

Review

TRPV1 in Dry Eye Disease

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Abstract

Dry eye disease (DED) is a prevalent ophthalmic ailment with intricate pathogenesis and that occurs primarily due to various factors which affect the ocular surface. DED is characterized by the disruption of tear film homeostasis, inflammatory reaction, and neuromyotonia. Transient receptor potential vanilloid 1 (TRPV1) is a versatile receptor that can be stimulated by heat, acid, capsaicin (CAP), hyperosmolarity, and numerous inflammatory agents. There is accumulating evidence that implicates TRPV1 in the initiation and progression of DED through its detection of hypertonic conditions and modulation of inflammatory pathways. In this article, we present a comprehensive review of the expression and function of the TRPV1 channel in tissues and cells associated with DED. In addition, we outline the potential mechanisms that implicate TRPV1 in the pathophysiology of DED. The aim of this review is to establish a theoretical basis for TRPV1 as a possible therapeutic target in DED, thereby encouraging further investigations into its role in DED.

Keywords: review; TRPV1; dry eye disease (DED)

1. Introduction

The 2017 TFOS DEWS II (Tear Film and Ocular Surface Society's Dry Eye Workshop II) report presents a comprehensive overview of dry eye disease (DED), a complex condition affecting the surface of the eyes. DED is typically associated with various eye symptoms and is characterized by altered tear film homeostasis. The primary reasons for the occurrence of DED are instability in the tear film, increased osmotic pressure, inflammation of the ocular surface, and abnormal nerve sensations [1]. The symptoms and signs of DED often differ between patients, and further research on the pathophysiology of this condition is required. Moreover, the treatment of DED has been controversial in recent years, starting first with the use of basic tear supplements [2], followed by well-studied anti-inflammatory immunosuppressive drugs such as cyclosporin A [3], and evolving into trials of multiple formulations [4]. A combination of physical therapy, local medication, and systemic medication can improve the quality of tear film to achieve effective treatment. However, this is becoming increasingly difficult due to the combined effects of video terminals, social environmental factors, psychological factors, etc. [5]. Several studies have reported that patients with DED experience corneal neuropathic pain, despite the apparent absence of corneal injury. This pain manifests as a spontaneous sensation of burning in the eye due to sensitivity to light, wind, heat, or cold [6,7]. Hence, there is an urgent need for further research into the pathophysiologic mechanisms of DED.

Both ionic and osmotic abnormalities have been linked to DED. Moreover, the interplay between inflammation and ion disturbances can alter the function of ocular

surface cells. The health of the cornea in DED has been investigated by studying the expression and activity of over 30 different types of ion channels, including voltage-gated, ligand-gated, mechanosensitive, aquaporin, chloride, and sodium-potassium-chloride co-transporters [8]. Increased expression or activity of Transient receptor potential vanilloid 1 (TRPV1) was recently associated with the pathogenesis of DED. Over the past 30 years, TRPV1 was shown to have crucial functions in sensing hypertonic environments and ocular surface injury, as well as in regulating inflammatory pathways. TRPV1 expression in organs and tissues associated with DED has recently attracted the attention of many researchers in this field. Although clinical trials with TRPV1-related targeted drugs have already begun for the treatment of DED [9], the relationship between TRPV1 and DED has yet to be reviewed. The aim of this paper was therefore to provide a comprehensive overview of TRPV1 expression and function in ocular tissues and cells, as well as its importance in the pathophysiology of DED. Future research directions involving the role of TRPV1 in DED will also be covered.

2. TRPV1 Channel

TRPV1 is one of the 7 major subfamilies of transient receptor potential (TRP) channels. It is categorized amongst the TRPV subfamilies as TRPV1-7. Other subfamilies with variations in amino acid sequence, topology and function include TRP ankyrin (TRPA), TRP canonical (TRPC), TRP melastatin (TRPM), TRP mucolipin 1-8 (TRPML1-8) and TRP polycystin (TRPP). (Fig. 1) [10,11]. TRPs are found in different tissues on the surface of the eye, including the corneal epithelium and endodermis, intersti-



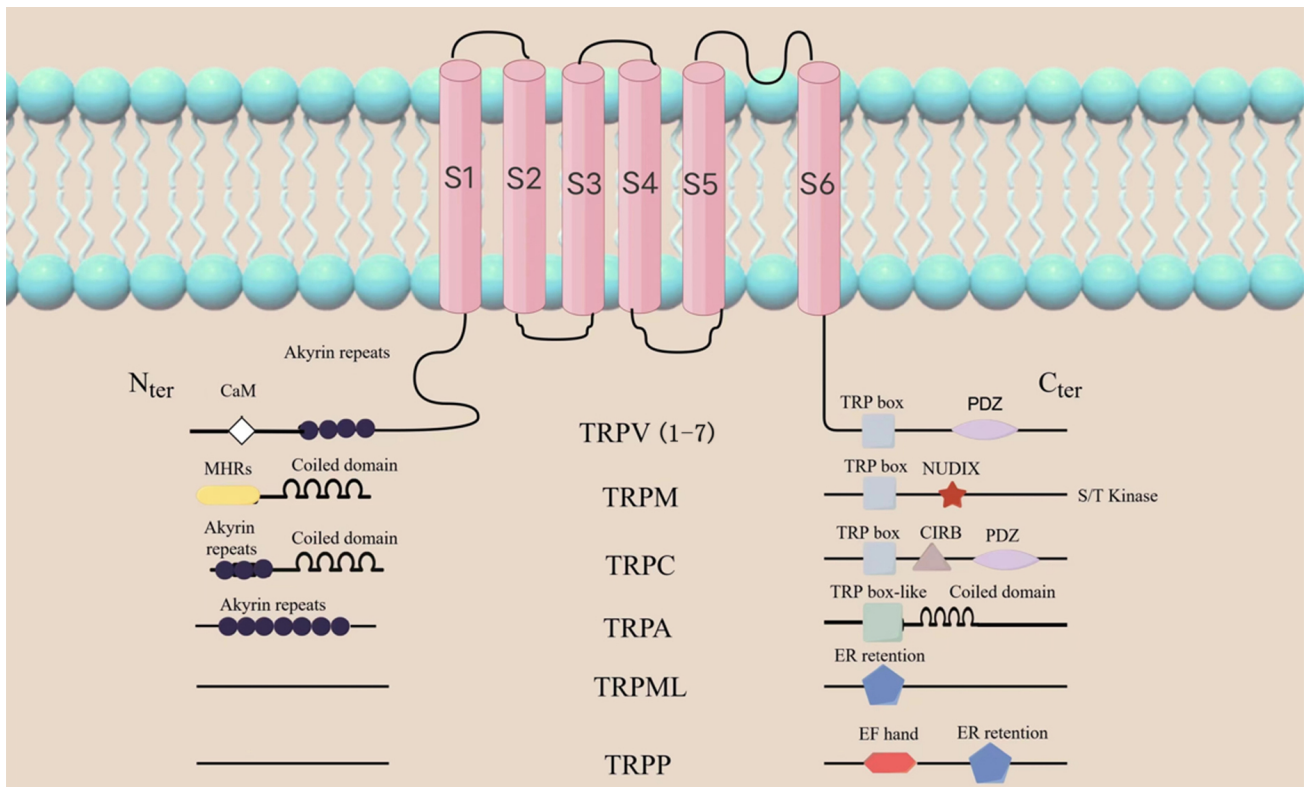


Fig. 1. General topology of the TRP channel superfamily. The structure of the TRP channel is shown as in the figure, among which TRPV1 consists of six transmembrane domains (S1-S6), with four subunits surrounding the ion channel at the N- and C-ends of the molecule. The N-terminal contains ankyrin repeat domains, while the C-terminal has a TRP domain and multiple calmodulin binding domains. TRP, transient receptor potential; TRPV, transient receptor potential vanilloid; TRPM, transient receptor potential melastatin; TRPC, transient receptor potential canonical; TRPA, transient receptor potential ankyrin; TRPML, transient receptor potential mucolipin; TRPP, transient receptor potential polycystic; PDZ, Postsynaptic density 95 / Discs large / ZO-1 domain; NUDIX, Nucleoside diphosphate sugar transferase 9 homology domain binding ADP ribose (ADPR) or ADPR-2'-phosphate (ADPRP); CIRB, calmodulin and inositol triphosphate receptor-binding site; EF, a canonical Ca^{2+} -binding domain; ER, an endoplasmic reticulum retention domain. (Created using Figdraw).

tial fibroblasts and nerve fibers, and the trigeminal ganglion (TG) [12–16]. TRPV1 helps to maintain tissue homeostasis and initiate the wound healing response, therefore making it the most studied of all the TRP channel [17]. Similar to the structure of potassium ion channels, TRPV1 consists of six transmembrane domains (S1-S6), with four subunits surrounding the ion channel at the N- and C-terminals of the molecule (Fig. 1). Caterina *et al.* [18] successfully cloned and isolated TRPV1 in 1997. TRPV1 was the first neuronal receptor found to be sensitive to capsaicin, and is thus commonly referred to as the capsaicin receptor. It is also a vanilloid compound, and is therefore also referred to as vanilloid receptor (VR1) [18].

Both excitable and non-excitable cells and tissues express TRPV1 channels. These have been linked to a number of physiological and pathological disorders, such as pain and nociception [19]. TRPV1 is a non-selective cation channel that functions as a multimodal nociceptor. It is triggered by a variety of substances, including inflammatory mediators (e.g., prostaglandin, histamine), acidity,

heat above 43 °C hyperosmolarity, and capsaicin [20–23]. TRPV1 can trigger a series of biological effects by mediating the influx of extracellular cations, including Ca^{2+} , Na^{+} and Mg^{2+} . TRPV1 therefore plays an important role in signal transduction between physical and chemical stimuli by converting mechanical stimuli, thermal stimuli, acidic stimuli, chemical stimuli, inflammatory mediators, and other stimulus signals into cellular chemical signals (Fig. 2). A notable feature of TRPV1 polymodal activation is the existence of non-overlapping activation pathways [20,21,24]. Of the six transmembrane regions (S1–S6), the S3/4 region is responsible for sensitivity [25]. Opening of the intracellular signaling pathways is dependent on pulling the S4–S5 linker away from the central pore [22]. Of the various TRPV1 stimulations, the capsaicin-channel interaction is the best understood in terms of both its location and the details of the molecular interactions [23,25–27]. The machinery for capsaicin activation is located mostly within the transmembrane region, with the S5/S6 region involved in regulating capsaicin-induced activation of TRPV1 [28].

