UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

PROGRAMA DE MAESTRÍA Y DOCTORADO EN CIENCIAS MÉDICAS, ODONTOLÓGICAS Y DE LA SALUD

ESTUDIO DE LAS RESPUESTAS NEUROINFLAMATORIAS EN EL GANGLIO TRIGÉMINO DE RATONES CON ENFERMEDAD DEL OJO SECO Y ANÁLISIS PROSPECTIVO DEL EFECTO TERAPÉUTICO DEL SECRETOMA DE CÉLULAS TRONCALES MESENQUIMALES EN EL COMPORTAMIENTO DEL DOLOR OCULAR

TESIS
QUE PARA OPTAR POR EL GRADO DE
MAESTRA EN CIENCIAS

PRESENTA:

ROCÍO ZAMORA ORTIZ

TUTORES:

YONATHAN OMAR GARFIAS BECERRA, FACULTAD DE MEDICINA, UNAM
STEPHANE MELIK PARSADANIANTZ, INSTITUTO DE LA VISIÓN. PARIS, FRANCIA

CIUDAD DE MÉXICO, JUNIO DE 2020

ROCÍO ZAMORA ORTIZ, M.D.

Study of neuroinflammatory responses in the mouse trigeminal ganglion associated with dry eye disease and prospective analysis of therapeutic effect of mesenchymal stem cell secretome on ocular pain behavior

Institut de la Vision
UMR S 968 INSERM, UMR 7210 CNRS, UPMC

Master Recherche de Sciences et Technologie

Mention Biologie Intégrative

Spécialité : NEUROSCIENCES

2020

ABSTRACT

Dry eye disease (DED) is one of the main causes of ophthalmology office visits and accompanying ocular pain affects the quality of life in patients suffering from. Today, no single therapeutic approach of drug is satisfactory, and no topical analgesic are available to alleviate chronic ocular pain. In first, we used a well characterized aqueous and lipid deficient DED model in order to characterize the neuroinflammatory response on trigeminal ganglion (TG) from DED mice through genetic strategies. In a second time, using the same preclinical model of DED, we also start a prospective analyze of therapeutic effect of mesenchymal stem cell secretome on ocular pain behavior. Indeed, mesenchymal stem cells (MSCs) are adult pluripotent stem cells with neuroprotective, neurotrophic and neuroregenerative properties. MSCs produce a set of proteins and growth factors with various properties such as anti-inflammatory, antifibrotic, anti-apoptotic or even immunomodulatory, and constituting their secretome, which can then exert a beneficial impact on the damaged tissue notably in ocular pain induced by dry eye.

1. Background

Dry eye disease (DED) is one of the main causes of ophthalmology office visits with a prevalence ranged from 5 to 40% in population over 40 years old [1, 2]. In 2017, the last TFOS DEWS II® report redefined DED as "a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles" [3]. One of the main symptoms of DED is ocular pain, which varies in intensity and duration, affecting the quality of life in around 60% of patients suffering from DED [1, 4]. Today, no single therapeutic approach of drug is satisfactory, and no topical analgesic are available to alleviate chronic ocular pain.

Cornea is the most densely innervated tissue in mammals, its sensory and autonomic innervation is supplied by the subepithelial plexus, made of thinly myelinated (A) or unmyelinated (C type) ciliary nerves. Corneal neurons are located in the ophthalmic branch of the trigeminal ganglion (TG). These sensory neurons represent about 1.3% to 2% of all TG neurons. Throughout the progress of DED severity, many significant biological processes occur at the peripheral nervous system leading to cellular and molecular changes in the TG. These changes include modulations of intracellular signal transduction, inflammation, glial-inflammatory cells communication and synaptic transmission [5].

Inflammation is the core mechanism of DED [6], and this has been supported by previous reports where inflammatory and immunomodulatory markers have been found in the epithelium of cornea and conjunctiva samples of patients suffering from DED [7].

Mesenchymal stem cells (MSCs) are adult pluripotent stem cells, present in all organism, distinguished by the expression of surface markers as CD73, CD90, CD29, and CD105 and with the capability of differentiation in several cell types (in vitro) [8, 9]. Today, cell therapies are considered a promising approach in the treatment of pain [10]. Indeed, increasingly evidence reported that MSCs exhibit neuroprotective, neurotrophic, neuroregenerative and immunomodulatory properties. The success of stem cells treatment has been described in rodent models of neuropathic pain[11] after nerved injury of peripheral nerves [12, 13, 14] and spinal cord [15].

One objective of cell therapy is to reduce the inflammatory response. MSCs produce a set of proteins and growth factors with various properties such as anti-inflammatory, antifibrotic, anti-apoptotic or even immunomodulatory, and constituting their secretome. In addition, they are little or non-immunogenic, this property facilitating their use in cell therapy studies. In ophthalmology, the first preclinical studies with MSCs began in 2007, in models of retinal degeneration. Since then, studies have extended to many ophthalmological pathologies: glaucoma, pathologies of the ocular surface or inflammatory and immune pathologies [16]. Evidence pointed that the modulatory activity of MSCs is owing to their ability to secrete several factors that can then exert a beneficial impact on the damaged tissue. These secretions include a broad spectrum of factors, collectively called secretome and extracellular vesicles containing functional proteins and RNAs, present in the medium where the stem cells are cultured [16]. Therapeutic approach of MSCs in the eye is very promising and have already been used in hypertension and glaucoma models [17,18], as well as for retinal degeneration pathologies (as retinitis pigmentosa) [19, 20]. However, its effect on ocular pain hasn't been evaluated until then.

The aim of this study is first to determine mRNA genes expression implicated in pain and inflammation process in preclinical model of Dry eye obtained by the excision of extraorbital lachrymal gland (ELG) and Harderian gland (HG) of adult male C57BL/6 mice. Then evaluate the clinical relevance of the effect of topical MSC secretome administration on corneal pain behavior induced by severe DED in mice. To depict the effect of bone marrow-derived MSCs secretome on ocular pain, stem cells were previously stimulated (or not) with TNF-α and INF-γ (100ng/ml) to obtain an anti-inflammatory profile.

2. Methods

2.1 Experimental Animals

Six to eight weeks-old C57BL/6 male mice were used (Janvier Labs, Le Genest Saint Isle, France) and housed in secure cages with free food and water gates, under controlled day-night 12/12 hours cycles, 22 ± 1$_o$C temperature and 60 ± 10% relative humidity. The study was performed in rigorous accordance with the institutional regulations on the care of animals used for research, approved by

the European Communities Council Directive 2010/63/UE (APAFIS #15012015081815454885 v2).

2.1.1 Surgical procedures

Unilateral (right side) ELG and HG gland excisions were performed under ketamine (80 mg/kg intraperitoneal, i.p.) and xylazine (8 mg/kg i.p.) anesthesia as previously described [21]. Before the surgery, a drop of lacrimal gel (Lubrital TM laboratory Dechra) was applied to both eyes. Under an operative microscope (Leica-Alcon II, Germany), an 8-mm skin incision on the temporal side was made to expose and remove the ELG. After dissociating the conjunctival tissue above the orbital cavity near the internal canthus, the HG was carefully removed. Complete removal was verified by inspecting the surgical area for any remaining glandular tissue. The skin incision was then sutured using 6.0 braided silk sutures (Vicryl 6-0, Ethicon, Scotland). A drop of iodine solution was applied onto the incision to avoid bacterial infection. For sham animals, an incision was made in the same zone without touching the glands. The mice were placed in warm (30°C) cages to recover from the surgery. A dose of 0.03µL of buprenorphine (1:10) was given to all mice on the surgical day and the first postsurgical day.

2.1.2 Drugs and topical instillation

MSCs secretome was obtained from MSCs Long Evans rat femurs. After culturing the MSCs until passage 5 and characterizing them, 2 types of secretomes were prepared: one without stimulation of MSCs (MEM, 1% Penicillium/Streptomycin) and the other one with MSCs priming by TNF-α and INF-γ (100ng/ml) in order to obtain an anti-inflammatory profile. Secretomes were concentrated X20 using the Amicon Filter Ultra-15 Ultracel 3K by centrifugation at 4000rpm for 1 hour. Both were instilled topically, 5µL twice a day, for 14 days (between days 8 and 21 post-surgery) in right eye.

There were four groups: sham group without treatment, instilled as the control treatment group with MEM-1% P/S, one treated group instilled with non-stimulated MSCs secretome (SNS) and one treated group with stimulated TNF-alfa /INF-gamma MSCs secretome (SSti).

2.2 Corneal Fluorescein Staining

In order to evaluate corneal integrity, fluorescein staining was performed on day 7 after surgery and before starting the treatment and on day 21, after the last instillation. A drop of 1% fluorescein sodium (Fluorescein Faure 0.5%; Novartis France) was applied in the right eye of lightly anesthetized mice. Eyes were examined under cobalt blue light, using slit lamp biomicroscopy. Digital images of corneal surface was captured by a digital camera using EyeSuiteTM software (Koeniz, Switzerland) and the defects were classified based on the Oxford Grading System and previous reports[21]: 0, no staining; 0.5, slight punctate staining; 1, diffuse punctate staining; 2, diffuse staining covering less than one third of the cornea; 3, diffuse staining covering more than one third of the cornea; and 4, staining covering more than two thirds of the cornea.

2.3 Eye closing ratio measurement

Spontaneous eye closure is a good index for monitoring spontaneous eye pain [21]. Spontaneous eye closure or the eye ratio is one of the quantitative measures of the grimace scale, which is used to monitor spontaneous pain behavior [23,24]. The eye closing ratio was calculated based on photographs and corresponds to the ratio height/width. The width is the distance between the internal and external canthus and height the distance between the edge of the upper eyelid and lower eyelids, going through the center of the cornea. Images were captured by a digital camera using EyeSuite™ software (Koeniz, Switzerland). The same millimetric scale was used in all the measurements.

2.4 Confocal microscopy

A laser-scanning *in vivo* confocal microscopy (IVCM, Heidelberg Retina Tomography (HTR) II/Rostock Cornea Module (RCM; Heidelberg Engineering GmbH, Heidelberg, Germany) was used to examine the entire corneal integrity of anesthetized mice [25]. A minimum of 100 serial TIFF images, covering an area of 400 x 400 µm, was acquired per animal. The first acquisition was in the superficial center of the tissue, determined manually as 0-40 µm for epithelium, 40-140 µm for the stroma and 140-150 µm for the endothelium, as previously described [26].

2.5 Measurement of mechanical corneal sensitivity

To evaluate mechanical corneal sensitivity, von Frey filaments test was performed at D0, D7 and D21. To achieve this purpose, various forces of calibrated von Frey filaments (0.008 to 0.04g) were applied to the center of the cornea of immobilized mice. The mechanical threshold corresponded to the eye-blink response [27].

2.6 Gene expression analysis of TG at day 21

2.6.1 Tissue preparation

21 days after lachrymal gland excision, anesthetized mice were transcardially perfused with 10 mL 0.9% NaCl solution. TG were carefully removed and stored at – 80°C until use.

2.6.2 RT-qPCR analysis

RNA extraction from the ipsilateral TG was performed with a NucleoSpin RNA Purification II kit (NucleoSpin RNA S, Germany). RNA quality and concentration were then measured by the NanoDrop method (Thermo Scientific, England). Then, reverse transcription was performed by iScript cDNA Synthesis Kit (BIO-RAD) according to the manufacturer's instructions. RT-qPCR was performed with SsoAdvanced Universal SYBR® Green supermix (Bio-Rad) and pain, neuropathic and inflammatory (SAB Target List) M96 well plate (Bio-Rad ref: 10034393). The GAPDH gene was used as the endogenous reference for each reaction; mRNA levels were calculated after normalization of the results for each sample with those for GAPDH mRNA. The $2^{-\Delta\Delta Ct}$ method was used to analyze the relative differences in specific mRNA levels between groups.

2.7 Statistical analysis of data

Statistical values are reported as mean ± standard error of the mean (SEM) and analyzed with Graph-Pad Prism version 7.00 (GraphPad Software, La Jolla California USA). The level of significance (α) was set to 0.05 in all cases. Data were evaluated by unpaired and two-tailed

Student's t test (two independent groups), or one-way ANOVA test (multiple independent groups) in case of equal variances, followed by post hoc Bonferroni test when $p < 0.05$. When variable ''time'' was considered, a bifactorial analysis was performed by two-way ANOVA.

