANOVA. N= 5 for sham and N=6 for DED mice.

4. Repeated topical instillations of DAMGO prevent ocular discomfort and mechanical allodynia in chronic DED mice.



Data as mean ± SEM. Two-way ANOVA. *: Significant difference with sham group. #: Significant difference with untreated DED group.

5. Repeated topical instillations of DAMGO reduced chemical hypersensitivity in chronic DED mice



Effects of repeated DAMGO treatment (from D7 to D21, twice/day) in chemical sensitivity. Chemical allodynia in DED mice treated with DAMGO or PBS quantified by capsaicin challenge test (palpebral closure time). Data as mean ± SEM. One-tail unpaired *t*-test (**C**). *: p < 0.05

Conclusions:

- Double gland excision significantly decreases tear production
- DED animals develops ocular discomfort mechanical allodynia
- MOR mRNA levels increase at the V1 branch of the TG
- Most TRPV1⁺ (polymodal) primary sensory neurons expressed MOR
- Repeated topical DAMGO reduces ocular discomfort and mechanical and chemical allodynia
- **Altogether, this data confirms MOR as a topical therapeutic target for the treatment of chronic ocular pain.**

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