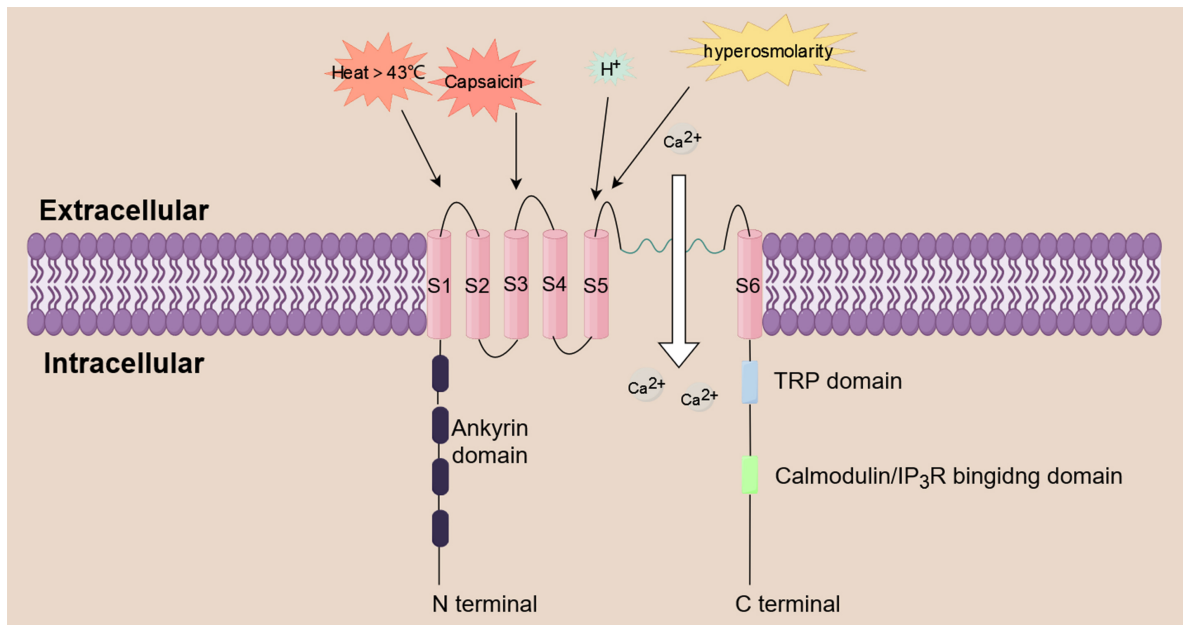


Fig. 2. Calcium influx induced by TRPV1. TRPV1 is a non-selective cation channel that functions as a multimodal nociceptor. It is triggered by a variety of substances, including prostaglandin, histamine, acidity, heat above 43 °C, hyperosmolarity, and capsaicin. TRPV1 triggers a series of biological effects by mediating the influx of extracellular Ca^{2+} . IP3R, inositol (1,4,5)-triphosphate receptor. (Created using Figdraw).

Other stimuli, such as Ca^{2+} and protons, are less well defined and are likely to act through multiple mechanisms or targets. Compared to chemical stimulations, physical stimulators of TRPV1 such as voltage, mechanical force and heat are still poorly understood, with the likely binding sites clustered in the outer pore region [29]. Previous studies have shown that both capsaicin- and hydrogen-induced receptor desensitization are dependent on calcium signaling, whereas thermal stimulation does not rely on the intracellular calcium level [30]. TRPV1 plays a crucial role in generating non-selective, cation-mediated outward integration currents. The entry of calcium through TRPV1 receptors and voltage-dependent calcium channels leads to the release of neuropeptides and excitatory amino acids [31]. In sensory neurons, TRPV1 can be activated at various hydrogen ion concentrations accompanied by sustained current excitation. Moreover, hydrogen ions can induce neuropeptide release in innervated tissues and in ischemic or inflamed tissues [32].

3. Expression of TRPV1 in Tissues and Cells Associated with DED

TRPV1 is widely expressed in various organs, including the eyes. Tissues and cells throughout the body utilize TRPV1 for diverse physiological and pathological functions, including coughing, pain, inflammation, itching, hearing, taste and apoptosis [33]. Here, we review the expression of TRPV1 in the anterior segment, corneal nerve fibers, TG and lacrimal gland, as well as its association with DED (Fig. 3).

3.1 Corneal Epithelium

Zhang *et al.* [12] first reported functional activity of TRPV1 in corneal epithelial cells in 2017. The TRPV1 channel has been identified in human corneal epithelial cells (HCEC), as well as in the corneal epithelial cells of rats and mice [12,34–36]. TRPV1 controls the entry of inflammatory mediators in the corneal epithelium, as well as the subsequent development of hyperalgesia in DED patients and in a mouse model of corneal wound healing [37]. Due to its ability to induce the release of inflammatory mediators, TRPV1 may therefore be crucial for maintaining tissue integrity and the function of HCEC [38].

3.2 Corneal Stroma

The structure of the corneal stromal layer plays a role in maintaining corneal transparency [12]. Primary cultured human corneal fibroblasts were found to express functional TRPV1 [13,39–41]. Recent studies have shown that keratinocytes also express TRPV1 [42,43].

3.3 Corneal Endothelium

The corneal endothelium is a single layer of postmitotic cells that maintains corneal transparency by regulating the flow of fluid from the stroma into the anterior chamber [44]. Several studies have shown expression of the TRPV1 channel in human and rabbit corneal endothelial cells [14,45]. Moreover, endothelial TRPV1 is thought to be responsive to temperature changes, thereby contributing to the regulation of Ca^{2+} homeostasis in the endothelium under different ambient conditions [14].

3.4 Corneal Nerve Fibers

The cornea is innervated by dense nerves that respond to a variety of sensations [46]. Corneal innervation comprises the sensory axons of trigeminal neurons, as well as sympathetic autonomic axons from postganglionic neurons in the superior cervical ganglion [47]. Nerve endings in the corneal epithelium can be divided into subgroups according to their morphology and to their molecular and functional phenotypes. Morphologically, the ends of corneal nerve fibers can be divided into simple, forked, and complex. In terms of their molecular phenotype, the branched and complex endings express glial cell line-derived neurotrophic factor family receptor alpha3 (GFR α 3) and calcitonin gene-related peptide (CGRP), respectively, while simple endings express both [48]. Corneal nerve fibers can also be classified into three major phenotypes based on their functional characteristics: polymodal nociceptors, pure mechano-nociceptors, and cold-sensing neurons [49,50]. Neurons that express TRPV1 are primarily multimodal nociceptors [48].

Due to its role in the structure and physiology of the cornea, the corneal nerve is involved in the pathophysiology of the cornea, including DED. First, the reflex arcs that control tear flow and blinking depend on sensory input from the ocular surface. Hence, the maintenance of adequate tear film depends on corneal nerves. Secondly, corneal nerves contribute to the integrity of the corneal epithelium and to local immune regulation [51–53], and are thus vital for ocular surface homeostasis. DED usually affects the cornea because of abnormalities in the tear film [54]. The pathophysiology of corneal nerve involvement in DED is manifested mainly through morphological changes, including a reduced density and the presence of deformity [55–57]. Somatosensory dysfunction of corneal nerves in DED is related to changes in basal tears and blinking, leading to further pathophysiological mechanisms of inflammation and corneal injury [49].

TRPV1 has been extensively described in the corneal nerve fibers of mice, guinea pigs and humans. It was first observed in small primary sensory neurons in the cornea, and later also in non-neuronal cell types. The expression of TRPV1 in the corneal sensory nerve fibers of mice and humans is consistent with the expression observed in non-corneal sensory nerve fibers [11,15,58].

3.5 Conjunctiva

TRPV1 is expressed in conjunctival epithelial cells [59]. The conjunctiva accounts for 85% of the total ocular surface area, with the conjunctival epithelium serving as an anatomical mechanical barrier. The integrity of this structure prevents entry by pathogens and contributes to the maintenance of tissue hydration. Disruption of epithelial cells in the conjunctiva of some types of DED is associated with increased osmotic pressure in the tear film, leading to impaired barrier function by the dense conjunctiva and

the triggering of inflammatory disorders [60,61]. Martínez-García *et al.* [45] confirmed the presence and activity of TRPV1 protein in the human conjunctiva. Subsequent research indicated that TRPV1-mediated calcium influx is inhibited by L-carnitine, leading to osmotic protection against hyperosmolarity. Therefore, the current evidence indicates that TRPV1 is crucial for controlling the osmotic pressure in conjunctival tissue [62].

3.6 Trigeminal Ganglion (TG)

Corneal sensitivity is innervated by the ocular branch of the TG. This is abundant in the cornea and facilitates the transmission of touch or pain from the outer tissues to the center. Impaired corneal sensitivity can lead to decreased blinking and tear reflexes (Fig. 4) [11]. TRPV1 is abundant in the TG and facilitates sensory transmission. TRPV1 stimulation in peripheral sensory neurons enhances action potential discharge and the release of neuropeptides such as CGRP, neurokinin, and substance P (SP). This results in the production of numerous immune cells and pro-inflammatory agents, thus creating a beneficial signaling feedback cycle that triggers TRPV1 activation and nociceptive signaling to the center [63,64].

Immunostaining studies found elevated levels of TRPV1 protein in the TG of rats with DED [65]. However, Yamazaki *et al.* [66] reported reduced levels of corneal TRPV1 protein in an animal model of DED created by removal of the extra-orbital lacrimal gland. Moreover, Hegarty *et al.* [16] reported that exposure to capsaicin suppressed natural blinking in rats. Interestingly, the corneal nerve terminal connected to the trigeminal nerve, which is stimulated by capsaicin, did not show TRPV1 expression [16]. These observations suggest possible inconsistencies in the expression of central TRPV1 [67].

3.7 Lacrimal Glands

Expression of TRPV1 in the epithelial cells of lacrimal glands was first demonstrated by Martínez-García *et al.* [45] in 2013. This observation suggests a potential role for TRPV1 in regulating both lacrimal secretion and Ca²⁺.