3. Results

3.1 Extraorbital lachrymal gland and Harderian gland excision induce superficial corneal epitheliopathy and reduced spontaneous eye opening

The oxford grade was measured with fluorescein staining at D7 after surgery, using a slit-lamp ophthalmoscope with cobalt blue filter and image analysis to determine corneal epithelium injury. Slit-lamp examination and fluorescein staining showed a significant difference on superficial punctate keratitis in operated animals (n = 14) (2.54 ± 0.14) compared sham group (n = 4) (1 ± 0) and at day 7 day after the surgery, (***P<0.001) (**Fig.1 and 2A**).

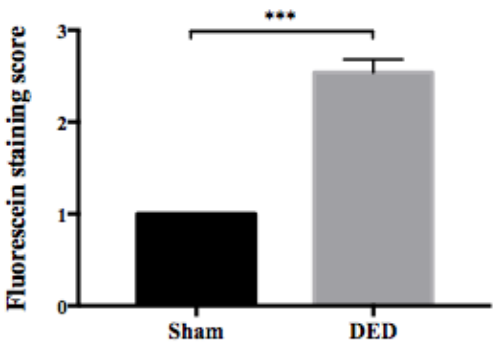


Figure 1:Corneal fluorescein test quantified at day 7 after surgery in sham and operated animals without treatment. Sham group n = 4, DED group n = 14. Results are expressed as the mean ± SEM.

The analysis of the *in vivo* confocal microscopy images showed no alteration in sham group during the study. In contrast, we reported some alterations in the corneal tissue in DED mice at day 7, before starting the treatment, notably in the epithelium corneal level (red asterisk), with the presence of dendritic cells (red arrows) and immune cells (yellow arrows) in the sub-basal plexus, and the presence of activated cells in their stroma (black arrows) (**Fig.2B**).

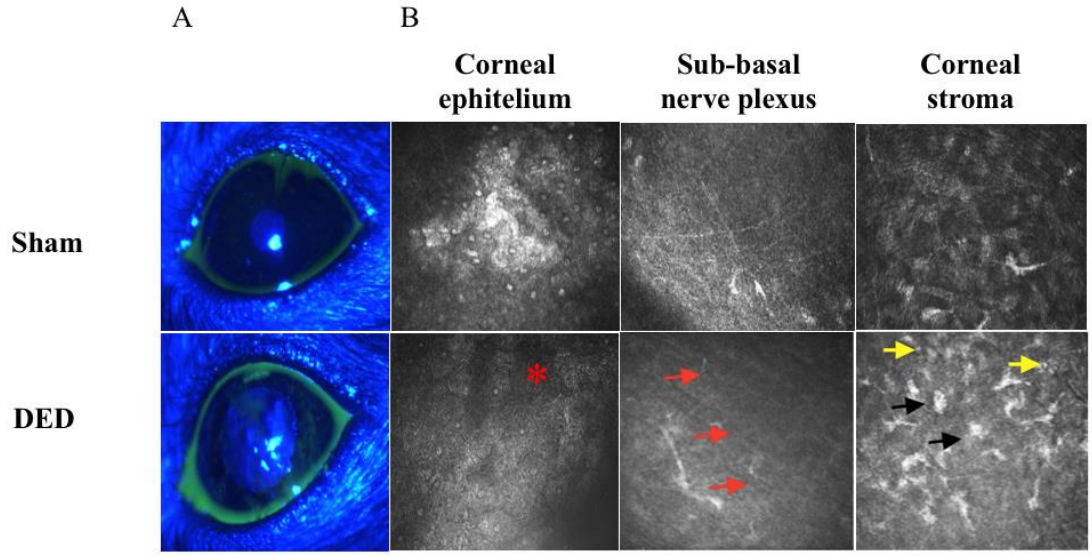


Figure 2. Corneal images acquired 7 days after surgery in sham and DED groups. (A) Corneal surface observed under slit lamp biomicroscopy examination with fluorescein. Superficial keratitis observed in DED mouse. (B) In vivo confocal microscopy. Note the alterations of the corneal epithelium (red asterisk), the long thin irregular dendritic cells in the sub- basal plexus (red arrows), small round hyper-reflective immune cells (yellow arrows), and the activated cells (black arrows) in the stroma of the DED mice.

The spontaneous eye closing ratio refers to the relation height/width in an open eye. Based on ocular photographs of lightly anesthetized mice, we considered the height as the distance between the eyelids' edges and the width as the distance between both medium and lateral canthus. The value for the sham animal was 0.74 ± 0.006, whereas for all the DED mice, at day 7, was 0.64 ± 0.004, showing a lower ratio in DED mice, which is considered an indicator of ophthalmic pain (**Fig.3**).

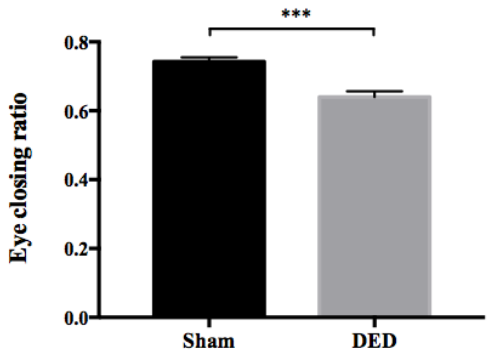
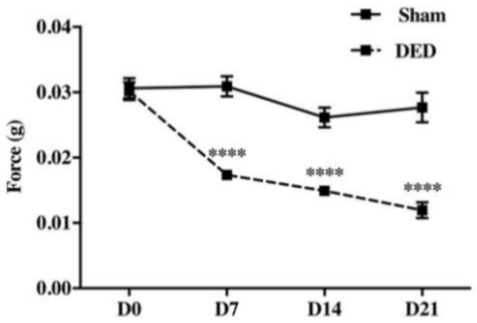


*Figure 2. Eye closing ratio of sham group and DED animals measure at day 7 after the surgery. A significant decrease in the ratio is showed in DED mice (***P<0.001).*

The corneal mechanical sensitivity was measured with von Frey filaments. At the beginning of the study (D0) we found not significantly difference between the sham animals and the DED group (0.031 ± 0.002 g vs 0.030 ± 0.001 g, P > 0.05). Sham animals remained stable during the study. The DED group showed a decreased in the corneal mechanical sensitivity by day 7 after surgery



and continued to decline until the end of the study (at D21: 0.028 ± 0.002 vs 0.012 ± 0.001 g, P < 0.0001), which reflected mechanical hypersensitivity (**Fig. 4**).

*Figure 4. Corneal mechanical sensitivity (g). The threshold was measured with von Frey filaments ****P < 0.0001 relative to the sham group. Results are expressed as the mean ± SEM.*

3.2 Extraorbital lachrymal gland and Harderian gland excisions induce upregulation of different pain and inflammatory related genes in the ipsilateral trigeminal ganglion 21 days after surgery

We evaluated by RT-qPCR analysis the expression of genes implicated in inflammatory and pain process at 21-day post-surgery in sham and DED mice. Thus, , our data showed that 28 of the 96 genes studied are significatively regulated by DED in the TG of DED mice when compared with sham mice.

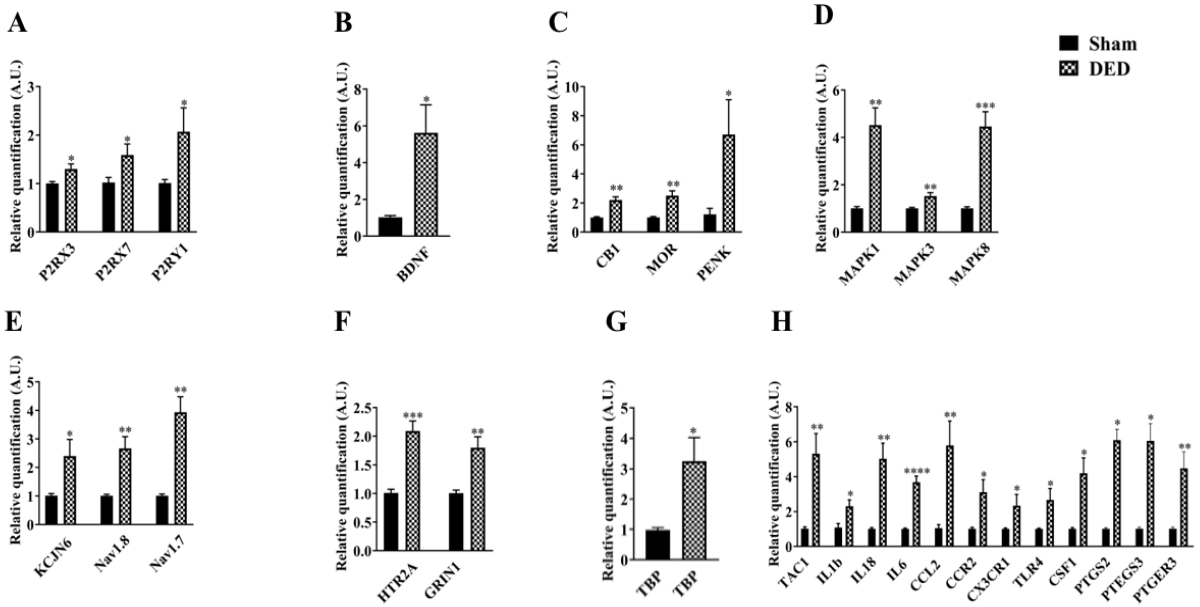


Figure 5: Real time qPCR analysis of TG from sham and DED animals at day 21 after surgery. (A), (B) Glial-inflammatory cell communications genes. (C) Pain inhibition related genes. (D) Intracellular signal transduction genes. (E) Voltage gated ion channels genes

.

3.2.1 Glial-inflammatory cells communication is increased in chronic DED

The mRNA expression levels of *P2RX3, P2RX7* and *P2RY1* in TG at 21 post-surgical day, dramatically increased by 29 %, 54.9 % and 103.96 % (P2RX3 :1.00 ± 0.03 vs 3.93 ± 1.29 ± 0.10; P2RX7: 1.02 ± 0.10 vs 1.58 ± 0.22; P2RY1: 1.01 ± 0.07 vs 2.06 ± 049, $P < 0.05$; **Fig. 5A**) in the TG of DED animals compared to sham animals. These data suggest that severe DED might provoke hyperalgesia mechanism by inducing an upregulation of these purinergic receptors.
Brain-derived neurotrophic factor (BDNF) plays a role in nociceptive hypersensitivity in the central nervous system. Its expression is induced by ATP which is controlled by purinergic receptors [28]. We showed that mRNA BDNF levels increased by 465 % in the TG of DED animals compared to sham animals (1.01 ± 0.09 vs 5.62 ± 1.52, $P < 0.05$; **Fig. 5B**).

3.2.2 Pain inhibition genes are upregulated in chronic DED

Mu opioid receptor (MOR), cannabinoid receptor 1 (CB1) and proenkephalin (PENK) are known to inhibit severe acute pain and modulate nociception [29, 30]. Thus, we observed that the mRNA levels of MOR, CB1 and PENK increased by 149 %, 121 % and 149 % in the TG of DED animals compared to sham animals (MOR :1.00 ± 0.05 vs 2.49 ± 0.33; CB1 :1.00 ± 0.05 vs 2.21 ± 0.21, $P < 0.01$ and PENK: 1.00 ± 0.05 vs 2.49 ± 0.33, $P < 0.05$; **Fig 5C**).

3.2.3 Increase of intracellular signal transduction at TG of DED animals

The mitogen-activated protein kinase (MAPK) are essential mediators of signal transduction and their activation leads to pain hypersensitivity[31]. MAPKs 1, 3 and 8 mRNA levels increased by 342 %, 49 % and 340 % (MAPK1: 1.02 ± 0.07 vs 4.51 ± 0.73, MAPK3: 1.02 ± 0.04 vs 1.52 ± 0.14, $P < 0.01$ and MAPK 8: 1.01 ± 0.06 vs 4.45 ± 0.62, $P < 0.001$; **Fig. 5D**) in TG of DED animals compared to sham animals.