4. TRPV1 in the Pathophysiology of DED

4.1 TRPV1 is a Hypertonic Osmotic Sensor

Research has demonstrated that TRPV1 functions as a hypertonic osmotic sensor. Therefore, inhibition of TRPV1 could provide osmotic protection and minimize ocular surface damage caused by hyperosmolality [68]. Osmo-protective agents are one of the conventional treatments for dry eyes. In conjunction with suitable solutes, they can stop hyperosmosis from harming the cornea [69]. Khajavi *et al.* [62] used reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemistry in a human conjunctiva-derived cell line (IOBA-NHC) to show that the osmo-protective effect of L-carnitine in hyperosmosis-induced HCE was due to the prevention of Ca²⁺ inflow

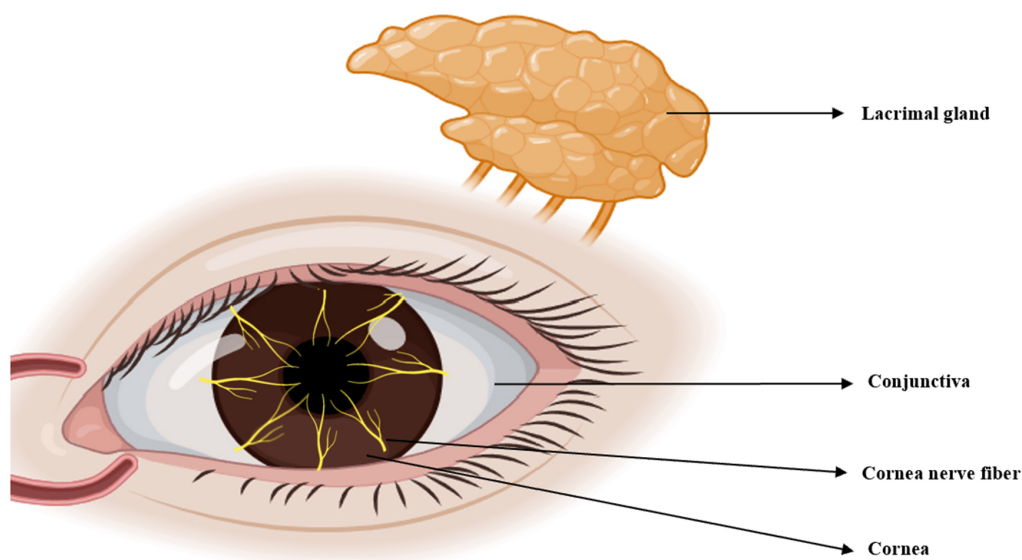


Fig. 3. TRPV1 in the human anterior surface and lacrimal gland. (Created using Figdraw).

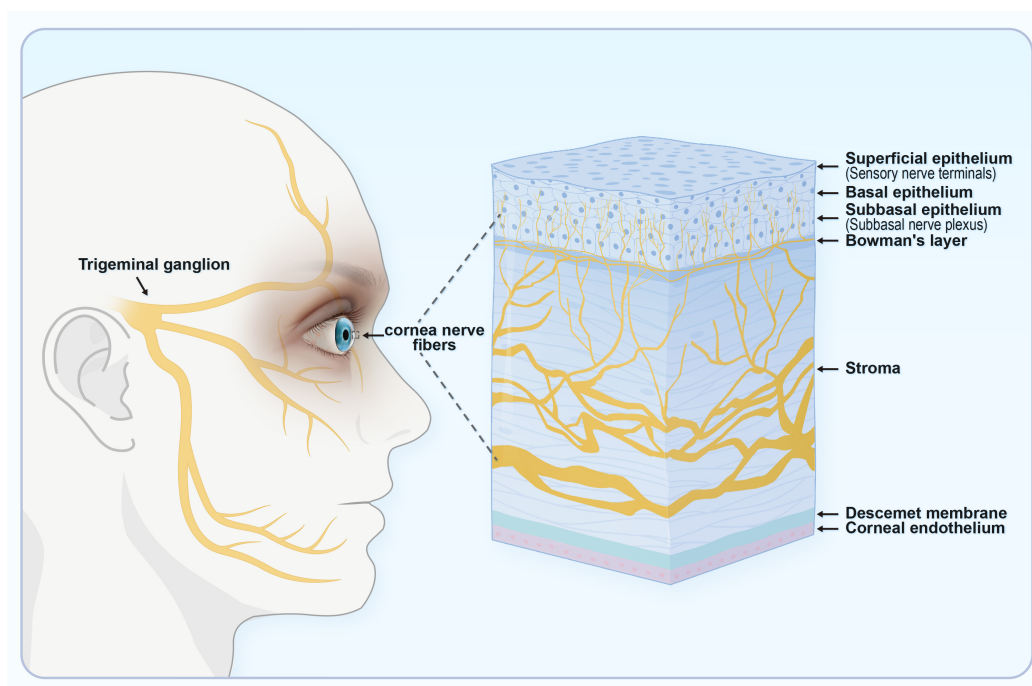


Fig. 4. The ocular sensory pathway. Corneal sensitivity is due to innervation by the ocular branch of the TG, which has abundant nerve endings in the cornea. TG, trigeminal ganglion. (Created using Figdraw).

and TRPV1 activation. Accumulating clinical data has shown the important role of osmo-protective agents (e.g., red tetrinol, taurine, trehalose, carboxymethyl cellulose, L-carnitine) in improving the vicious cycle of physiology and pathology in DED [70]. One of these osmo-protective molecules, L-carnitine, is a naturally occurring antioxidant. It is thought to be essential in preventing hyperosmotic, media-induced cell contraction by facilitating Na^+ -dependent co-transporters. These can inhibit some inflammatory pathways on the surface of the eye, includ-

ing TRPV1-related pathways [71,72]. Lucius *et al.* [68] recently showed that both L-carnitine (an osmo-protector) and capsaicin (a TRPV1 antagonist) can block the hyperosmotic, stress-induced increase in Ca^{2+} inflow and cell size reduction in HCEC.

4.2 TRPV1 Participates in Ocular Surface Injury Sensation

The cornea has the highest density of nociceptor nerve endings in the body, and is therefore the organ most able to

produce pain [73,74]. Slow pain sensation is attributed to sensory nerve fibers called C fibers, which constitute approximately 80% of the nerve fibers in the cornea. The remaining corneal nerve fibers, known as A δ fibers, are the protagonists of acute pain [34,75,76]. The expression of TRPV1 in corneal nerve fibers plays a reflexively protective role. Studies with gold chloride (AuCl) staining showed a significant reduction in corneal nerve fibers after treatment of mice with capsaicin. Further research found that treatment of neonate rats with capsaicin reduced intraepithelial nerve fibers or endings in the adults, as manifested by reduced eye rubbing [11,77]. Several studies have found that TRPV1 acts as a receptor in the fundamental process of peripheral sensitization. When allergic reaction-related mediators such as histamine and bradykinin are stimulated intraocularly, activation of the TRPV1 receptor triggers the release of neuropeptides such as CGRP, neurokinin, and SP. Pro-inflammatory peptides released directly into the surrounding tissues by activated sensory neurons also improve SP and TRPV1 expression by binding to TRPV1 receptors. Eventually, this results in hurt and pain sensations [34,74]. More recently, it was reported that development of ocular surface pain resulting from persistent intermittent hypoxia may be linked to TRPV1-dependent pathways [78]. Further evidence that TRPV1 is important in ocular sensation comes from the observation that sensory neurons in TRPV1^{-/-} mice are less susceptible to acute heat stimuli, capsaicin, resinous interferon, and protons [10].

Following injection into newborn rats, capsaicin binds to the TRPV1 receptor, resulting in a range of symptoms including neurogenic inflammation, visceral hyper-reflexia, and pain [79]. When administered topically, capsaicin produces a strong burning sensation due to it being a TRPV1 agonist. Following this burning sensation, mucous membranes exposed to capsaicin do not respond negatively to harmful stimuli for a considerable length of time. Moreover, TRPV1-related ocular pain can be caused by many commonly used chemicals, including some found in shampoos and soaps [80,81]. Rats treated with resiniferatoxin (RTX), a strong TRPV1 agonist, used less eye wipes than rats treated with capsaicin (CAP). This continued for several days without interfering with the ability of the cornea to blink, or with healing of the epithelium. Consequently, RTX is regarded as a safe and efficient medication for managing pain associated with eye disorders and surgery [82]. In cases of allergic conjunctivitis (AC), Acosta *et al.* [83] showed that the TRPV1 antagonist capsazepine could reverse the increased blink rate, tear film rupture rate, and multi-mode nociceptor sensitization to CO₂ response. In addition, capsazepine attenuated the lower activity of hot and cold receptors caused by allergies. These results indicate that TRPV1 is also involved in the allergic reaction process that damages the eyes [83].

Reduced tear production in dry eye causes inflammation, sensitization of nociceptor nerve endings and ther-

moreceptors, and long-term alterations in the molecular, structural, and functional pathways of the trigeminal nerve, which in turn causes ocular pain [7]. Research on DED suggests that TRPV1 is likely to underlie the increased corneal cold-sensitive, multimode nociceptor response in chronic tear insufficiency. This is reflected in the enhancement of corneal excitability to cold stimulation [83]. An increased number of corneal neurons expressing TRPV1 and GCRP was observed following lacrimal gland excision (LGE) in a mouse model. The increased expression of TRPV1 in corneal neurons following LGE may activate neuroprotective systems after long-term injury [84]. Amplification of pain and neurogenic inflammation is largely dependent on the sensitization mechanism of GPCRS via the TRPV1 channel [33]. Research using animal models has shown that DED can upregulate genes in the TG that are linked to inflammatory and neuropathic pain. This upregulation can be prevented by repeat treatment with the TRPV1 inhibitor capsazepine, thereby decreasing the sensation of eye pain [38].

4.3 TRPV1 Participates in Inflammation of the Ocular Surface

Multiple anterior segment tissues and nerve fibers are impacted by TRPV1-mediated inflammatory damage [33]. TRPV1 perceives a range of environmental stimuli and is triggered by these to stimulate cellular Ca²⁺ influx, which in turn induces the activation of mitogen-activated protein kinase (MAPK) and nuclear factor (NF)- κ B, leading to increased production of pro-inflammatory cytokines [35]. This phenomenon resembles the hyperosmolar stress observed in the tears of individuals with dry eye conditions [72,85,86]. Zhang *et al.* [35] reported that application of capsaicin to HCEC promotes the secretion of pro-inflammatory cytokines, specifically IL-6 and IL-8, leading to dry eye. Activation of MAPK is a determining factor in the inflammatory response of HCEC, since capsazepine and several inhibitors of the MAPK pathway can suppress the secretion of IL-6 and IL-8. TRPV1 therefore participates in inflammation of the cornea [35].