Electrical excitation of peripheral somatosensory nerves is controlled by potassium and sodium ion channels[32,,33]. The level of mRNA coding for potassium channel 2 (KCJN6) increased by 136.63 % the TG of DED animals compared to sham animals (KCJN6: 1.01 ± 0.07 vs 2.39 ± 0.58, $P < 0.05$; Fig. 6). In addition, the levels of sodium channel 1.8 (Nav 1.8) and 1.7 (Nav 1.7) mRNA increased by 166 % and 293 % (Nav1.8: 1.00 ± 0.05 vs 2.66 ± 0.40; Nav1.7: 1.00 ± 0.06 vs 3.93 ± 0.53, $P < 0.01$; **Fig. 5E**) in the TG of DED animals compared to sham animals.

3.2.5 Markers of synaptic transmission increases in chronic DED

An increase of electrical excitation increases synaptic transmission. We investigated serotonin and glutamate receptors expression in the TG of DED and sham animals. Therefore, the levels of 5-Hydroxytryptamine Receptor 2A (HTR2A) and Glutamate [NMDA] receptor subunit zeta-1 (GRIN1) mRNA increased by 109 % and 79 % (HTR2A: 1.00 ± 0.06 vs 2.09 ± 0.17, $P < 0.001$; GRIN1: 1.00 ± 0.05 vs 1.79 ± 0.18, $P < 0.01$; **Fig. 5F**) in TG of DED animals.

3.2.6 TATA box binding protein gene is overexpressed in DED mice

The levels TATA-binding protein (TBP) mRNA increased by 235.05 % in TG of DED animals compared to sham animals (0.97 ± 0.08 vs 3.25 ± 0.77, $P < 0.01$; **Fig. 5G**).

3.2.7 Genes related to Inflammation are highly expressed in TG of DED animals

The mRNA levels of prostaglandin-endoperoxide synthase 2 (PTGS2) also known as cyclooxygenase-2 (COX-2), prostaglandin E synthase 3 (PTGES3) and prostaglandin E receptor 3 (PTGER3) increased by 501 %, 501 % and 342 % (PTGS2: 1.01 ± 0.07 vs 6.08 ± 0.62; PTGES3: 1.01 ± 0.09 vs 6.05 ± 0.98, $P < 0.05$ and PTGER3: 1.01 ± 0.08 vs 4.47 ± 0.94, $P < 0.01$; Fig. 5H) in TG of DED animals compared to sham animals.

Moreover, the levels of tachykinin Precursor 1 (TAC1), colony stimulating factor 1 (CSF1) and toll like receptor 4 (TLR4) mRNA increased by 421 %, 315 % and 163 % (TAC1: 1.02 ± 0.10 vs 5.31 ± 1.15, $P < 0.01$; CSF1: 1.01 ± 0.06 vs 4.19 ± 0.87, $P < 0.05$ and TLR4 :1.00 ± 0.04 vs 2.66

± 0.65, P < 0.05; **Fig. 5H**) in TG of DED animals compared to sham animals. Besides, the levels of interleukins IL1B, IL18 and IL6 mRNA increased by 113 %, 398 % and 263 % (IL1B: 1.08 ± 0.23 vs 2.30 ± 0.36, P < 0.05; IL18:1.01 ± 0.07 vs 5.03 ± 0.89, P < 0.01 and IL6 :1.00 ± 0.05 vs 3.67 ± 0.35, P < 0.0001; **Fig. 5H**) in TG of DED animals. Furthermore, the levels of the chemokine (C-C motif) ligand 2 (CCL2), C-C chemokine receptor type 2 (CCR2) and CX3C chemokine receptor 1 (CX3CR1) mRNA expression increased by 450%, 207 % and 132 % (CCL2: 1.05 ± 0.19 vs 5.78 ± 1.39, P < 0.01; CCR2: 1.01 ± 0.07 vs 3.10 ± 0.71, P < 0.05; CX3CR1: 1.00 ± 0.05 vs 2.34 ± 0.64, P < 0.05; **Fig. 5H**) in TG of DED animals.

These results highlight an upregulation of the genes implicated in pain conduction and modulation of pain responses in TG of DED animals.

3.3 Analysis of therapeutic effect of MSC secretome topical treatment on severe DED mice cornea

Twice daily topical instillations of MSC secretome or MEM 1% P/S was applied topically from 7 days after extraorbital and Harderian lacrimal glands excision, during 14 consecutive days. Sham group, with healthy corneas, was not treated. DED animals were separated into 3 treatment groups: the control treatment group with MEM-1% P/S, one group instilled with non-stimulated MSCs secretome (SNS) and one group treated with stimulated TNF-alfa and INF-gamma MSCs secretome (SSti).

By slit-lamp examination with fluorescein staining after 14 days of treatment, at D21 we observed a statistically difference in all treated DED animals according to the Oxford score, compared with the first measurement at D7, before starting the treatment (SSti 1.20 ± 0.020, SNS 1.00 ± 0.27 and MEM P/S 1.25 ± 0.25; ***P>0.001) and compared with DED animals without treatment (2.55±0.33; *P<0.05, **P<0.01, ***P<0.001) **(Fig.6).**

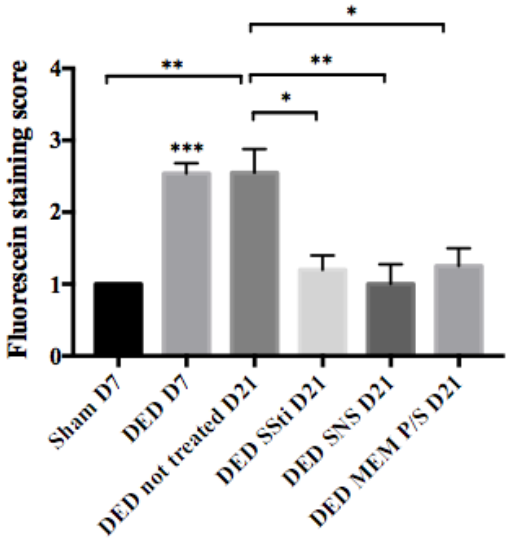


*Figure 6. Fluorescein staining was examined at D7 after surgery, before starting treatment in DED mice (Sham group n = 4 (0.88 ±0.13), DED group n=14 (2.54 ± 0.14));(***P<0.001) and also at D21 (14 days after treatment) showing a remarkable decrease in all treated mice (DED not treated n = 10 (2.55 ±0.33), SSti* n *= 5 (2.6 ± 0.25), SNS* n *= 5 (2.4 ± 0.25), MEMP/S* n *= 4 (2.5 ± 0.29)). Results are expressed as the mean ± SEM.*

The eye closing ratio of sham animals (0.75 ± 0.010) showed an improve in the different DED treated groups at the end of the study (SSti 0.76 ± 0.003, SNS 0.73 ± 0.006 and MEM group 0.703 ± 0.011) when comparing with the previous measurement at day 7, showing a statistically difference between day 7 and day 21 measures (***P<0.001) and also compared with DED animals not treated at day 21(0.65±0.022; *P<0.05, **P<0.01) (**Fig. 7**).

The mechanical allodynia was tested using von Frey filaments on treated mice. At D7 after surgery, DED mice showed mechanical hypersensitivity compared with sham animals. After the topical administration of treatment, the three groups showed an increase in the mechanical threshold, what can be interpreted as a recovery on normal corneal sensitivity (**P<0.01). Although we reported no difference between SSti, SNS and MEM groups, the statistical significance was higher in relation to DED mice not treated at day 21 (***P<0.001, ****P<0.0001) (**Fig. 8**).

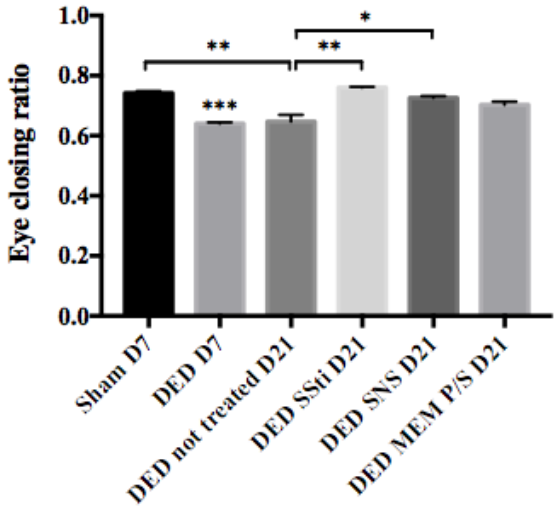


*Figure 7. Eye closing ratio. Measurement on D7 showing a significant difference between sham group and DED animals (sham 0.74 ± 0.006 and DED mice 0.64 ± 0.004); (***P<0.001) Note the raise on the ratio of treated groups at D21 (Ssti 0.76 ± 0.003, SNS 0.73±0.006 and MEM group 0.703± 0.011); compared with DED not treated animals (0.65±0.022; *P<0.05, **P<0.01).*

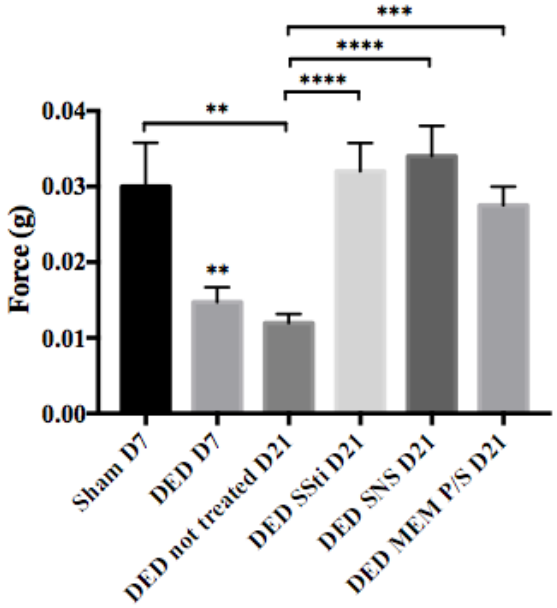


*Figure 8. Corneal mechanical sensitivity. The threshold was measured with von Frey filaments (A) At day 7 after surgery, DED animals showed an important increase in the mechanical threshold when compared with sham animals (sham 0.275 ± 0.005 and DED mice 0.015 ± 0.005) ;(***P < 0.001). (B) At day 21 , after 14 days of treatment, note the improvement on the corneal sensitivity of all treated DED mice (Ssti 0.032 ± 0.004, SNS 0.034 ± 0.004 and MEM P/S 0.039 ± 0.006);(**P<0.01) when compared with not treated DED animals (0.011±0.001; ***P<0.001, ****P<0.0001). Results are expressed as the mean ± SEM.*

4. DISCUSSION

4.1 Effect of the excision of ELG and HG glands compared with a sham group

DED symptomatology has a broad range of complaints in addition to visual ones, that includes dysesthesias, referred as “dryness”, “burning” and “aching”, between others[34]. The clinical

manifestations of DED are well known as factors that impact quality of life, assessed in severe cases, as debilitating as angina pectoris [35].

Different rodent models of DED have been studied, for example with the topical use of benzalkonium chloride[36] , the intraglandular administration of botulinum toxin[37] and the excision of extra and intraorbital lacrimal glands[38].

In this study, clinically, we observed that the excision of ELG and HG glands triggered an increased in behavioral pain when measured with eye closing ratio and corneal mechanical threshold, as well as a rise on the corneal fluorescein staining scale.

It has been considered that inflammation plays a main role in the physiopathology of DED, held by research on different tissue culture, animal models and patients results[39]. We observed a significative difference on the fluorescein score since the day 7 after surgery when compared sham and DED animals. We also found important differences in DED animals on the *in vivo* confocal microscopy as early as the 7 day after surgery [21] according to previous studies: epithelial alterations, dendritic cells in sub-basal nerve layer and activated and immune cells in the anterior stroma. In accordance with previous DED models, the immune and inflammatory response has been described with IVCM findings like activated keratocytes and cell infiltration[40]. The increased number of hyper-reflective structures in the stroma is thought that correspond to inflammatory cell infiltration and activation that could been triggered by the epithelial injury[41].

4.2 Effect of DED on the expression of gene implicated in inflammatory and pain process in TG at D21 after surgery in mice

In this study, we systematically investigated the gene expression related to neuropathic pain and inflammation on TG of a mice model of pain induced by severe DED, at day 21. Once we evaluated the different mRNA expression between sham and DED group, our data showed that 28 of the 96 genes studied might play a pivotal role in neuroinflammatory mechanisms related to this specific type of chronic pain. Thus, a better understanding of pain biomarkers is required not only to

improve the evaluation and diagnosis techniques, but to develop prognosis supports and to reach new and better therapeutic targets related to the expression of genes manipulation.

4.2.1 Glial inflammatory cells communication

Adenosine triphosphate (ATP) connects with to the purinergic signaling system including P2X and P2Y receptors and activates inflammatory cells. Besides, activation of purinergic receptors contributes to acute nociception and maintaining of nociceptive sensitivity[42]. Therefore, we investigated the mRNA levels of P2RX3, P2RX7 and P2RY1 in the TG of DED animals and sham animals. Our data showed that the excision of ELG and HG generates a severe aqueous deficient dry eye that increase some markers associated with inflammation and pain at day 21 after the surgery on the TG of those animals. In the family of P2X purinoreceptors, P2X3 receptors were firstly characterized in dorsal root ganglia neurons, and it was reported an important role in neuropathic and inflammatory pain transduction[43]. It has been found that the signaling from different soluble factors, such as BDNF, is effective in rising P2X3 receptor function and promoting phosphorylation [44]. The P2RX7 receptor is a member of a family of extracellular ATP (eATP)-gated ion channels expressed in immune cells, where its function is to activate the inflammatory cascade. This receptor is found documented in pain processes and has been investigated as a target in the treatment of infectious and inflammatory diseases[45].

4.2.2 Pain inhibition genes

CB1 or type 1 cannabinoid receptor (CB_1) is a primary constituent of the endocannabinoid system. Its receptor abundance, as well as its function, are dynamically regulated according the CB1 gene expression when there is a pathological condition or in response to drug exposure, including cannabinoids themselves[46]. Activation of cannabinoid receptors (CB1R and CB2R) induces potent anti-allodynic effects in rodent models of pain [47,48]. However, it is little known about regulation of CB1 gene expression. This increase expression of CB1, MOR and PENK genes may cause an increase of their receptor's activation, although in the other hand it may not explain why analgesia is decreased during neuropathic pain.

The mu opioid receptor (MOR) plays a key role in mediating the major clinical effects of analgesics, such as the effects of morphine [49]. In contrast to a previous study that reported a

decrease MOR mRNA in dorsal root ganglia following a chronic nerve injury[†][50], we found an increase in MOR mRNA expression. The study of different neuropathic models may explain and underlie those differences.

Proenkephalin (PENK) -derived peptides are involved in responses as pain and neuromodulation, as they control the expression of endogenous opioid peptide [51]. This is the first study that describes the mRNA levels of PENK in TG in a DED model. The increased expression in the results suggest it as a promising therapeutic target.

4.2.3 Intracellular signal transduction

Signaling pathways for neuropathic pain related to DED include the gene mitogen-activated protein kinase (MAPK) family [4]. The gene mitogen-activated protein kinase-1 (*MAPK1*), which belongs to the MAP kinase family and is also known as extracellular signal-regulated protein kinase-2 (ERK2), a pain response modulation gene MAPK3, and the cell apoptosis closed related MAPK8 were evaluated in this study. Quantification of MAPK1, MAPK3 and MAPK8 gene expression showed a significant increase in pathologic tissue of DED mice compared with normal tissue of sham, suggesting that the regulation of MAPK signaling has a potential therapeutic target for the treatment of neuropathic pain in DED, since the stimulation of endocannabinoids receptors is associated with the activation of MAPKs pathways with increased activity of different transcription factors[52].

4.2.4 Voltage ion channels

G protein-gated inwardly rectifying potassium channel (GIRK) are associated in regulation of excitatory and inhibitory neurotransmission by decreasing cell excitability through the membrane hyperpolarization with the outward potassium current induced by their opening [53]. GIRK 2 or KCNJ6[‡] is a G protein coupled channel that mediates a significant component of analgesia as it has been associated with opioid antinociception on postsynaptic inhibition[54].

† This decreased expression of the MOR may partially explain why opioid analgesia is decreased during neuropathic pain.

‡ KCNJ6: potassium inwardly rectifying channels, subfamily J, member 6.

It has been reported that KCNJ6 is expressed in dorsal root ganglia (DRG), where is selective for small unmyelinated non-peptidergic and large myelinated neurons[55]. Genetic analyses suggest that GIRK2 gene variation may predict analgesic effectiveness (GIRK2)[56]. In our study, the genetic expression of KCNJ6 was significantly increased in TG of DED mice at D21 when compared with sham group.

Voltage-gated sodium (Nav) channels are essential contributors for action potentials in excitable cells such as neurons and they are well known for their main role as analgesic targets[33]. Nine subtypes of Nav (Nav1.1–Nav1.9) are known, and each one has been described with a different physiological function in dorsal root ganglion neurons[57]. The Nav1.7 channel is highly expressed in small nociceptive neurons while the Nav1.8 channel is mainly expressed by small primary sensory neurons[58]. These channels, Nav1.7 and Nav1.8, play important roles in the development of inflammatory and neuropathic pain and therefore they have been underlined as potential molecular targets for its management [58]. In contrast to $Na_v1.7$ channels, which may play a role as threshold channels in peripheral sensory neurons, $Na_v1.8$ channels have been found to carry most of the sodium current responsible for the rising phase of the action potential[59].

Nerve injury has been found to trigger upregulation of Nav1.7 in DRG neurons and rearrangement of Nav1.7 channels, rising neuropathic pain in mice[60].

Additionally, endogenous opioids contribute to pain insensitivity in mice with Nav1.7 lack, which suggest the existence of compensatory regulation mechanisms in Nav1.7 deficient mice [61].
Gain-of-function modifications in Nav1.8 have been described in patients with neuropathic pain, which had an enhanced channel response to depolarization and hyperexcitability in DRG neurons, including reduced current threshold, increased firing frequency, and spontaneous activity[62].
Both sodium channels genes, Nav1.7 and Nav 1.8, showed a substantial increase in our DED murine model at day 21 after surgery, highlighting them as significant molecular targets.

4.2.5 Synaptic transmission genes

In our study, we found a relevant increase in the measurement of HTR2A and GRIN1 genes. The detection of the mRNA synaptic transmission genes in rodent TG untreated model guided us to

develop new approaches to further define their clinic relevant. Serotonin or 5-hydroxytryptamine (5-HT) has been identified as a central neurotransmitter of the antinociceptive system of the spinal cord[63]. From the metabotropic serotonin receptor (5-HT2R) family, which contains three isoforms[64], the 5-HT2AR is a G protein-coupled receptor (GPCR) that leads a rise in calcium release from intracellular stores[65]. The multiple interactions of 5-HT2A receptors form protein complexes, with molecules such as: the metabotropic glutamate mGlu2 receptor, dopamine D2 receptor, cannabinoid CB1 receptor, 5-HT1A receptor, the ghrelin receptor (growth hormone secretagogue receptor 1α, GHS1αR), the melatonin MT2 receptor, and the N-methyl-D-aspartate (NMDA)-gated ion channel subunit GluN2A [66]. This biological interaction is not completely elucidated but has led to identify the HT2A as a mail participant in allodynia circuits [67], and it has been reported the involvement of HTR2A gene polymorphisms on the sensitivity to chronic pain disorders [68, 69].

The GRIN1 gene encodes for the heteromeric glutamate-gated calcium ionotropic channel, the subunit zeta-1 protein of the N-methyl-D-aspartate (NMDA1) receptor, which has a fundamental role in the brain on synaptic physiology[70].

Activation of NMDARs has been described as a key for the developed and maintenance of chronic pain, known as central sensitization, due to its capability of increase calcium influx and the consequent trigger of intracellular signaling pathways, which magnify the response to nociceptive stimuli. The overload of calcium has been associated with neuron loss[71], and extra-synaptic NMDAR activation may suppress survival-promoting genes[72]. A recent study reported that NMDAR deletion at dorsal horn synapses offered neuroprotection by blocking the transition from acute to chronic pain and without disturb of nociception[73].

4.2.6 TATA box binding protein

The regulation of transcription initiation has been proposed the main way of gene expression control. The pre-initiation complex or PIC assembly is in charge of the loading of RNA polymerase II (Pol II) onto DNA[74]. The TATA-box binding protein (TBP), a single protein of intermediate size but a ubiquitous general transcription factor, was identified as a component of the TFIID factor,

one of the leading eight PIC factors[§][75]. TBP first characterization was as an activity that attaches to TATA mRNA box genes and nucleates the arrangement of the Pol II – PIC, but now is known that transcription from RNA polymerase I, II and III promoters requires TBP in different complexes, even the processes are not well understood[76].

It has become clear that the increase or decrease in TBP amount will be reflected either in gene expression or gene activity[39,77]. When neuronal and glial cells were cocultured it was reported that mutant TBP promoted a markedly inflammatory pathways activation in astrocytes, which caused neurotoxicity[78]. Further studies are necessary to explain the implication of this TBP gene rise expression on inflammation and pain on TG due to DED.

4.2.7 Inflammation related genes in DED

In a deep gene analysis reported, six genes were identified as main related with pain: FOS, IL6, TNF, TAC1, IL8, and KNG1[79]. In the present study, 12 genes related to inflammation are reported, two of them were identified as essential in the pain gene analysis and we reported a statistically significant higher value of mRNA in TG of DED mice: IL6, and TAC1.

Interleukin 6 (IL6) was the second central gene consider as a key element of pain[79]. It is one of the main cytokines of acute phase response and its over-expression has been broadly studied in association with a wide range of diseases as well as its regulatory function in many types of pathological pain, including nerve injuries[80].

TAC1 belong to the tachykinin gene family. It encodes different neuropeptides, such as Substance P, which contributes to neurogenic inflammation process and its presence in C fibers suggest the role of TAC1 onto inflammatory pain[81] .

Two important members of the IL-1 cytokine family were found statistically highly expressed in the DED group: IL-18 and IL-1 beta. IL-18 function was firstly described as a factor that induces IFN-γ production from Th1 cells. At present, it is known that has a stimulating role in both innate

[§] TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH, Pol II, and Mediator.

and acquired immunity[82]. On the other hand, IL-1 beta has already been studied as a potent pronociceptive agent in the periphery and CNS [83]. Likewise, its release as result of corneal injury-induced ocular pain has been documented[26].

We observed that mice suffering from DED showed at D21 an upregulation of CCL2 and CCR2 proinflammatory markers in the TG, with respect with sham control animals.
Evidence suggest that sensory neurons express the chemokine CCL2 and its receptor CCR2[84]. The CCL2-CCR2 axis has been implicated in the pathogenesis of a broad spectrum of diseases including neurological diseases, where directly excite nociceptive neurons and stimulate pain behavior[84].

Recently, the microglial activation of TLR4 and CCR2 signaling pathways showed to promote mechanical allodynia during repeated social defeat, a mouse model of psychosocial stress that activates microglia and rise the neuroinflammatory response[85]. Toll-like receptor 4 (TRL4) has been identified as a receptor in the microglia involved in the induction and preservation of pain in rodent models of neuropathy[86]. We found an increased mRNA expression of TRL4 gene in our murine DED model.

In this study, the pain induced by DED was accompanied by a significant increase in the levels of CX3CR1 and CSF1. The CX3CL1(fractalkine) is a soluble chemokine produced by neurons and astrocytes[87], and its receptor CX3CR1, which expression has been reported on microglial cells in the spinal cord of rats with neuropathic pain through a nerve injury induced model[88].

CSF1 is a cytokine that acts on macrophage-lineage cells. Increased levels of CSF1 are found in different neurologic pathologies including nerve injury[89]. CSF1 over-expression was described to contribute in microglial numbers and inflammatory responses[90].