Neurogenic inflammation is a particularly important mechanism by which TRPV1 causes inflammation of the ocular surface in DED [87]. Guzmán *et al.* [88] found that hyperosmolarity (THO) in conjunctival epithelial cells activates nuclear factor- κ B signaling and increases the recruitment and maturation of dendritic cells through the activation of TRPV1. As a result, the density of intraepithelial neurons and terminals in the cornea decreases, while the number of activated and memory CD4 T-cells in the eye-draining lymph nodes increases [88]. By using blockers and agonists, Guzmán *et al.* [89] found this mechanism also occurred in sympathetic eye disease. Following ocular surface injury, the TRPV1 channel in corneal sensory nerve endings is usually activated and signals are transmitted to the central nervous system, mostly the hypothalamus. These signals

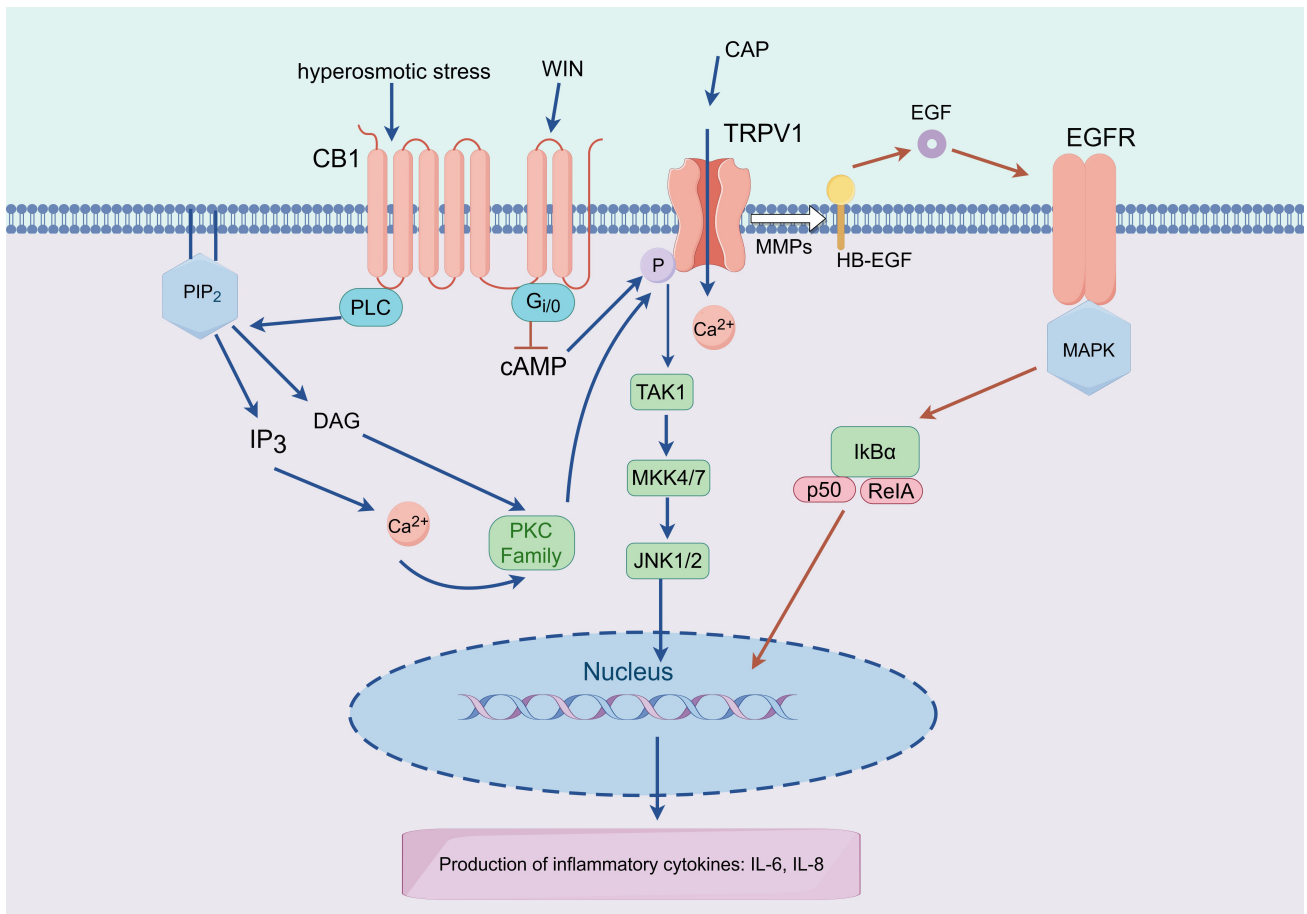


Fig. 5. Working model of TRPV1-CB1 and TRPV1-EGFR interactions that mediate expression of proinflammatory cytokines. Lipid precursors (DAG) are produced by PLC activity and CB1 stimulation, which then stimulate kinases (PKC) and the release of intracellular Ca^{2+} stores (IP3). Moreover, Gi/o activation reduces the synthesis of cAMP, which inhibits PKA-mediated increases in the TRPV1 phosphorylation state. This results in reduced expression of TRPV1-induced pro-inflammatory cytokines. Conversely, the TRPV1-EGFR interaction has the opposite effect. TRPV1 stimulation causes EGFR to be transactivated through MMP-dependent HB-EGF shedding. This is followed by EGFR-independent NF- κ B stimulation, MAPK activation, and NF- κ B activation. Activated NF- κ B then moves to the nucleus where it induces the synthesis of IL-6 and IL-8. EGFR, epidermal growth factor receptor. (Created using Figdraw).

cause the release of SP and activate pre-sympathetic neurons located in the PVH and LHA, thereby activating the NF- κ B pathway in ocular epithelial cells [90]. The TRPV1 channel acts as a receptor to detect tissue injury, while SP is released as an effector of the sympathetic response.

TRPV1 and the GPCR cannabinoid receptor type 1 (CB1) coexist and interact functionally in HCEC, with TRPV1 mediating Ca^{2+} signaling. Activation of CB1 prevents TRPV1-induced inflammation in corneal epithelial cells. This in turn causes the release of intracellular stored Ca^{2+} and the synthesis of inositol (1,4,5) triphosphate (IP3) [33]. As a result, the TRP subunit-based and store-operated Ca^{2+} channels subsequently open. The promotion of diacylglycerol (DAG) synthesis through phospholipase C (PLC) activity stimulates kinases (PKC) and releases intracellular Ca^{2+} from storage. TRPV1 is phosphorylated following its interaction with CB1, leading to the release of IL-6 and IL-8 from HCEC (Fig. 5) [65,91,92].

However, it is unclear whether TRPV1 is activated directly by the CB1 agonist WIN, or whether CB1 induces TRPV1 activation through cytoplasmic changes in Ca^{2+} . Under inflammatory conditions, the decrease in CB1 inhibition can also reduce TRPV1 activity in primary sensory neurons [93].

4.4 TRPV1 is Involved in the Regeneration of Corneal Epithelial Cells

The corneal epithelium is most susceptible to damage during dry eye conditions. It serves as a protective shield against damaging stimuli, and epithelial cells are maintained by the migration of basal cells to the upper layers of the tissue to replace terminally differentiated cells at the surface [36]. Corneal epithelial injury can promote wound healing by inducing the release of several growth factors, including EGF. Capsaicin activates TRPV1 in corneal epithelial cells, which then further activates EGFR by increas-

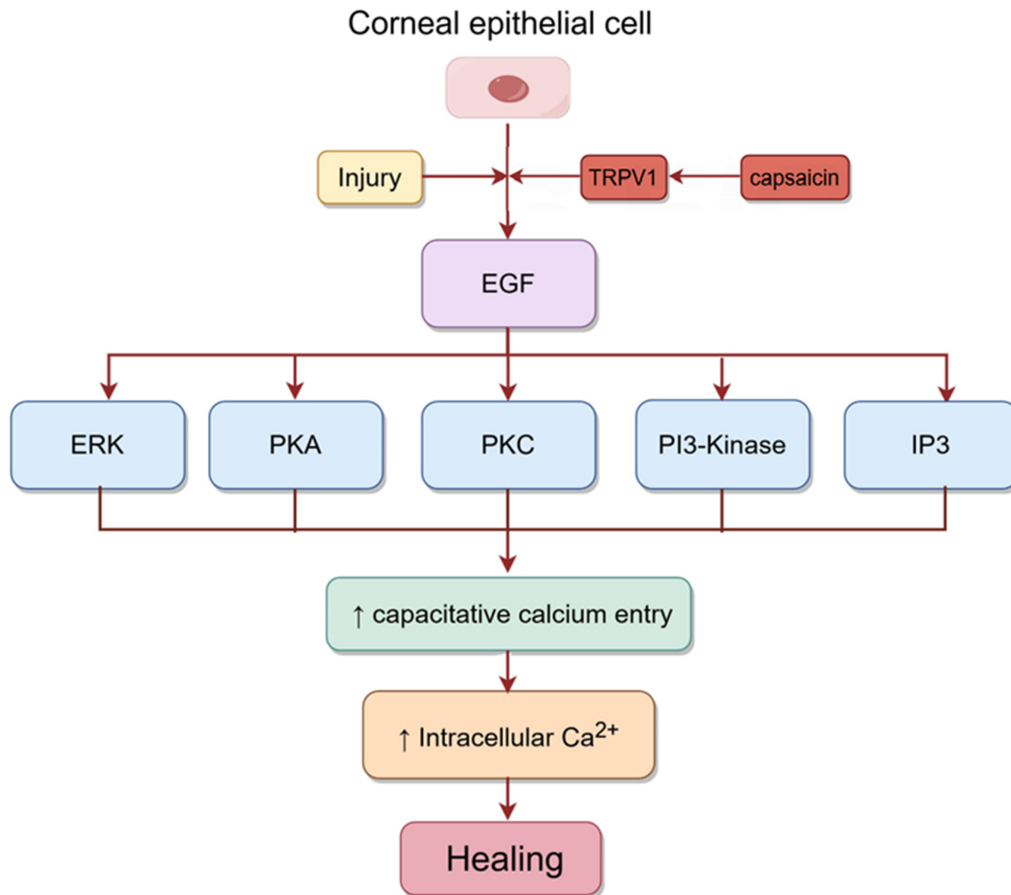


Fig. 6. Signaling pathways involved in corneal epithelial healing. Injury to corneal epithelial cells activates TRPV1, which then activates EGFR by mediating the release of EGF. Subsequently, the rise in intracellular Ca^{2+} triggers the MAPK downstream signaling cascade, including PKA, PKC, ERK, IP3 and PI3K. Activation of these MAPK signals can promote the proliferation and migration of epithelial cells. ERK, extracellular signal-regulated kinase; PKA, protein kinase A; PKC, protein kinase C; EGF, endothelial growth factor; IP3, inositol (1,4,5)-triphosphate; PI3K, phosphatidylinositol-3 kinase. (Created using Figdraw).