Lastly, the mRNA levels of PTGS2, PTGES3, and PTGER3, which contributes to prostaglandin synthesis, were significantly higher in TG of DED animals than to their sham counterparts. COX-2, known as the prostaglandin synthase cyclooxygenase-2, catalyzes the first two steps prostaglandin synthesis and it is encoded by the PTGS2 gene, and even it can be found in both non-

inflammatory and inflammatory environments, the main expression of this gene is controlled by pro-inflammatory stimuli [91]. PTGS2 can be induced by cytokines and there are numerous signaling pathways that activates its promoter, which contains binding sites for TATA-box. It has been characterized as a therapeutic target with a basic function in pain response that presents diverse variants that are associated with pharmacodynamics[92]. One of the four known prostaglandin E2 receptor is PTGER3. It has been reported, regarding pain, that its expression provides endogenous antinociception, showing an opposite effect of the other PTGE2 receptors. Furthermore, PTGE3 receptor was found vastly expressed in spinal cord and sensory DRG of rats[93].

Chronic neuropathic pain caused by DED remains a major therapeutic challenge and new proposals are been studied; in example, the enhancement of endogenous opioid peptide concentration in anterior segment tissues[94], and the use of topic cannabinoids[95]. However, the interaction of the genes involved is required, because the final effect not depends only of the aim interaction of the ligands with the proteins, but also the molecular environment. In example, studies suggest that glial cells, mostly microglia, exert a critical task in opioid tolerance and opioid induced hyperalgesia by the activation of TLR4[96], while ion channels, such as KCNJ6, are also basic for opioids analgesic effects achievements[97]. The injury to nervous system that conducts to neuropathic pain may result in neural cells excitability, where the expression and correct function of ion channels expressed by them is the core of the promising ion channels blockers onto sensory nociceptive neurons[98]. Thus, the opioid binding to MOR ligands is not enough to achieve a satisfactory analgesic response. In this long-sought need for improved pharmacological responses, gathering research in ionic channels physiology had led to potential analgesic treatments.

4.3 Effect of the instillation of MSC secretome twice a day during 14 days in a DED characterized model

This study showed a potential anti- inflammatory effect of MSCs secretome, a set of several factors that have been proved to produce a positive impact on damage tissue, in a rodent model of corneal pain generated by DED. Our data indicates a clinical improvement in mice with DED induced by the excision of ELG and HG lacrimal glands. However, we could not find any significative difference between the groups treated with secretome SSti and SNS when compared with MEM

P/S instillation. This could be explained due to the topical administration of a medium that contributes with aminoacids, glucose and vitamins in addition to supplemented antibiotics (penicillin and streptomycin) in a severely dry corneal surface. Due to Covid-19 pandemic we neither could replicated this study in a new group of animals nor could be able to finish the complete molecular study. Our experiments could be improved with a higher number of mice, taking measurements more frequently and added a MEM without P/S treated DED group; and according to the results, the topical dose could be increased.

Current DED management help to improve the symptoms associated with DED[99], but antinociceptive treatments for this disease remains a therapeutic challenge. Nowadays, some treatments use anti-inflammatory and immunosuppressive actions of autologous serum which aims to promote tissue repair by providing growth factors[100].

Different reports have demonstrated the effect of MSCs *in vitro* and *in vivo* models of DED, showing in both cases, beneficial outcomes on inflammation. In one *in vitro* model, the stimulation of corneal human cells through pro-inflammatory cytokines TNF-alfa and IFN-gamma in culture with the secretome or in coculture with MSCs showed a decrease in the expression of inflammatory markers [101]. Two *in vivo* studies have shown the success of MSCs in decreasing inflammation reaction in DED models [102,103]. Several studies have proved the promising effect of MSCs and their secreted factors as the responsible of tissue repair [104, 105, 106,]. Hence, our study assessed a new topical treatment by considering the potential effect of MSCs secretome based on the background of precedent acute inflammatory models and a recent study that have focused the value of MSCs drafts in dry eye . Also, there have been reported in severe DED murine models the effect of MSCs when they have been applied through different ways, systemically or periorbital and the improve was significative on the lacrimal gland function in both cases[44].

Even it has been proved that MSC transplantation significantly improves the regenerative capacity of damaged lacrimal gland structures to 62% of total tissue[107], the risk of side effects of bone marrow transplant hasn't been reported. The higher amount of viable acinar structures after MSC draft corroborates the therapeutic potential of this type of therapy[107].

Using the secretome without the risk of secondary effects of drafts, based on the proposal that the trophic factors on the MSCs secretion are the responsible of the therapeutic effects[108,109,110], emerges as a promising approach for the restorative therapy of pain in patients with severe DED.

4.4 Limitations of the current study

Several limitations of this study should be considered. Research of ocular pain as a symptom of DED has lately been started. Therefore, the biomarkers related, and the curative treatment remain unknown. The number of the mice where less than the half of our main model, thus additional validation studies are needed to corroborate our findings.
We are describing for the first time the genes overexpressed in a rodent DED model TG. The molecular mechanism and associated signaling pathways of this pathology still remains largely unknown. What has become evident is that the expression levels of these genes and the protein framework are critical for the neuropathological outcomes. Despite the limitation that the analysis in this study is considered preliminary and exploratory, we present novel meaningful candidate genes associated with chronic pain in dry eye disease.

Considering that gene expression is controlled by a large amount of transcription factors and epigenetic mechanisms, there is a long way to walk, however, we have started analyzing how peripheral nerve injury alters the expression levels of many genes. Moreover, it is well known that chronic pain due to severe DED is associated with neural damage. However, the complete gene expression in the TG is still undetermined. With the increase of injury time, compared with the sham group, the gene expression profile of our mice model exposed to an induced DED, showed on TG significantly upregulation at mRNA level; it is necessary to confirm the protein expression in further studies, to understand if the up and downregulation is consistent to their gene expression at mRNA level.

Finally, the effect of topical administration of MSCs secretome in our DED model was not completely evaluated because of the quarantine due to Covid-19. The therapeutic implications of these outcomes remain to be determined. Finally, the use of topical MSCs secretome has not yet

been officially evaluated in controlled trials as treatments of ocular pain generated by DED, and consequently, it is not possible to discuss the potential therapeutic dose.

5. BIBLIOGRAPHY

(1) Réaux-Le Goazigo, A.; Labbé, A.; Baudouin, C.; Melik Parsadaniantz, S. [Towards a better understanding of chronic ocular pain]. *Med. Sci. MS* **2017**, *33* (8–9), 749–757. https://doi.org/10.1051/medsci/20173308020.

(2) Stapleton, F.; Alves, M.; Bunya, V. Y.; Jalbert, I.; Lekhanont, K.; Malet, F.; Na, K.-S.; Schaumberg, D.; Uchino, M.; Vehof, J.; Viso, E.; Vitale, S.; Jones, L. TFOS DEWS II Epidemiology Report. *Ocul. Surf.* **2017**, *15* (3), 334–365. https://doi.org/10.1016/j.jtos.2017.05.003.

(3) Craig, J. P.; Nichols, K. K.; Akpek, E. K.; Caffery, B.; Dua, H. S.; Joo, C.-K.; Liu, Z.; Nelson, J. D.; Nichols, J. J.; Tsubota, K.; Stapleton, F. TFOS DEWS II Definition and Classification Report. *Ocul. Surf.* **2017**, *15* (3), 276–283. https://doi.org/10.1016/j.jtos.2017.05.008.

(4) Belmonte, C.; Nichols, J. J.; Cox, S. M.; Brock, J. A.; Begley, C. G.; Bereiter, D. A.; Dartt, D. A.; Galor, A.; Hamrah, P.; Ivanusic, J. J.; Jacobs, D. S.; McNamara, N. A.; Rosenblatt, M. I.; Stapleton, F.; Wolffsohn, J. S. TFOS DEWS II Pain and Sensation Report. *Ocul. Surf.* **2017**, *15* (3), 404–437. https://doi.org/10.1016/j.jtos.2017.05.002.

(5) Melik Parsadaniantz, S.; Rostène, W.; Baudouin, C.; Réaux-Le Goazigo, A. [Understanding chronic ocular pain]. *Biol. Aujourdhui* **2018**, *212* (1–2), 1–11. https://doi.org/10.1051/jbio/2018017.

(6) Yamaguchi, T. Inflammatory Response in Dry Eye. *Invest. Ophthalmol. Vis. Sci.* **2018**, *59* (14), DES192–DES199. https://doi.org/10.1167/iovs.17-23651.

(7) Barabino, S.; Montaldo, E.; Solignani, F.; Valente, C.; Mingari, M. C.; Rolando, M. Immune Response in the Conjunctival Epithelium of Patients with Dry Eye. *Exp. Eye Res.* **2010**, *91* (4), 524–529. https://doi.org/10.1016/j.exer.2010.07.008.

(8) da Silva Meirelles, L.; Chagastelles, P. C.; Nardi, N. B. Mesenchymal Stem Cells Reside in Virtually All Post-Natal Organs and Tissues. *J. Cell Sci.* **2006**, *119* (Pt 11), 2204–2213. https://doi.org/10.1242/jcs.02932.

(9) Dominici, M.; Le Blanc, K.; Mueller, I.; Slaper-Cortenbach, I.; Marini, F.; Krause, D.; Deans, R.; Keating, A.; Prockop, D.; Horwitz, E. Minimal Criteria for Defining Multipotent Mesenchymal Stromal Cells. The International Society for Cellular Therapy Position Statement. *Cytotherapy* **2006**, *8* (4), 315–317. https://doi.org/10.1080/14653240600855905.

(10) Ren, J.; Liu, N.; Sun, N.; Zhang, K.; Yu, L. Mesenchymal Stem Cells and Their Exosomes: Promising Therapeutics for Chronic Pain. *Curr. Stem Cell Res. Ther.* **2019**, *14* (8), 644–653. https://doi.org/10.2174/1574888X14666190912162504.

(11) Franchi, S.; Castelli, M.; Amodeo, G.; Niada, S.; Ferrari, D.; Vescovi, A.; Brini, A. T.; Panerai, A. E.; Sacerdote, P. Adult Stem Cell as New Advanced Therapy for Experimental Neuropathic Pain Treatment. *BioMed Res. Int.* **2014**, *2014*, 470983. https://doi.org/10.1155/2014/470983.

(12) Yoo, S. H.; Lee, S. H.; Lee, S.; Park, J. H.; Lee, S.; Jin, H.; Park, H. J. The Effect of Human Mesenchymal Stem Cell Injection on Pain Behavior in Chronic Post-Ischemia Pain Mice. *Korean*