ing the release of EGF. The PLC-IP3 cascade subsequently reduces intracellular Ca^{2+} storage. Ca^{2+} influx is increased by opening the Ca^{2+} channels for storage processes [17]. Activation of the downstream MAPK signaling cascade is triggered by the rise in intracellular Ca^{2+} , followed by increased transcription and activation of PKA, PKC, Janus kinase/signalizing (JAK/STAT) and phosphatidylinositol-3 kinase/protein kinase B (PI3K/Akt). Activation of these MAPK signals can promote the proliferation and migration of epithelial cells (Fig. 6) [11]. Research has shown that $\text{TRPV1}^{-/-}$ mice exhibit delayed re-epithelialization and restoration of tissue transparency. The ability of TRPV1 to promote cell proliferation and migration is also linked to the increased expression of SP and IL-6, which are two co-activators of growth factor-induced wound healing [35]. Inhibition of TRPV1 leads to a reduction in mucin and goblet cells, apoptosis of corneal epithelial cells, and impairment of ocular surface moisture. Eventually, the elevated os-

motric pressure at the surface of the eye becomes worse, thus creating a vicious cycle of dry eyes and setting up mechanisms that perpetuate the disease.

The loss or blocking of TRPV1 expression in mice with alkali burns inhibits the production of TGF1 and other proinflammatory factors, resulting in severe and long-lasting corneal inflammation and scarring [39]. Inactivation of TRPV1 may therefore be a potential therapeutic target to improve inflammatory/fibrous wound healing [41].

5. Co-expression and Crosstalk Between TRPV1 with TRPM8

The sensation of cold pain in the cornea requires TRPV1 activity and SP release. The ion channels TRPV1 and TRPM8 are essential for detecting pain and temperature. Indeed, the sensation of cold pain depends on the colocalization of these factors [94,95]. The sensation of cold pain in the eye is typically indicated by feelings of intense

irritation and burning, leading to speculation the burning feeling may be due to thermal channels like TRPV1. Li *et al.* [96] reported that nearly half of all neurons that stained positively for TRPM8 (TRPM8⁺) also exhibited immunohistochemical staining for TRPV1 in retrograde labeled neurons originating from the cornea. Moreover, the increased expression of TRPM8 in TRPV1-positive neurons was also observed in DED. The cold response was reduced by a strong and specific TRPV1 antagonist (AMG9810) [96]. These results show that TRPV1 contributes to the production of SP by making cold-responsive TRPM8⁺ neurons more sensitive. SP is therefore necessary for TRPM8⁺ neurons and post-synaptic neurons to react to cold discomfort. Li *et al.* [96] suggested the existence of a communication channel between TRPV1 and TRPM8, with the implication being that GPCRs could potentially regulate both TRPM8 and TRPV1. This is supported by the observation that activated GPCRs can effectively hinder the activity of both TRPM8 and TRPV1 [97]. Interactions between TRPV1 and TRPM8 have also been implicated in DED. By activating TRPM8 directly at room temperature, thyronamine prevents CAP from stimulating TRPV1 and offers protection against dry eye when applied to the cornea [98]. Using an *in vitro* model of dry eye, the endogenous thyroid hormone metabolite 3-iodothyramine can also activate TRPM8 to reduce IL-6 production following capsaicin-mediated activation of TRPV1 in HCEC at room temperature [99].

Previous research indicated that VEGF-induced Ca²⁺ signaling in the human corneal stroma is influenced by crosstalk between TRPM8, TRPV1, and the VEGF receptor. Türker *et al.* [42] reported that CPZ is able to suppress the VEGF-induced increase in Ca²⁺ transient. Conversely, the selective TRPM8 antagonist AMTB enhances the VEGF signaling pathway. Based on these results, it was inferred that the VEGF-stimulated increase in Ca²⁺ influx and its possible ionic flow arise due to communication between the VEGF receptor and TRPV1 [42].

6. TRPV1 as a Therapeutic Target

The presence of TRPV1 in tears collected from dry eye patients after exposure to hypertonic conditions suggests that it may be a potential drug target for reducing osmotic pressure at the ocular surface [100]. Osmo-protectants maintain cell volume and stabilize cell structure when cells are exposed to high osmotic pressure. These small organic molecules play a crucial role in restoring cellular balance under osmotic stress [70]. Their osmo-protective function is thought to be associated with the uptake of Na⁺-dependent co-transporters that inhibit some of the inflammatory pathways on the eye surface caused by TRPV1 [71,72].

Activation of TRPV1 could potentially accelerate the healing of corneal epithelium, whereas its inhibition could help to reduce stromal opacity and hyperosmolar inflammation. These properties make TRPV1 a potential target

for drug development [34,35,93]. The contrasting wound healing effects indicate that TRPV1 antagonists should be restricted to cases of deep interstitial injuries, whereas TRPV1 agonists may be effective for superficial epithelial damage.

Many patients with moderate to severe dry eye syndrome experience symptoms such as redness, burning, itching, and pain. TRPV1 inhibitors are therefore often designed to relieve neuropathic and inflammatory pain caused by DED. Tivanisiran is a synthetic, RNA-interfering, oligonucleotide intraocular infusion designed to silence human TRPV1 (proto-SYL1001, Sylentis). It has been shown to improve ocular congestion and tear quality in humans, reduce eye discomfort, and avoid ocular surface damage (congestion and corneal staining). In a Phase II trial, Tivanisiran (11.25 mg/mL) significantly reduced eye pain scores, improved conjunctival congestion from abnormal to normal in 50% of patients, and extended the tear film rupture time by two seconds [9]. Most current research is focused on the development of TRPV1 antagonists to relieve neuropathic and inflammatory pain. Paradoxically, TRPV1 agonists also have analgesic properties. For example, a concentrated (8%) version of capsaicin called Qutenza is approved in the United States for the treatment of postherpetic neuralgia. Results from a Phase 2B clinical trial suggest that topical application or injection of capsaicin may lead to long-term dysfunction of TRPV1-expressing sensory neurons due to excessive activation of the TRPV1 channel. TRPV1 can cause desensitization when exposed to capsaicin over a long time period, although the mechanism behind this de-functionalization is unclear [92,101]. Bates *et al.* [82] proposed that TRPV1 agonists could be used to treat post-operative or post-injury ocular pain. Their reasoning was based on the observation that direct application of capsaicin to the cornea in cats reduced pain sensitivity. However, administration of the powerful TRPV1 agonist RTX eliminated the eye rubbing reaction caused by capsaicin without affecting the mechanical sensitivity of the cornea [82]. Fakhri *et al.* [38] also found that topical infusion of an isolated ocular formulation of capsaicin (10 µM) twice daily for two weeks in a mouse model of severe DED reduced the corneal response to hot, cold, and acidic stimuli. Chronic administration of capsaicin in an animal model of DED decreased the expression of genes in the TG associated with neuropathic and inflammatory pain, thereby reducing corneal pain [38].

Based on studies of gabapentin (GBT) use for neuropathic pain, experiments in rabbits showed that GBT eye drops not only have analgesic and anti-inflammatory properties, but also stimulate the secretion of tears [102,103]. Systemic administration of GBT has been used to relieve neuropathic pain in glaucoma patients caused by high intraocular pressure [104]. Biggs *et al.* [105] found that GBT could enter cells more quickly (500 times faster than via the transporter) through the activated TRPV1 channel to

exert an analgesic effect [105]. TRPV1 expression is up-regulated at the ocular surface and TG of patients with dry eye [65,84]. If used for local treatment of DED, it is therefore worth exploring whether GBT has a stronger effect on relieving neuropathic pain via up-regulated TRPV1.

7. Discussion

The cornea is comprised of the corneal epithelium, stroma, endothelium, and nerve fibers. It is currently the subject of extensive studies into the distribution of TRPV1 in eye tissues and cells associated with dry eye. The distribution of TRPV1 in the conjunctiva has mainly been studied in the context of allergic conjunctivitis (AC). Children with AC and dry eye share characteristics such as unstable tear films. The strong similarities between DED and AC, and the significant overlap in their symptoms likely reflects common mechanistic aspects of the two disorders [106,107]. However, more experimental studies are needed to ascertain TRPV1 expression, distribution, and function in patients with DED. For example, it has yet to be confirmed whether TRPV1 is expressed in the meibomian gland. Although some studies have confirmed the involvement of other TRP channels in tear secretion, little research has been done on the expression of TRPV1 in the lacrimal gland. There are two main reasons for the lack of studies on TRPV1 in lacrimal glands. TRPV1 is a multimode nociceptor that was first observed in neurons. Most research on DED has therefore focused on the role of TRPV1 in neuro-regulation. Moreover, most studies have used lacrimal gland resection to create animal models of DED, and hence the expression of TRPV1 in this tissue could not be evaluated. In addition, TRPV4 has the same role in the lacrimal gland as it does in the salivary gland, which is to regulate the ANO1 transporter through Ca^{2+} influx and thereby enhance lacrimal secretion [100]. However, the specific mechanism involving crosstalk between TRP channels in the lacrimal glands and DED requires further study.

The distribution and expression of TRPV1 channels are clearly different between animal and human tissues. This may underlie functional differences, thereby limiting the use of animal models in TRPV1 research. Whether functional differences exist between different physiological and pathological states also requires further study [67].

Immune dysregulation is involved in the development of dry eye, and hence the immune function of TRPV1 in allergic diseases has been extensively studied. During AC, activated TRPV1 can induce inflammatory cells to infiltrate into tissues and increase Th2 cytokine levels [108]. In the absence of other pathogenic factors, corneal nerves may be sensitive to immune-driven damage mediated by Th1 CD4^{+} T cells [109]. However, in dry eye, corneal neuropathy involving TRPV1 does not appear to be associated with changes in CD4^{+} T cells at the ocular surface, since TRPV1-KO does not alter the number of CD4^{+} T

cells in the conjunctiva. Therefore, the TRPV1 signaling pathway in corneal tissue, rather than CD4^{+} T cells, is involved in the progression of corneal neuropathy in DED [110]. An explanation for this may be that inflammatory neuroimmune pathology is specific to certain disease etiologies [111]. These findings have potential therapeutic implications for ocular surface disorders. Furthermore, the function of TRPV1 in dry eye immune disorders also warrants further study.

TRPV1 and TRPM8 are the most commonly studied TRP channels in dry eye, with co-expression of TRPV1 and other TRP subchannels. TRPV1 appears to respond to thermal stimulation and hyper-osmosis, whereas TRPM8 responds to cold stimulation. However, the two channels are co-expressed and interact with each other. Hence, the expression and characteristics of both TRPV1 and TRPM8 should be assessed when studying dry eye, and other TRP channels such as TRPA1 should also be considered.

Under environmental stimuli, the activation of TRPV1 triggers a basic response that has adaptive functions in preventing or reducing functional damage to tissue. The ocular surface expresses TRPV1 in order to adapt to different conditions. This is evident when capsaicin is applied to the eyes, resulting in intense pain. Due to the growing reliance on video display terminals and the ongoing process of urbanization, various daily life stressors can activate corneal TRPV1 channels, thereby contributing to the increasing worldwide prevalence of dry eye syndrome [112]. DED can be characterized as abnormal sensations in response to minor stimuli. Corneal paraesthesia is one of the main symptoms of DED and has become a considerable social burden due to its increasing prevalence. Based on current research with TRPV1-related targeted therapy, both TRPV1 agonists and antagonists have potential value not only for inhibiting corneal sensitivity, but also for inhibiting eye pain. The improvement of symptoms after using TRPV1 antagonists can be explained by the decreased response of the ocular surface to stimulation. This further reduces the release of inflammatory mediators such as SP, thereby relieving pain. With regard to the efficacy of agonists, there could be additional explanations based on the principle of desensitization therapy, although further research is needed to identify the specific reasons. Agonist-mediated inhibition of TRPV1 activity via down-regulation initially aggravates pain due to the activation of TRPV1, but this diminishes over time. New and more selective TRPV1 antagonists are being developed to block nociception. This requires the screening of many candidates, as it must be shown that any reduction in the functional expression of TRPV1 results in localized and targeted effects, with no systemic response. Moreover, it is now clear that the use of TRPV1 antagonists to restore corneal function should be limited to patients with severe injury, and not those with epithelial injury. This distinction must be made because it is only after damage to the basement membrane that TGF- β can enter the matrix and induce

corneal scarring by deactivating TRPV1. Otherwise, the suppression of TRPV1 activation during mild injury does not promote the healing process in epithelial cells. However, potential challenges arise in the development of medications for DED because of the multiple roles of activated TRPV1 following injury. TRPV1 is expressed in normal corneal tissue, and when down-regulated by factors such as estrogen, this reduces corneal sensitivity, response to stimuli, and pain perception. This may decrease the production of tears, blink rate, and eye wiping response. Another potential risk during the treatment of neuropathic pain in dry eye is that TRPV1 inhibitors may weaken the self-defense behavior of the ocular surface due to reduced blinking frequency and eye rubbing response. This may reduce pain symptoms, but also lead to increased ocular surface damage. Finally, targeted therapy involving TRPV1 may not only be beneficial for dry eyes, but could also be extended to other indications such as surgery, eye injections, or pain related to the wearing of contact lenses. This will be an interesting direction for future research. Based on the interaction between TRPV1 and VEGF, TRPV1 could also be explored further as a potential therapeutic target for corneal neovascularization diseases.

Author Contributions

YG and JWJ designed the study. YG and JWJ provided help and advice on grammar. QQG wrote the manuscript and ZS provided help to the revision. ZS performed the acquisition and interpretation of data. QQG and YG designed the figures. All authors contributed to the editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Craig JP, Nichols KK, Akpek EK, Caffery B, Dua HS, Joo CK, *et al.* TFOS DEWS II Definition and Classification Report. *The Ocular Surface*. 2017; 15: 276–283.
- [2] Fallacara A, Vertuani S, Panozzo G, Pecorelli A, Valacchi G, Manfredini S. Novel Artificial Tears Containing Cross-Linked Hyaluronic Acid: An In Vitro Re-Epithelialization Study. *Molecules (Basel, Switzerland)*. 2017; 22: 2104.
- [3] de Paiva CS, Pflugfelder SC, Ng SM, Akpek EK. Topical cyclosporine A therapy for dry eye syndrome. *The Cochrane Database of Systematic Reviews*. 2019; 9: CD010051.
- [4] Vigo L, Senni C, Pellegrini M, Vagge A, Ferro Desideri L, Carones F, *et al.* Effects of a New Formulation of Multiple-Action Tear Substitute on Objective Ocular Surface Parameters and Ocular Discomfort Symptoms in Patients with Dry Eye Disease. *Ophthalmology and Therapy*. 2022; 11: 1441–1447.
- [5] Jones L, Downie LE, Korb D, Benitez-Del-Castillo JM, Dana R, Deng SX, *et al.* TFOS DEWS II Management and Therapy Report. *The Ocular Surface*. 2017; 15: 575–628.
- [6] Galor A, Moein HR, Lee C, Rodriguez A, Felix ER, Sarantopoulos KD, *et al.* Neuropathic pain and dry eye. *The Ocular Surface*. 2018; 16: 31–44.
- [7] Levitt AE, Galor A, Chowdhury AR, Felix ER, Sarantopoulos CD, Zhuang GY, *et al.* Evidence that Dry Eye Represents a Chronic Overlapping Pain Condition. *Molecular Pain*. 2017; 13: 1744806917729306.
- [8] Ashok N, Khamar P, D’Souza S, Gijs M, Ghosh A, Sethu S, *et al.* Ion channels in dry eye disease. *Indian Journal of Ophthalmology*. 2023; 71: 1215–1226.
- [9] Moreno-Montañés J, Bleau AM, Jimenez AI. Tivansiran, a novel siRNA for the treatment of dry eye disease. *Expert Opinion on Investigational Drugs*. 2018; 27: 421–426.
- [10] Liao M, Cao E, Julius D, Cheng Y. Structure of the TRPV1 ion channel determined by electron cryo-microscopy. *Nature*. 2013; 504: 107–112.
- [11] Eguchi H, Hiura A, Nakagawa H, Kusaka S, Shimomura Y. Corneal Nerve Fiber Structure, Its Role in Corneal Function, and Its Changes in Corneal Diseases. *BioMed Research International*. 2017; 2017: 3242649.
- [12] Zhang F, Yang H, Wang Z, Mergler S, Liu H, Kawakita T, *et al.* Transient receptor potential vanilloid 1 activation induces inflammatory cytokine release in corneal epithelium through MAPK signaling. *Journal of Cellular Physiology*. 2007; 213: 730–739.
- [13] Yang Y, Yang H, Wang Z, Mergler S, Wolosin JM, Reinach PS. Functional TRPV1 expression in human corneal fibroblasts. *Experimental Eye Research*. 2013; 107: 121–129.
- [14] Mergler S, Valtink M, Coulson-Thomas VJ, Lindemann D, Reinach PS, Engelmann K, *et al.* TRPV channels mediate temperature-sensing in human corneal endothelial cells. *Experimental Eye Research*. 2010; 90: 758–770.
- [15] Gunthorpe MJ, Benham CD, Randall A, Davis JB. The diversity in the vanilloid (TRPV) receptor family of ion channels. *Trends in Pharmacological Sciences*. 2002; 23: 183–191.
- [16] Hegarty DM, Hermes SM, Largent-Milnes TM, Aicher SA. Capsaicin-responsive corneal afferents do not contain TRPV1 at their central terminals in trigeminal nucleus caudalis in rats. *Journal of Chemical Neuroanatomy*. 2014; 61-62: 1–12.
- [17] Mergler S, Valtink M, Takayoshi S, Okada Y, Miyajima M, Saika S, *et al.* Temperature-sensitive transient receptor potential channels in corneal tissue layers and cells. *Ophthalmic Research*. 2014; 52: 151–159.