J. Pain **2020**, *33* (1), 23–29. https://doi.org/10.3344/kjp.2020.33.1.23.
(13) Zhang, E. J.; Song, C. H.; Ko, Y. K.; Lee, W. H. Intrathecal Administration of Mesenchymal Stem Cells Reduces the Reactive Oxygen Species and Pain Behavior in Neuropathic Rats. *Korean J. Pain* **2014**, *27* (3), 239–245. https://doi.org/10.3344/kjp.2014.27.3.239.
(14) Siniscalco, D.; Giordano, C.; Galderisi, U.; Luongo, L.; de Novellis, V.; Rossi, F.; Maione, S. Long-Lasting Effects of Human Mesenchymal Stem Cell Systemic Administration on Pain-like Behaviors, Cellular, and Biomolecular Modifications in Neuropathic Mice. *Front. Integr. Neurosci.* **2011**, *5*, 79. https://doi.org/10.3389/fnint.2011.00079.
(15) Vaquero, J.; Zurita, M.; Oya, S.; Santos, M. Cell Therapy Using Bone Marrow Stromal Cells in Chronic Paraplegic Rats: Systemic or Local Administration? *Neurosci. Lett.* **2006**, *398* (1–2), 129–134. https://doi.org/10.1016/j.neulet.2005.12.072.
(16) Roubeix, C.; Denoyer, A.; Brignole-Baudouin, F.; Baudouin, C. [Mesenchymal stem cell therapy, a new hope for eye disease]. *J. Fr. Ophtalmol.* **2015**, *38* (8), 764–775. https://doi.org/10.1016/j.jfo.2015.05.003.
(17) Manuguerra-Gagné, R.; Boulos, P. R.; Ammar, A.; Leblond, F. A.; Krosl, G.; Pichette, V.; Lesk, M. R.; Roy, D.-C. Transplantation of Mesenchymal Stem Cells Promotes Tissue Regeneration in a Glaucoma Model through Laser-Induced Paracrine Factor Secretion and Progenitor Cell Recruitment. *Stem Cells Dayt. Ohio* **2013**, *31* (6), 1136–1148. https://doi.org/10.1002/stem.1364.
(18) Roubeix, C.; Godefroy, D.; Mias, C.; Sapienza, A.; Riancho, L.; Degardin, J.; Fradot, V.; Ivkovic, I.; Picaud, S.; Sennlaub, F.; Denoyer, A.; Rostene, W.; Sahel, J. A.; Parsadaniantz, S. M.; Brignole-Baudouin, F.; Baudouin, C. Intraocular Pressure Reduction and Neuroprotection Conferred by Bone Marrow-Derived Mesenchymal Stem Cells in an Animal Model of Glaucoma. *Stem Cell Res. Ther.* **2015**, *6*, 177. https://doi.org/10.1186/s13287-015-0168-0.
(19) Mead, B.; Berry, M.; Logan, A.; Scott, R. A. H.; Leadbeater, W.; Scheven, B. A. Stem Cell Treatment of Degenerative Eye Disease. *Stem Cell Res.* **2015**, *14* (3), 243–257. https://doi.org/10.1016/j.scr.2015.02.003.
(20) Huo, D.-M.; Dong, F.-T.; Yu, W.-H.; Gao, F. Differentiation of Mesenchymal Stem Cell in the Microenviroment of Retinitis Pigmentosa. *Int. J. Ophthalmol.* **2010**, *3* (3), 216–219. https://doi.org/10.3980/j.issn.2222-3959.2010.03.08.
(21) Fakih, D.; Zhao, Z.; Nicolle, P.; Reboussin, E.; Joubert, F.; Luzu, J.; Labbé, A.; Rostène, W.; Baudouin, C.; Mélik Parsadaniantz, S.; Réaux-Le Goazigo, A. Chronic Dry Eye Induced Corneal Hypersensitivity, Neuroinflammatory Responses, and Synaptic Plasticity in the Mouse Trigeminal Brainstem. *J. Neuroinflammation* **2019**, *16* (1), 268. https://doi.org/10.1186/s12974-019-1656-4.
(22) Bağbaba, A.; Şen, B.; Delen, D.; Uysal, B. S. An Automated Grading and Diagnosis System for Evaluation of Dry Eye Syndrome. *J. Med. Syst.* **2018**, *42* (11), 227. https://doi.org/10.1007/s10916-018-1086-3.
(23) Langford, D. J.; Bailey, A. L.; Chanda, M. L.; Clarke, S. E.; Drummond, T. E.; Echols, S.; Glick, S.; Ingrao, J.; Klassen-Ross, T.; Lacroix-Fralish, M. L.; Matsumiya, L.; Sorge, R. E.; Sotocinal, S. G.; Tabaka, J. M.; Wong, D.; van den Maagdenberg, A. M. J. M.; Ferrari, M. D.; Craig, K. D.; Mogil, J. S. Coding of Facial Expressions of Pain in the Laboratory Mouse. *Nat. Methods* **2010**, *7* (6), 447–449. https://doi.org/10.1038/nmeth.1455.
(24) Matsumiya, L. C.; Sorge, R. E.; Sotocinal, S. G.; Tabaka, J. M.; Wieskopf, J. S.; Zaloum, A.; King, O. D.; Mogil, J. S. Using the Mouse Grimace Scale to Reevaluate the Efficacy of Postoperative Analgesics in Laboratory Mice. *J. Am. Assoc. Lab. Anim. Sci. JAALAS* **2012**, *51* (1), 42–49.

(25) Liang, H.; Baudouin, C.; Faure, M.-O.; Lambert, G.; Brignole-Baudouin, F. Comparison of the Ocular Tolerability of a Latanoprost Cationic Emulsion versus Conventional Formulations of Prostaglandins: An in Vivo Toxicity Assay. *Mol. Vis.* **2009**, *15*, 1690–1699.
(26) Launay, P.-S.; Reboussin, E.; Liang, H.; Kessal, K.; Godefroy, D.; Rostene, W.; Sahel, J.-A.; Baudouin, C.; Melik Parsadaniantz, S.; Reaux Le Goazigo, A. Ocular Inflammation Induces Trigeminal Pain, Peripheral and Central Neuroinflammatory Mechanisms. *Neurobiol. Dis.* **2016**, *88*, 16–28. https://doi.org/10.1016/j.nbd.2015.12.017.
(27) Ren, K. An Improved Method for Assessing Mechanical Allodynia in the Rat. *Physiol. Behav.* **1999**, *67* (5), 711–716. https://doi.org/10.1016/s0031-9384(99)00136-5.
(28) Klein, K.; Aeschlimann, A.; Jordan, S.; Gay, R.; Gay, S.; Sprott, H. ATP Induced Brain-Derived Neurotrophic Factor Expression and Release from Osteoarthritis Synovial Fibroblasts Is Mediated by Purinergic Receptor P2X4. *PloS One* **2012**, *7* (5), e36693. https://doi.org/10.1371/journal.pone.0036693.
(29) Madariaga-Mazón, A.; Marmolejo-Valencia, A. F.; Li, Y.; Toll, L.; Houghten, R. A.; Martinez-Mayorga, K. Mu-Opioid Receptor Biased Ligands: A Safer and Painless Discovery of Analgesics? *Drug Discov. Today* **2017**, *22* (11), 1719–1729. https://doi.org/10.1016/j.drudis.2017.07.002.
(30) Donvito, G.; Nass, S. R.; Wilkerson, J. L.; Curry, Z. A.; Schurman, L. D.; Kinsey, S. G.; Lichtman, A. H. The Endogenous Cannabinoid System: A Budding Source of Targets for Treating Inflammatory and Neuropathic Pain. *Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.* **2018**, *43* (1), 52–79. https://doi.org/10.1038/npp.2017.204.
(31) Ji, G.; Liu, D.; Liu, J.; Gao, H.; Yuan, X.; Shen, G. P38 Mitogen-Activated Protein Kinase up-Regulates NF-KappaB Transcriptional Activation through RelA Phosphorylation during Stretch-Induced Myogenesis. *Biochem. Biophys. Res. Commun.* **2010**, *391* (1), 547–551. https://doi.org/10.1016/j.bbrc.2009.11.095.
(32) Du, X.; Gamper, N. Potassium Channels in Peripheral Pain Pathways: Expression, Function and Therapeutic Potential. *Curr. Neuropharmacol.* **2013**, *11* (6), 621–640. https://doi.org/10.2174/1570159X113119990042.
(33) Cummins, T. R.; Dib-Hajj, S. D.; Black, J. A.; Waxman, S. G. Sodium Channels and the Molecular Pathophysiology of Pain. *Prog. Brain Res.* **2000**, *129*, 3–19. https://doi.org/10.1016/S0079-6123(00)29002-X.
(34) Galor, A.; Moein, H.-R.; Lee, C.; Rodriguez, A.; Felix, E. R.; Sarantopoulos, K. D.; Levitt, R. C. Neuropathic Pain and Dry Eye. *Ocul. Surf.* **2018**, *16* (1), 31–44. https://doi.org/10.1016/j.jtos.2017.10.001.
(35) Schiffman, R. M.; Walt, J. G.; Jacobsen, G.; Doyle, J. J.; Lebovics, G.; Sumner, W. Utility Assessment among Patients with Dry Eye Disease. *Ophthalmology* **2003**, *110* (7), 1412–1419. https://doi.org/10.1016/S0161-6420(03)00462-7.
(36) Lin, Z.; Liu, X.; Zhou, T.; Wang, Y.; Bai, L.; He, H.; Liu, Z. A Mouse Dry Eye Model Induced by Topical Administration of Benzalkonium Chloride. *Mol. Vis.* **2011**, *17*, 257–264.
(37) Suwan-apichon, O.; Rizen, M.; Rangsin, R.; Herretes, S.; Reyes, J. M. G.; Lekhanont, K.; Chuck, R. S. Botulinum Toxin B-Induced Mouse Model of Keratoconjunctivitis Sicca. *Invest. Ophthalmol. Vis. Sci.* **2006**, *47* (1), 133–139. https://doi.org/10.1167/iovs.05-0380.
(38) Shinomiya, K.; Ueta, M.; Kinoshita, S. A New Dry Eye Mouse Model Produced by Exorbital and Intraorbital Lacrimal Gland Excision. *Sci. Rep.* **2018**, *8*. https://doi.org/10.1038/s41598-018-19578-6.
(39) Wei, Y.; Asbell, P. A. The Core Mechanism of Dry Eye Disease Is Inflammation. *Eye Contact Lens* **2014**, *40* (4), 248–256. https://doi.org/10.1097/ICL.0000000000000042.

(40) Salomon, E.; Jiucheng, H.; Na, L.; Nicolas G, B.; Isi, E.; Haydee EP, B. Comparative in Vivo High-Resolution Confocal Microscopy of Corneal Epithelium, Sub-Basal Nerves and Stromal Cells in Mice with and without Dry Eye after Photorefractive Keratectomy. *Clin. Experiment. Ophthalmol.* **2007**, *35* (6), 545–549.
(41) Hong, J. W.; Liu, J. J.; Lee, J. S.; Mohan, R. R.; Mohan, R. R.; Woods, D. J.; He, Y. G.; Wilson, S. E. Proinflammatory Chemokine Induction in Keratocytes and Inflammatory Cell Infiltration into the Cornea. *Invest. Ophthalmol. Vis. Sci.* **2001**, *42* (12), 2795–2803.
(42) Calovi, S.; Mut-Arbona, P.; Sperlágh, B. Microglia and the Purinergic Signaling System. *Neuroscience* **2019**, *405*, 137–147. https://doi.org/10.1016/j.neuroscience.2018.12.021.
(43) Chen, L.; Liu, Y.; Yue, K.; Ru, Q.; Xiong, Q.; Ma, B.; Tian, X.; Li, C. Differential Expression of ATP-Gated P2X Receptors in DRG between Chronic Neuropathic Pain and Visceralgia Rat Models. *Purinergic Signal.* **2016**, *12* (1), 79–87. https://doi.org/10.1007/s11302-015-9481-4.
(44) Xiang, Z.; Bo, X.; Burnstock, G. Localization of ATP-Gated P2X Receptor Immunoreactivity in Rat Sensory and Sympathetic Ganglia. *Neurosci. Lett.* **1998**, *256* (2), 105–108. https://doi.org/10.1016/s0304-3940(98)00774-5.
(45) Young, C. N. J.; Górecki, D. C. P2RX7 Purinoceptor as a Therapeutic Target-The Second Coming? *Front. Chem.* **2018**, *6*, 248. https://doi.org/10.3389/fchem.2018.00248.
(46) Laprairie, R. B.; Kelly, M. E. M.; Denovan-Wright, E. M. The Dynamic Nature of Type 1 Cannabinoid Receptor (CB(1)) Gene Transcription. *Br. J. Pharmacol.* **2012**, *167* (8), 1583–1595. https://doi.org/10.1111/j.1476-5381.2012.02175.x.
(47) Deng, L.; Guindon, J.; Vemuri, V. K.; Thakur, G. A.; White, F. A.; Makriyannis, A.; Hohmann, A. G. The Maintenance of Cisplatin- and Paclitaxel-Induced Mechanical and Cold Allodynia Is Suppressed by Cannabinoid CB_2 Receptor Activation and Independent of CXCR4 Signaling in Models of Chemotherapy-Induced Peripheral Neuropathy. *Mol. Pain* **2012**, *8*, 71. https://doi.org/10.1186/1744-8069-8-71.
(48) Vera, G.; Cabezos, P. A.; Martín, M. I.; Abalo, R. Characterization of Cannabinoid-Induced Relief of Neuropathic Pain in a Rat Model of Cisplatin-Induced Neuropathy. *Pharmacol. Biochem. Behav.* **2013**, *105*, 205–212. https://doi.org/10.1016/j.pbb.2013.02.008.
(49) Kieffer, B. L.; Evans, C. J. Opioid Tolerance-in Search of the Holy Grail. *Cell* **2002**, *108* (5), 587–590. https://doi.org/10.1016/s0092-8674(02)00666-9.
(50) Lee, C.-Y.; Perez, F. M.; Wang, W.; Guan, X.; Zhao, X.; Fisher, J. L.; Guan, Y.; Sweitzer, S. M.; Raja, S. N.; Tao, Y.-X. Dynamic Temporal and Spatial Regulation of Mu Opioid Receptor Expression in Primary Afferent Neurons Following Spinal Nerve Injury. *Eur. J. Pain Lond. Engl.* **2011**, *15* (7), 669–675. https://doi.org/10.1016/j.ejpain.2010.11.018.
(51) Le Merrer, J.; Becker, J. A. J.; Befort, K.; Kieffer, B. L. Reward Processing by the Opioid System in the Brain. *Physiol. Rev.* **2009**, *89* (4), 1379–1412. https://doi.org/10.1152/physrev.00005.2009.
(52) Ghonghadze, M.; Pachkoria, K.; Okujava, M.; Antelava, N.; Gongadze, N. ENDOCANNABINOIDS RECEPTORS MEDIATED CENTRAL AND PERIPHERAL EFFECTS (REVIEW). *Georgian Med. News* **2020**, No. 298, 137–143.
(53) Kano, H.; Toyama, Y.; Imai, S.; Iwahashi, Y.; Mase, Y.; Yokogawa, M.; Osawa, M.; Shimada, I. Structural Mechanism Underlying G Protein Family-Specific Regulation of G Protein-Gated Inwardly Rectifying Potassium Channel. *Nat. Commun.* **2019**, *10* (1), 2008. https://doi.org/10.1038/s41467-019-10038-x.
(54) Lötsch, J.; Prüss, H.; Veh, R. W.; Doehring, A. A KCNJ6 (Kir3.2, GIRK2) Gene Polymorphism Modulates Opioid Effects on Analgesia and Addiction but Not on Pupil Size.

Pharmacogenet. Genomics **2010**, *20* (5), 291–297. https://doi.org/10.1097/FPC.0b013e3283386bda.
(55) Lyu, C.; Mulder, J.; Barde, S.; Sahlholm, K.; Zeberg, H.; Nilsson, J.; Århem, P.; Hökfelt, T.; Fried, K.; Shi, T.-J. S. G Protein-Gated Inwardly Rectifying Potassium Channel Subunits 1 and 2 Are down-Regulated in Rat Dorsal Root Ganglion Neurons and Spinal Cord after Peripheral Axotomy. *Mol. Pain* **2015**, *11*, 44. https://doi.org/10.1186/s12990-015-0044-z.
(56) Tsantoulas, C. Emerging Potassium Channel Targets for the Treatment of Pain. *Curr. Opin. Support. Palliat. Care* **2015**, *9* (2), 147–154. https://doi.org/10.1097/SPC.0000000000000131.
(57) Chang, W.; Berta, T.; Kim, Y. H.; Lee, S.; Lee, S.-Y.; Ji, R.-R. Expression and Role of Voltage-Gated Sodium Channels in Human Dorsal Root Ganglion Neurons with Special Focus on Nav1.7, Species Differences, and Regulation by Paclitaxel. *Neurosci. Bull.* **2018**, *34* (1), 4–12. https://doi.org/10.1007/s12264-017-0132-3.
(58) Dib-Hajj, S. D.; Black, J. A.; Waxman, S. G. Voltage-Gated Sodium Channels: Therapeutic Targets for Pain. *Pain Med. Malden Mass* **2009**, *10* (7), 1260–1269. https://doi.org/10.1111/j.1526-4637.2009.00719.x.
(59) Black, J. A.; Nikolajsen, L.; Kroner, K.; Jensen, T. S.; Waxman, S. G. Multiple Sodium Channel Isoforms and Mitogen-Activated Protein Kinases Are Present in Painful Human Neuromas. *Ann. Neurol.* **2008**, *64* (6), 644–653. https://doi.org/10.1002/ana.21527.
(60) Hameed, S. Nav1.7 and Nav1.8: Role in the Pathophysiology of Pain. *Mol. Pain* **2019**, *15*, 1744806919858801. https://doi.org/10.1177/1744806919858801.
(61) Minett, M. S.; Pereira, V.; Sikandar, S.; Matsuyama, A.; Lolignier, S.; Kanellopoulos, A. H.; Mancini, F.; Iannetti, G. D.; Bogdanov, Y. D.; Santana-Varela, S.; Millet, Q.; Baskozos, G.; MacAllister, R.; Cox, J. J.; Zhao, J.; Wood, J. N. Endogenous Opioids Contribute to Insensitivity to Pain in Humans and Mice Lacking Sodium Channel Nav1.7. *Nat. Commun.* **2015**, *6*, 8967. https://doi.org/10.1038/ncomms9967.
(62) Sopacua, M.; Hoeijmakers, J. G. J.; Merkies, I. S. J.; Lauria, G.; Waxman, S. G.; Faber, C. G. Small-Fiber Neuropathy: Expanding the Clinical Pain Universe. *J. Peripher. Nerv. Syst. JPNS* **2019**, *24* (1), 19–33. https://doi.org/10.1111/jns.12298.
(63) Mense, S. Neurobiological Concepts of Fibromyalgia--the Possible Role of Descending Spinal Tracts. *Scand. J. Rheumatol. Suppl.* **2000**, *113*, 24–29. https://doi.org/10.1080/030097400446599.
(64) Julius, D.; Huang, K. N.; Livelli, T. J.; Axel, R.; Jessell, T. M. The 5HT2 Receptor Defines a Family of Structurally Distinct but Functionally Conserved Serotonin Receptors. *Proc. Natl. Acad. Sci. U. S. A.* **1990**, *87* (3), 928–932. https://doi.org/10.1073/pnas.87.3.928.
(65) Hannon, J.; Hoyer, D. Molecular Biology of 5-HT Receptors. *Behav. Brain Res.* **2008**, *195* (1), 198–213. https://doi.org/10.1016/j.bbr.2008.03.020.
(66) Felsing, D. E.; Anastasio, N. C.; Miszkiel, J. M.; Gilbertson, S. R.; Allen, J. A.; Cunningham, K. A. Biophysical Validation of Serotonin 5-HT2A and 5-HT2C Receptor Interaction. *PloS One* **2018**, *13* (8), e0203137. https://doi.org/10.1371/journal.pone.0203137.
(67) Alba-Delgado, C.; Mountadem, S.; Mermet-Joret, N.; Monconduit, L.; Dallel, R.; Artola, A.; Antri, M. 5-HT2A Receptor-Induced Morphological Reorganization of PKCγ-Expressing Interneurons Gates Inflammatory Mechanical Allodynia in Rat. *J. Neurosci. Off. J. Soc. Neurosci.* **2018**, *38* (49), 10489–10504. https://doi.org/10.1523/JNEUROSCI.1294-18.2018.
(68) Nicholl, B. I.; Holliday, K. L.; Macfarlane, G. J.; Thomson, W.; Davies, K. A.; O'Neill, T. W.; Bartfai, G.; Boonen, S.; Casanueva, F. F.; Finn, J. D.; Forti, G.; Giwercman, A.; Huhtaniemi, I. T.; Kula, K.; Punab, M.; Silman, A. J.; Vanderschueren, D.; Wu, F. C. W.; McBeth, J.; European Male Ageing Study Group. Association of HTR2A Polymorphisms with Chronic Widespread Pain

and the Extent of Musculoskeletal Pain: Results from Two Population-Based Cohorts. *Arthritis Rheum.* **2011**, *63* (3), 810–818. https://doi.org/10.1002/art.30185.
(69) Yildiz, S. H.; Ulaşli, A. M.; Özdemir Erdoğan, M.; Dikici, Ö.; Arikan Terzi, E. S.; Dündar, Ü.; Solak, M. Assessment of Pain Sensitivity in Patients With Chronic Low Back Pain and Association With HTR2A Gene Polymorphism. *Arch. Rheumatol.* **2017**, *32* (1), 3–9. https://doi.org/10.5606/ArchRheumatol.2017.5846.
(70) Ohba, C.; Shiina, M.; Tohyama, J.; Haginoya, K.; Lerman-Sagie, T.; Okamoto, N.; Blumkin, L.; Lev, D.; Mukaida, S.; Nozaki, F.; Uematsu, M.; Onuma, A.; Kodera, H.; Nakashima, M.; Tsurusaki, Y.; Miyake, N.; Tanaka, F.; Kato, M.; Ogata, K.; Saitsu, H.; Matsumoto, N. GRIN1 Mutations Cause Encephalopathy with Infantile-Onset Epilepsy, and Hyperkinetic and Stereotyped Movement Disorders. *Epilepsia* **2015**, *56* (6), 841–848. https://doi.org/10.1111/epi.12987.
(71) Hara, M. R.; Snyder, S. H. Cell Signaling and Neuronal Death. *Annu. Rev. Pharmacol. Toxicol.* **2007**, *47*, 117–141. https://doi.org/10.1146/annurev.pharmtox.47.120505.105311.
(72) Hardingham, G. E.; Fukunaga, Y.; Bading, H. Extrasynaptic NMDARs Oppose Synaptic NMDARs by Triggering CREB Shut-off and Cell Death Pathways. *Nat. Neurosci.* **2002**, *5* (5), 405–414. https://doi.org/10.1038/nn835.
(73) Inquimbert, P.; Moll, M.; Latremoliere, A.; Tong, C.-K.; Whang, J.; Sheehan, G. F.; Smith, B. M.; Korb, E.; Athié, M. C. P.; Babaniyi, O.; Ghasemlou, N.; Yanagawa, Y.; Allis, C. D.; Hof, P. R.; Scholz, J. NMDA Receptor Activation Underlies the Loss of Spinal Dorsal Horn Neurons and the Transition to Persistent Pain after Peripheral Nerve Injury. *Cell Rep.* **2018**, *23* (9), 2678–2689. https://doi.org/10.1016/j.celrep.2018.04.107.
(74) Patel, A. B.; Louder, R. K.; Greber, B. J.; Grünberg, S.; Luo, J.; Fang, J.; Liu, Y.; Ranish, J.; Hahn, S.; Nogales, E. Structure of Human TFIID and Mechanism of TBP Loading onto Promoter DNA. *Science* **2018**, *362* (6421). https://doi.org/10.1126/science.aau8872.
(75) Schier, A. C.; Taatjes, D. J. Structure and Mechanism of the RNA Polymerase II Transcription Machinery. *Genes Dev.* **2020**, *34* (7–8), 465–488. https://doi.org/10.1101/gad.335679.119.
(76) Hernandez, N. TBP, a Universal Eukaryotic Transcription Factor? *Genes Dev.* **1993**, *7* (7B), 1291–1308. https://doi.org/10.1101/gad.7.7b.1291.
(77) Johnson, S. A. S.; Dubeau, L.; Kawalek, M.; Dervan, A.; Schönthal, A. H.; Dang, C. V.; Johnson, D. L. Increased Expression of TATA-Binding Protein, the Central Transcription Factor, Can Contribute to Oncogenesis. *Mol. Cell. Biol.* **2003**, *23* (9), 3043–3051. https://doi.org/10.1128/mcb.23.9.3043-3051.2003.
(78) Yang, Y.; Yang, S.; Guo, J.; Cui, Y.; Tang, B.; Li, X.-J.; Li, S. Synergistic Toxicity of Polyglutamine-Expanded TATA-Binding Protein in Glia and Neuronal Cells: Therapeutic Implications for Spinocerebellar Ataxia 17. *J. Neurosci. Off. J. Soc. Neurosci.* **2017**, *37* (38), 9101–9115. https://doi.org/10.1523/JNEUROSCI.0111-17.2017.
(79) Rezaei Tavirani, M.; Rezaei Tavirani, S.; Zadeh-Esmaeel, M.-M.; Ali Ahmadi, N. Introducing Critical Pain-Related Genes: A System Biology Approach. *Basic Clin. Neurosci.* **2019**, *10* (4), 401–408. https://doi.org/10.32598/bcn.9.10.310.
(80) Zhou, Y.-Q.; Liu, Z.; Liu, Z.-H.; Chen, S.-P.; Li, M.; Shahveranov, A.; Ye, D.-W.; Tian, Y.-K. Interleukin-6: An Emerging Regulator of Pathological Pain. *J. Neuroinflammation* **2016**, *13* (1), 141. https://doi.org/10.1186/s12974-016-0607-6.
(81) Shanley, L.; Lear, M.; Davidson, S.; Ross, R.; MacKenzie, A. Evidence for Regulatory Diversity and Auto-Regulation at the TAC1 Locus in Sensory Neurones. *J. Neuroinflammation* **2011**, *8*, 10. https://doi.org/10.1186/1742-2094-8-10.
(82) Yasuda, K.; Nakanishi, K.; Tsutsui, H. Interleukin-18 in Health and Disease. *Int. J. Mol.*