- [18] Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*. 1997; 389: 816–824.
- [19] Amaya-Rodriguez CA, Carvajal-Zamorano K, Bustos D, Alegria-Arcos M, Castillo K. A journey from molecule to physiology and *in silico* tools for drug discovery targeting the transient receptor potential vanilloid type 1 (TRPV1) channel. *Frontiers in Pharmacology*. 2024; 14: 1251061.
- [20] Yang F, Cui Y, Wang K, Zheng J. Thermosensitive TRP channel pore turret is part of the temperature activation pathway. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107: 7083–7088.
- [21] Jordt SE, Tominaga M, Julius D. Acid potentiation of the capsaicin receptor determined by a key extracellular site. *Proceedings of the National Academy of Sciences of the United States of America*. 2000; 97: 8134–8139.
- [22] Gao Y, Cao E, Julius D, Cheng Y. TRPV1 structures in nanodiscs reveal mechanisms of ligand and lipid action. *Nature*. 2016; 534: 347–351.
- [23] Darré L, Domene C. Binding of Capsaicin to the TRPV1 Ion Channel. *Molecular Pharmaceutics*. 2015; 12: 4454–4465.
- [24] Matta JA, Ahern GP. Voltage is a partial activator of rat thermosensitive TRP channels. *The Journal of Physiology*. 2007; 585: 469–482.
- [25] Gavva NR, Klionsky L, Qu Y, Shi L, Tamir R, Edenson S, *et al.* Molecular determinants of vanilloid sensitivity in TRPV1. *The Journal of Biological Chemistry*. 2004; 279: 20283–20295.
- [26] Yang F, Xiao X, Cheng W, Yang W, Yu P, Song Z, *et al.* Structural mechanism underlying capsaicin binding and activation of the TRPV1 ion channel. *Nature Chemical Biology*. 2015; 11: 518–524.
- [27] Jordt SE, Julius D. Molecular basis for species-specific sensitivity to “hot” chili peppers. *Cell*. 2002; 108: 421–430.
- [28] Welch JM, Simon SA, Reinhart PH. The activation mechanism of rat vanilloid receptor 1 by capsaicin involves the pore domain and differs from the activation by either acid or heat. *Proceedings of the National Academy of Sciences of the United States of America*. 2000; 97: 13889–13894.
- [29] Ma L, Yang F, Vu S, Zheng J. Exploring functional roles of TRPV1 intracellular domains with unstructured peptide-insertion screening. *Scientific Reports*. 2016; 6: 33827.
- [30] Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, *et al.* The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron*. 1998; 21: 531–543.
- [31] Koplas PA, Rosenberg RL, Oxford GS. The role of calcium in the desensitization of capsaicin responses in rat dorsal root ganglion neurons. *The Journal of Neuroscience: the Official Journal of the Society for Neuroscience*. 1997; 17: 3525–3537.
- [32] Reeh PW, Kress M. Molecular physiology of proton transduction in nociceptors. *Current Opinion in Pharmacology*. 2001; 1: 45–51.
- [33] Veldhuis NA, Poole DP, Grace M, McIntyre P, Bunnett NW. The G protein-coupled receptor-transient receptor potential channel axis: molecular insights for targeting disorders of sensation and inflammation. *Pharmacological Reviews*. 2015; 67: 36–73.
- [34] Fakhri D, Migeon T, Moreau N, Baudouin C, Réaux-Le Goazigo A, Mélik Parsadaniantz S. Transient Receptor Potential Channels: Important Players in Ocular Pain and Dry Eye Disease. *Pharmaceutics*. 2022; 14: 1859.
- [35] Pan Z, Wang Z, Yang H, Zhang F, Reinach PS. TRPV1 activation is required for hypertonicity-stimulated inflammatory cytokine release in human corneal epithelial cells. *Investigative Ophthalmology & Visual Science*. 2011; 52: 485–493.
- [36] Mergler S, Garreis F, Sahlmüller M, Reinach PS, Paulsen F, Pleyer U. Thermosensitive transient receptor potential channels in human corneal epithelial cells. *Journal of Cellular Physiology*. 2011; 226: 1828–1842.
- [37] Harrell CR, Feulner L, Djonov V, Pavlovic D, Volarevic V. The Molecular Mechanisms Responsible for Tear Hyperosmolarity-Induced Pathological Changes in the Eyes of Dry Eye Disease Patients. *Cells*. 2023; 12: 2755.
- [38] Fakhri D, Guerrero-Moreno A, Baudouin C, Réaux-Le Goazigo A, Parsadaniantz SM. Capsazepine decreases corneal pain syndrome in severe dry eye disease. *Journal of Neuroinflammation*. 2021; 18: 111.
- [39] Tomoyose K, Okada Y, Sumioka T, Miyajima M, Flanders KC, Shirai K, *et al.* Suppression of In Vivo Neovascularization by the Loss of TRPV1 in Mouse Cornea. *Journal of Ophthalmology*. 2015; 2015: 706404.
- [40] Zheng Y, Huang Q, Zhang Y, Geng L, Wang W, Zhang H, *et al.* Multimodal roles of transient receptor potential channel activation in inducing pathological tissue scarification. *Frontiers in Immunology*. 2023; 14: 1237992.
- [41] Okada Y, Reinach PS, Shirai K, Kitano A, Kao WWY, Flanders KC, *et al.* TRPV1 involvement in inflammatory tissue fibrosis in mice. *The American Journal of Pathology*. 2011; 178: 2654–2664.
- [42] Türker E, Garreis F, Khajavi N, Reinach PS, Joshi P, Brockmann T, *et al.* Vascular Endothelial Growth Factor (VEGF) Induced Downstream Responses to Transient Receptor Potential Vanilloid 1 (TRPV1) and 3-Iodothyronamine (3-T₁AM) in Human Corneal Keratocytes. *Frontiers in Endocrinology*. 2018; 9: 670.
- [43] Turan E, Valtink M, Reinach PS, Skupin A, Luo H, Brockmann T, *et al.* L-carnitine suppresses transient receptor potential vanilloid type 1 activity and myofibroblast transdifferentiation in human corneal keratocytes. *Laboratory Investigation; a Journal of Technical Methods and Pathology*. 2021; 101: 680–689.
- [44] Okumura N, Koizumi N. Regeneration of the Corneal Endothelium. *Current Eye Research*. 2020; 45: 303–312.
- [45] Martínez-García MC, Martínez T, Pañeda C, Gallego P, Jiménez AI, Merayo J. Differential expression and localization of transient receptor potential vanilloid 1 in rabbit and human eyes. *Histology and Histopathology*. 2013; 28: 1507–1516.
- [46] Shaheen BS, Bakir M, Jain S. Corneal nerves in health and disease. *Survey of Ophthalmology*. 2014; 59: 263–285.
- [47] Al-Aqaba MA, Dhillon VK, Mohammed I, Said DG, Dua HS. Corneal nerves in health and disease. *Progress in Retinal and Eye Research*. 2019; 73: 100762.
- [48] Alamri A, Bron R, Brock JA, Ivanusic JJ. Transient receptor potential cation channel subfamily V member 1 expressing corneal sensory neurons can be subdivided into at least three subpopulations. *Frontiers in Neuroanatomy*. 2015; 9: 71.
- [49] Belmonte C. Pain, Dryness, and Itch Sensations in Eye Surface Disorders Are Defined By a Balance Between Inflammation and Sensory Nerve Injury. *Cornea*. 2019; 38: S11–S24.
- [50] Belmonte C, Nichols JJ, Cox SM, Brock JA, Begley CG, Bereiter DA, *et al.* TFOS DEWS II pain and sensation report. *The Ocular Surface*. 2017; 15: 404–437.
- [51] Galletti JG, de Paiva CS. The ocular surface immune system through the eyes of aging. *The Ocular Surface*. 2021; 20: 139–162.
- [52] Müller LJ, Marfurt CF, Kruse F, Tervo TMT. Corneal nerves: structure, contents and function. *Experimental Eye Research*. 2003; 76: 521–542.
- [53] Sabatino F, Di Zazzo A, De Simone L, Bonini S. The Intriguing Role of Neuropeptides at the Ocular Surface. *The Ocular Surface*. 2017; 15: 2–14.
- [54] Willcox MDP, Argüeso P, Georgiev GA, Holopainen JM, Laurie GW, Millar TJ, *et al.* TFOS DEWS II Tear Film Report. *The Ocular Surface*. 2017; 15: 366–403.
- [55] Alhathem A, Cavalcanti B, Hamrah P. In vivo confocal microscopy in dry eye disease and related conditions. *Seminars in*

- Ophthalmology. 2012; 27: 138–148.
- [56] Cruzat A, Qazi Y, Hamrah P. *In Vivo* Confocal Microscopy of Corneal Nerves in Health and Disease. *The Ocular Surface*. 2017; 15: 15–47.
 - [57] Labetoulle M, Baudouin C, Calonge M, Merayo-Llodes J, Boboridis KG, Akova YA, *et al.* Role of corneal nerves in ocular surface homeostasis and disease. *Acta Ophthalmologica*. 2019; 97: 137–145.
 - [58] Puja G, Sonkodi B, Bardoni R. Mechanisms of Peripheral and Central Pain Sensitization: Focus on Ocular Pain. *Frontiers in Pharmacology*. 2021; 12: 764396.
 - [59] Mogi M, Mendonza AE, Chastain J, Demirs JT, Medley QG, Zhang Q, *et al.* Ocular Pharmacology and Toxicology of TRPV1 Antagonist SAF312 (Libvatrep). *Translational Vision Science & Technology*. 2023; 12: 5.
 - [60] Messmer EM. The pathophysiology, diagnosis, and treatment of dry eye disease. *Deutsches Arzteblatt International*. 2015; 112: 71–81; quiz 82.
 - [61] Perez VL, Stern ME, Pflugfelder SC. Inflammatory basis for dry eye disease flares. *Experimental Eye Research*. 2020; 201: 108294.
 - [62] Khajavi N, Reinach PS, Skrzypski M, Lude A, Mergler S. L-carnitine reduces in human conjunctival epithelial cells hypertonic-induced shrinkage through interacting with TRPV1 channels. *Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology*. 2014; 34: 790–803.
 - [63] Koroleva K, Svitko S, Ananev A, Buglinina A, Bogatova K, Yakovleva O, *et al.* Effects of Nitric Oxide on the Activity of P2X and TRPV1 Receptors in Rat Meningeal Afferents of the Trigeminal Nerve. *International Journal of Molecular Sciences*. 2023; 24: 7519.
 - [64] Masuoka T, Yamashita Y, Nakano K, Takechi K, Niimura T, Tawa M, *et al.* Chronic Tear Deficiency Sensitizes Transient Receptor Potential Vanilloid 1-Mediated Responses in Corneal Sensory Nerves. *Frontiers in Cellular Neuroscience*. 2020; 14: 598678.
 - [65] Bereiter DA, Rahman M, Thompson R, Stephenson P, Saito H. TRPV1 and TRPM8 Channels and Nocifensive Behavior in a Rat Model for Dry Eye. *Investigative Ophthalmology & Visual Science*. 2018; 59: 3739–3746.
 - [66] Yamazaki R, Yamazoe K, Yoshida S, Hatou S, Inagaki E, Okano H, *et al.* The Semaphorin 3A inhibitor SM-345431 preserves corneal nerve and epithelial integrity in a murine dry eye model. *Scientific Reports*. 2017; 7: 15584.
 - [67] Vereertbrugghen A, Galletti JG. Corneal nerves and their role in dry eye pathophysiology. *Experimental Eye Research*. 2022; 222: 109191.
 - [68] Lucius A, Chhatwal S, Valtink M, Reinach PS, Li A, Pleyer U, *et al.* L-Carnitine Suppresses Transient Receptor Potential Vanilloid Type 1 Activation in Human Corneal Epithelial Cells. *International Journal of Molecular Sciences*. 2023; 24: 11815.
 - [69] Messmer EM. Osmoprotection as a new therapeutic principle. *Der Ophthalmologe: Zeitschrift Der Deutschen Ophthalmologischen Gesellschaft*. 2007; 104: 987–990.
 - [70] Baudouin C, Aragona P, Messmer EM, Tomlinson A, Calonge M, Boboridis KG, *et al.* Role of hyperosmolarity in the pathogenesis and management of dry eye disease: proceedings of the OCEAN group meeting. *The Ocular Surface*. 2013; 11: 246–258.
 - [71] Hua X, Deng R, Li J, Chi W, Su Z, Lin J, *et al.* Protective Effects of L-Carnitine Against Oxidative Injury by Hyperosmolarity in Human Corneal Epithelial Cells. *Investigative Ophthalmology & Visual Science*. 2015; 56: 5503–5511.
 - [72] Hua X, Su Z, Deng R, Lin J, Li DQ, Pflugfelder SC. Effects of L-carnitine, erythritol and betaine on pro-inflammatory markers in primary human corneal epithelial cells exposed to hyperosmotic stress. *Current Eye Research*. 2015; 40: 657–667.
 - [73] Al-Aqaba MA, Fares U, Suleman H, Lowe J, Dua HS. Architecture and distribution of human corneal nerves. *The British Journal of Ophthalmology*. 2010; 94: 784–789.
 - [74] Belmonte C, Aracil A, Acosta MC, Luna C, Gallar J. Nerves and sensations from the eye surface. *The Ocular Surface*. 2004; 2: 248–253.
 - [75] Réaux-Le Goazigo A, Labbé A, Baudouin C, Melik Parsadaniantz S. Towards a better understanding of chronic ocular pain. *Medicine Sciences: M/S*. 2017; 33: 749–757.
 - [76] Belmonte C, Acosta MC, Merayo-Llodes J, Gallar J. What Causes Eye Pain? *Current Ophthalmology Reports*. 2015; 3: 111–121.
 - [77] Hiura A, Nakagawa H. Capsaicin-resistant, nonspecific acetylcholinesterase (NsAChE) reactive nerve fibers in the rat cornea: a quantitative and developmental study. *Okajimas Folia Anatomica Japonica*. 2004; 81: 75–84.
 - [78] Kishimoto S, Katagiri A, Oyamaguchi A, Sato H, Toyoda H, Niwa H, *et al.* Enhanced Ocular Surface and Intraoral Nociception via a Transient Receptor Potential Vanilloid 1 Mechanism in a Rat Model of Obstructive Sleep Apnea. *Neuroscience*. 2022; 483: 66–81.
 - [79] Jeong KY. Changes in TRPV1-Mediated Physiological Function in Rats Systemically Treated With Capsaicin on the Neonate. *International Journal of Molecular Sciences*. 2020; 21: 3143.
 - [80] Fattori V, Hohmann MSN, Rossaneis AC, Pinho-Ribeiro FA, Verri WA. Capsaicin: Current Understanding of Its Mechanisms and Therapy of Pain and Other Pre-Clinical and Clinical Uses. *Molecules (Basel, Switzerland)*. 2016; 21: 844.
 - [81] Forsby A, Norman KG, El Andaloussi-Lilja J, Lundqvist J, Walczak V, Curren R, *et al.* Using novel in vitro NociOcular assay based on TRPV1 channel activation for prediction of eye sting potential of baby shampoos. *Toxicological Sciences: an Official Journal of the Society of Toxicology*. 2012; 129: 325–331.
 - [82] Bates BD, Mitchell K, Keller JM, Chan CC, Swaim WD, Yaskovich R, *et al.* Prolonged analgesic response of cornea to topical resiniferatoxin, a potent TRPV1 agonist. *Pain*. 2010; 149: 522–528.
 - [83] Acosta CM, Luna C, Quirce S, Belmonte C, Gallar J. Changes in sensory activity of ocular surface sensory nerves during allergic keratoconjunctivitis. *Pain*. 2013; 154: 2353–2362.
 - [84] Sullivan C, Lee J, Bushey W, Demers D, Dinsdale S, Lowe K, *et al.* Evidence for a phenotypic switch in corneal afferents after lacrimal gland excision. *Experimental Eye Research*. 2022; 218: 109005.
 - [85] Liu H, Begley C, Chen M, Bradley A, Bonanno J, McNamara NA, *et al.* A link between tear instability and hyperosmolarity in dry eye. *Investigative Ophthalmology & Visual Science*. 2009; 50: 3671–3679.
 - [86] Luo L, Li DQ, Pflugfelder SC. Hyperosmolarity-induced apoptosis in human corneal epithelial cells is mediated by cytochrome c and MAPK pathways. *Cornea*. 2007; 26: 452–460.
 - [87] Lasagni Vitar RM, Rama P, Ferrari G. The two-faced effects of nerves and neuropeptides in corneal diseases. *Progress in Retinal and Eye Research*. 2022; 86: 100974.
 - [88] Guzmán M, Miglio M, Keitelman I, Shiromizu CM, Sabbione F, Fuentes F, *et al.* Transient tear hyperosmolarity disrupts the neuroimmune homeostasis of the ocular surface and facilitates dry eye onset. *Immunology*. 2020; 161: 148–161.
 - [89] Guzmán M, Miglio MS, Zgajnar NR, Colado A, Almejún MB, Keitelman IA, *et al.* The mucosal surfaces of both eyes are immunologically linked by a neurogenic inflammatory reflex involving TRPV1 and substance P. *Mucosal Immunology*. 2018; 11: 1441–1453.
 - [90] Lasagni Vitar RM, Fonteyne P, Chaabane L, Rama P, Ferrari

- G. A Hypothalamic-Controlled Neural Reflex Promotes Corneal Inflammation. *Investigative Ophthalmology & Visual Science*. 2021; 62: 21.
- [91] Reinach PS, Mergler S, Okada Y, Saika S. Ocular transient receptor potential channel function in health and disease. *BMC Ophthalmology*. 2015; 15: 153.
- [92] Yang Y, Yang H, Wang Z, Varadaraj K, Kumari SS, Mergler S, *et al.* Cannabinoid receptor 1 suppresses transient receptor potential vanilloid 1-induced inflammatory responses to corneal injury. *Cellular Signalling*. 2013; 25: 501–511.
- [93] Yang H, Wang Z, Capó-Aponte JE, Zhang F, Pan Z, Reinach PS. Epidermal growth factor receptor transactivation by the cannabinoid receptor (CB1) and transient receptor potential vanilloid 1 (TRPV1) induces differential responses in corneal epithelial cells. *Experimental Eye Research*. 2010; 91: 462–471.
- [94] Yang JM, Wei ET, Kim SJ, Yoon KC. TRPM8 Channels and Dry Eye. *Pharmaceuticals (Basel, Switzerland)*. 2018; 11: 125.
- [95] Izquierdo C, Martín-Martínez M, Gómez-Monterrey I, González-Muñoz R. TRPM8 Channels: Advances in Structural Studies and Pharmacological Modulation. *International Journal of Molecular Sciences*. 2021; 22: 8502.
- [96] Li F, Yang W, Jiang H, Guo C, Huang AJW, Hu H, *et al.* TRPV1 activity and substance P release are required for corneal cold nociception. *Nature Communications*. 2019; 10: 5678.
- [97] Damann N, Voets T, Nilius B. TRPs in our senses. *Current Biology*. 2008; 18: R880–R889.
- [98] Khajavi N, Reinach PS, Slavi N, Skrzypski M, Lucius A, Strauß O, *et al.* Thyronamine induces TRPM8 channel activation in human conjunctival epithelial cells. *Cellular Signalling*. 2015; 27: 315–325.
- [99] Lucius A, Khajavi N, Reinach PS, Köhrle J, Dhandapani P, Huimann P, *et al.* 3-Iodothyronamine increases transient receptor potential melastatin channel 8 (TRPM8) activity in immortalized human corneal epithelial cells. *Cellular Signalling*. 2016; 28: 136–147.
- [100] Liu Y, Lyu Y, Wang H. TRP Channels as Molecular Targets to Relieve Endocrine-Related Diseases. *Frontiers in Molecular Biosciences*. 2022; 9: 895814.
- [101] Moran MM. TRP Channels as Potential Drug Targets. *Annual Review of Pharmacology and Toxicology*. 2018; 58: 309–330.
- [102] Anfuso CD, Olivieri M, Fidilio A, Lupo G, Rusciano D, Pezzino S, *et al.* Gabapentin Attenuates Ocular Inflammation: *In vitro* and *In vivo* Studies. *Frontiers in Pharmacology*. 2017; 8: 173.
- [103] Cammalleri M, Amato R, Olivieri M, Pezzino S, Bagnoli P, Dal Monte M, *et al.* Effects of Topical Gabapentin on Ocular Pain and Tear Secretion. *Frontiers in Pharmacology*. 2021; 12: 671238.
- [104] Kavalieratos CS, Dimou T. Gabapentin therapy for painful, blind glaucomatous eye: case report. *Pain Medicine (Malden, Mass.)*. 2008; 9: 377–378.
- [105] Biggs JE, Stemkowski PL, Knaus EE, Chowdhury MA, Balanyi K, Smith PA. Suppression of network activity in dorsal horn by gabapentin permeation of TRPV1 channels: implications for drug access to cytoplasmic targets. *Neuroscience Letters*. 2015; 584: 397–402.
- [106] Dogru M, Gunay M, Celik G, Aktas A. Evaluation of the tear film instability in children with allergic diseases. *Cutaneous and Ocular Toxicology*. 2016; 35: 49–52.
- [107] Kalangara JP, Galor A, Levitt RC, Felix ER, Alegret R, Sarantopoulos CD. Burning Eye Syndrome: Do Neuropathic Pain Mechanisms Underlie Chronic Dry Eye? *Pain Medicine (Malden, Mass.)*. 2016; 17: 746–755.
- [108] Wilson SR, Gerhold KA, Bifulck-Fisher A, Liu Q, Patel KN, Dong X, *et al.* TRPA1 is required for histamine-independent, Mas-related G protein-coupled receptor-mediated itch. *Nature Neuroscience*. 2011; 14: 595–602.
- [109] Vereertbrugghen A, Pizzano M, Sabbione F, Keitelman IA, Shiromizu CM, Aguilar DV, *et al.* An ocular Th1 immune response promotes corneal nerve damage independently of the development of corneal epitheliopathy. *Journal of Neuroinflammation*. 2023; 20: 120.
- [110] Pizzano M, Vereertbrugghen A, Cernutto A, Sabbione F, Keitelman IA, Shiromizu CM, *et al.* Ocular TRPV1 deficiency protects corneal nerve damage from dry eye-induced nerve damage. *bioRxiv*. 2023. (preprint)
- [111] Royer DJ, Echegaray-Mendez J, Lin L, Gmyrek GB, Mathew R, Saban DR, *et al.* Complement and CD4⁺ T cells drive context-specific corneal sensory neuropathy. *eLife*. 2019; 8: e48378.
- [112] Abdulmannan DM, Naser AY, Ibrahim OK, Mahmood AS, Alyoussef Alkrad J, Sweiss K, *et al.* Visual health and prevalence of dry eye syndrome among university students in Iraq and Jordan. *BMC Ophthalmology*. 2022; 22: 265.