Sci. **2019**, *20* (3). https://doi.org/10.3390/ijms20030649.
(83) Ren, K.; Torres, R. Role of Interleukin-1beta during Pain and Inflammation. *Brain Res. Rev.* **2009**, *60* (1), 57–64. https://doi.org/10.1016/j.brainresrev.2008.12.020.
(84) O'Connor, T.; Borsig, L.; Heikenwalder, M. CCL2-CCR2 Signaling in Disease Pathogenesis. *Endocr. Metab. Immune Disord. Drug Targets* **2015**, *15* (2), 105–118. https://doi.org/10.2174/1871530315666150316120920.
(85) Sawicki, C. M.; Kim, J. K.; Weber, M. D.; Faw, T. D.; McKim, D. B.; Madalena, K. M.; Lerch, J. K.; Basso, D. M.; Humeidan, M. L.; Godbout, J. P.; Sheridan, J. F. Microglia Promote Increased Pain Behavior through Enhanced Inflammation in the Spinal Cord during Repeated Social Defeat Stress. *J. Neurosci. Off. J. Soc. Neurosci.* **2019**, *39* (7), 1139–1149. https://doi.org/10.1523/JNEUROSCI.2785-18.2018.
(86) Scholz, J.; Woolf, C. J. The Neuropathic Pain Triad: Neurons, Immune Cells and Glia. *Nat. Neurosci.* **2007**, *10* (11), 1361–1368. https://doi.org/10.1038/nn1992.
(87) Lindia, J. A.; McGowan, E.; Jochnowitz, N.; Abbadie, C. Induction of CX3CL1 Expression in Astrocytes and CX3CR1 in Microglia in the Spinal Cord of a Rat Model of Neuropathic Pain. *J. Pain Off. J. Am. Pain Soc.* **2005**, *6* (7), 434–438. https://doi.org/10.1016/j.jpain.2005.02.001.
(88) Ni, C.-M.; Ling, B.-Y.; Xu, X.; Sun, H.-P.; Jin, H.; Zhang, Y.-Q.; Cao, H.; Xu, L. CX3CR1 Contributes to Streptozotocin-Induced Mechanical Allodynia in the Mouse Spinal Cord. *J. Zhejiang Univ. Sci. B* **2020**, *21* (2), 166–171. https://doi.org/10.1631/jzus.B1900439.
(89) De, I.; Nikodemova, M.; Steffen, M. D.; Sokn, E.; Maklakova, V. I.; Watters, J. J.; Collier, L. S. CSF1 Overexpression Has Pleiotropic Effects on Microglia in Vivo. *Glia* **2014**, *62* (12), 1955–1967. https://doi.org/10.1002/glia.22717.
(90) Vogel, L. K.; Sæbø, M.; Høyer, H.; Kopp, T. I.; Vogel, U.; Godiksen, S.; Frenzel, F. B.; Hamfjord, J.; Bowitz-Lothe, I. M.; Johnson, E.; Kure, E. H.; Andersen, V. Intestinal PTGS2 MRNA Levels, PTGS2 Gene Polymorphisms, and Colorectal Carcinogenesis. *PloS One* **2014**, *9* (8), e105254. https://doi.org/10.1371/journal.pone.0105254.
(91) Chun, K.-S.; Surh, Y.-J. Signal Transduction Pathways Regulating Cyclooxygenase-2 Expression: Potential Molecular Targets for Chemoprevention. *Biochem. Pharmacol.* **2004**, *68* (6), 1089–1100. https://doi.org/10.1016/j.bcp.2004.05.031.
(92) Thorn, C. F.; Grosser, T.; Klein, T. E.; Altman, R. B. PharmGKB Summary: Very Important Pharmacogene Information for PTGS2. *Pharmacogenet. Genomics* **2011**, *21* (9), 607–613. https://doi.org/10.1097/FPC.0b013e3283415515.
(93) Natura, G.; Bär, K.-J.; Eitner, A.; Boettger, M. K.; Richter, F.; Hensellek, S.; Ebersberger, A.; Leuchtweis, J.; Maruyama, T.; Hofmann, G. O.; Halbhuber, K.-J.; Schaible, H.-G. Neuronal Prostaglandin E2 Receptor Subtype EP3 Mediates Antinociception during Inflammation. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110* (33), 13648–13653. https://doi.org/10.1073/pnas.1300820110.
(94) Reaux-Le Goazigo, A.; Poras, H.; Ben-Dhaou, C.; Ouimet, T.; Baudouin, C.; Wurm, M.; Melik Parsadaniantz, S. Dual Enkephalinase Inhibitor PL265: A Novel Topical Treatment to Alleviate Corneal Pain and Inflammation. *Pain* **2019**, *160* (2), 307–321. https://doi.org/10.1097/j.pain.0000000000001419.
(95) Thapa, D.; Cairns, E. A.; Szczesniak, A.-M.; Toguri, J. T.; Caldwell, M. D.; Kelly, M. E. M. The Cannabinoids Δ8THC, CBD, and HU-308 Act via Distinct Receptors to Reduce Corneal Pain and Inflammation. *Cannabis Cannabinoid Res.* **2018**, *3* (1), 11–20. https://doi.org/10.1089/can.2017.0041.
(96) Corder, G.; Tawfik, V. L.; Wang, D.; Sypek, E. I.; Low, S. A.; Dickinson, J. R.; Sotoudeh, C.; Clark, J. D.; Barres, B. A.; Bohlen, C. J.; Scherrer, G. Loss of μ Opioid Receptor Signaling in Nociceptors, but Not Microglia, Abrogates Morphine Tolerance without Disrupting Analgesia.

Nat. Med. **2017**, *23* (2), 164–173. https://doi.org/10.1038/nm.4262.
(97) Nockemann, D.; Rouault, M.; Labuz, D.; Hublitz, P.; McKnelly, K.; Reis, F. C.; Stein, C.; Heppenstall, P. A. The K(+) Channel GIRK2 Is Both Necessary and Sufficient for Peripheral Opioid-Mediated Analgesia. *EMBO Mol. Med.* **2013**, *5* (8), 1263–1277. https://doi.org/10.1002/emmm.201201980.
(98) Payne, C. E.; Brown, A. R.; Theile, J. W.; Loucif, A. J. C.; Alexandrou, A. J.; Fuller, M. D.; Mahoney, J. H.; Antonio, B. M.; Gerlach, A. C.; Printzenhoff, D. M.; Prime, R. L.; Stockbridge, G.; Kirkup, A. J.; Bannon, A. W.; England, S.; Chapman, M. L.; Bagal, S.; Roeloffs, R.; Anand, U.; Anand, P.; Bungay, P. J.; Kemp, M.; Butt, R. P.; Stevens, E. B. A Novel Selective and Orally Bioavailable Nav 1.8 Channel Blocker, PF-01247324, Attenuates Nociception and Sensory Neuron Excitability. *Br. J. Pharmacol.* **2015**, *172* (10), 2654–2670. https://doi.org/10.1111/bph.13092.
(99) Marshall, L. L.; Roach, J. M. Treatment of Dry Eye Disease. *Consult. Pharm. J. Am. Soc. Consult. Pharm.* **2016**, *31* (2), 96–106. https://doi.org/10.4140/TCP.n.2016.96.
(100) Wang, L.; Cao, K.; Wei, Z.; Baudouin, C.; Labbé, A.; Liang, Q. Autologous Serum Eye Drops versus Artificial Tear Drops for Dry Eye Disease: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Ophthalmic Res.* **2019**. https://doi.org/10.1159/000505630.
(101) Wen, L.; Zhu, M.; Madigan, M. C.; You, J.; King, N. J. C.; Billson, F. A.; McClellan, K.; Sutton, G.; Petsoglou, C. Immunomodulatory Effects of Bone Marrow-Derived Mesenchymal Stem Cells on pro-Inflammatory Cytokine-Stimulated Human Corneal Epithelial Cells. *PloS One* **2014**, *9* (7), e101841. https://doi.org/10.1371/journal.pone.0101841.
(102) Beyazyıldız, E.; Pınarlı, F. A.; Beyazyıldız, O.; Hekimoğlu, E. R.; Acar, U.; Demir, M. N.; Albayrak, A.; Kaymaz, F.; Sobacı, G.; Delibaşı, T. Efficacy of Topical Mesenchymal Stem Cell Therapy in the Treatment of Experimental Dry Eye Syndrome Model. *Stem Cells Int.* **2014**, *2014*, 250230. https://doi.org/10.1155/2014/250230.
(103) Lee, M. J.; Ko, A. Y.; Ko, J. H.; Lee, H. J.; Kim, M. K.; Wee, W. R.; Khwarg, S. I.; Oh, J. Y. Mesenchymal Stem/Stromal Cells Protect the Ocular Surface by Suppressing Inflammation in an Experimental Dry Eye. *Mol. Ther. J. Am. Soc. Gene Ther.* **2015**, *23* (1), 139–146. https://doi.org/10.1038/mt.2014.159.
(104) Squillaro, T.; Peluso, G.; Galderisi, U. Clinical Trials With Mesenchymal Stem Cells: An Update. *Cell Transplant.* **2016**, *25* (5), 829–848. https://doi.org/10.3727/096368915X689622.
(105) Xi, J.; Yan, X.; Zhou, J.; Yue, W.; Pei, X. Mesenchymal Stem Cells in Tissue Repairing and Regeneration: Progress and Future. *Burns Trauma* **2013**, *1* (1), 13–20. https://doi.org/10.4103/2321-3868.113330.
(106) Uccelli, A.; Moretta, L.; Pistoia, V. Mesenchymal Stem Cells in Health and Disease. *Nat. Rev. Immunol.* **2008**, *8* (9), 726–736. https://doi.org/10.1038/nri2395.
(107) Dietrich, J.; Ott, L.; Roth, M.; Witt, J.; Geerling, G.; Mertsch, S.; Schrader, S. MSC Transplantation Improves Lacrimal Gland Regeneration after Surgically Induced Dry Eye Disease in Mice. *Sci. Rep.* **2019**, *9* (1), 18299. https://doi.org/10.1038/s41598-019-54840-5.
(108) An, H.-Y.; Shin, H.-S.; Choi, J.-S.; Kim, H. J.; Lim, J.-Y.; Kim, Y.-M. Adipose Mesenchymal Stem Cell Secretome Modulated in Hypoxia for Remodeling of Radiation-Induced Salivary Gland Damage. *PloS One* **2015**, *10* (11), e0141862. https://doi.org/10.1371/journal.pone.0141862.
(109) Shigemoto-Kuroda, T.; Oh, J. Y.; Kim, D.-K.; Jeong, H. J.; Park, S. Y.; Lee, H. J.; Park, J. W.; Kim, T. W.; An, S. Y.; Prockop, D. J.; Lee, R. H. MSC-Derived Extracellular Vesicles Attenuate Immune Responses in Two Autoimmune Murine Models: Type 1 Diabetes and Uveoretinitis. *Stem Cell Rep.* **2017**, *8* (5), 1214–1225. https://doi.org/10.1016/j.stemcr.2017.04.008.

(110) Lim, J.-Y.; Yi, T.; Choi, J.-S.; Jang, Y. H.; Lee, S.; Kim, H. J.; Song, S. U.; Kim, Y.-M. Intraglandular Transplantation of Bone Marrow-Derived Clonal Mesenchymal Stem Cells for Amelioration of Post-Irradiation Salivary Gland Damage. *Oral Oncol.* **2013**, *49* (2), 136–143. https://doi.org/10.1016/j.oraloncology.2012.08.010.