Research Correspondence

Patients with neurotrophic keratitis demonstrate alterations in ocular surface expression of transient receptor potential (TRP) channels

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To the Editor,

Neurotrophic keratopathy (NK), a rare corneal disease caused by trigeminal nerve damage, leads to impaired corneal sensitivity, blinking and tear reflexes, and corneal epithelium breakdown with poor healing, which may progress to corneal ulcer, melting and perforation [1].

Recent studies have highlighted the key role of transient receptor potential (TRP) channels in cellular and molecular changes within primary sensory neurons underlying nociception, wound healing, dry eye, scarring, and immune response in the cornea [2].

TRPs are widely expressed in eye tissues [2]. In the cornea, polymodal nociceptors have been identified to have small ramifying nerve endings that express TRPV1, while cold thermoreceptors exhibit large complex endings positive for TRPM8 [3]. TRP expression is not restricted to neural elements in the eye, but also extends to supporting non-neuronal cells. Specifically, TRPV1/4 and TRPM8 were detected in human corneal and conjunctival epithelial cells [2]. In vitro and in vivo studies showed that TRPV1/4 and TRPM8 have a role in modulating inflammation and would healing in the ocular surface [2].

Sensory nerves and corneal and conjunctival supporting cells are anatomically and functionally related and are able to mutually modulate each other by release of neuromediators and growth factors [1]. However, it is not known if ocular surface diseases with neurosensory etiology such as NK and Sjogren's Syndrome (SS) may be associated with different expression of TRPs on epithelial cells.

Therefore, the purpose of this prospective, cross-sectional study was to evaluate conjunctival expression of TRPV1/4 and TRPM8 in patients with NK, and compare results to patients with SS-related dry eye disease (SS-DED) and healthy subjects. Furthermore, relationships between NK and SS clinical features with TRPs' levels were evaluated. The study was prospectively reviewed by the Ethics Committee of the Sapienza University of Rome (rif. 2339/26.01.2012). The research followed the Tenets of the Declaration of Helsinki, and informed consent was obtained from all subjects of the study.

Thirty patients including 10 patients (8 females and 2 males) diagnosed with NK (n=10 eyes), 10 patients (10 females and 0 males) diagnosed with SS-DED (n=10 eyes), and 10 (8 females and 2 males) healthy controls (n=10 eyes) were recruited for this study between 2021 and 2022 at the Department of Sense Organs, Sapienza University of Rome.

All NK eyes was classified as stage 1 according to the Mackie classification [1], while all SS-DED eyes were graded as severity 2 using the International Dry Eye Workshop (DEWS) classification [4].

Exclusionary criteria were: any systemic or ocular co-morbidities that could affect the ocular surface, contact lens use, ocular trauma or surgeries. Also excluded were subjects with punctual plugs, used eye drops other than artificial tears in the previous 30 days, used any systemic medication that could cause alterations to the ocular surface, or who were pregnant. All participants underwent medical history collection, completion of OSDI questionnaire, complete ophthalmological examination including assessment of central corneal

sensitivity by Cochet Bonnet esthesiometer (Luneau Ophtalmologie, France), corneal fluorescein staining score (CFS) according to the NEI scale, lacrimal function tests by Schirmer test without anesthesia and fluorescein tear break-up time (FTBUT). Conjunctival impression cytology was carried out in all included eyes by applying a sterile methylcellulose cell culture insert (Millicell-CM, 0.4 μm, Ø12 mm,Millipore) to the nasal and temporal bulbar conjunctiva. The biological samples were anonymously encoded and stored at −20 °C for Western blot analysis. Images from Western blots were analyzed with ImageJ software for Windows. The following antibodies were used: anti-TRPV1 (NBP1-97417, Novus Biologicals, CO, USA), anti-TRPV4 (NBP2-41262, Novus Biologicals, CO, USA), and anti-TRPM8 (ab3243, Abcam, UK). Actin (sc-47778, Santa Cruz Biotechnology,CA,USA) was used as reference to normalize protein loading in conjunctival impression cytology.

Descriptive statistical analysis was performed in all collected data. Continuous variables were presented as mean ± standard deviation. Intergroup differences were evaluated using the independent Student's t-Test with Levene's correction and one-way Anova. The correlations between TRPV1/4, TRPM8 expression profiles and clinical parameters were calculated by using the Spearman Rho test. The analysis was conducted using SPSS software version 29.0 (SPSS Inc., IBM,Chicago,IL,USA). P values lower than 0.05 were considered statistically significant.

The mean age of the patients was 59.0 ± 12.45 (50.0–80.0 years) in NK, 68.4 ± 8.41 (56.0–77.0 years) in SS-DED, and 60.2 ± 20.86 (31.0–80.0 years) in controls, respectively (p=0.40). In NK group, the etiology of disease was mainly postherpetic (n=6, 60%), followed by neurosurgical (n=2, 20%) and ocular surgery (n=2, 20%). In SS-DED group, two patients were diagnosed with primary SS whereas in 8 cases the disease was secondary to rheumatologic (n=6; 75%) and thyroid disorders (n=2, 25%).

The analysis of conjunctival expression of TRPV1/4 and TRPM8 receptors showed a statistically significant increase of TRPV1 expression in NK compared to SS-DED (2.77±1.12 Vs 0.65±0.37; p=0.004) and to the healthy control population (1.00±0.38; p=0.01).

TRPV4 showed a trend of increasing conjunctival expression in NK (1.11±0.41) compared to SS-DED (0.64±0.24) and controls (1.0±0.29), but this did not reach statistically significant values (p=0.06 and p=0.65, respectively).

TRPM8 expression was reduced in NK (0.51 ±0.24) compared to SS-DED (0.59±0.62) and controls (0.96± 0.36), but these differences were also not statistically relevant (p=0.78 and p=0.05 respectively). A statistically significant correlation was demonstrated between the increase of conjunctival expression of TRPV1 and reduction of corneal sensitivity (r_s = -0.52, p=0.02, R^2 = 0.33). Results are summarized in Figure 1.

These findings are consistent with previous in vitro and in vivo studies demonstrating that TRPV1 and TRPV4 have a primary role in regulating the inflammatory response and promoting wound healing in the ocular surface [2,5,6]. Specifically, TRPV1 activation on the trigeminal ganglia contributed to nociception experienced in a rat model of dry eye [2]. Moreover, TRPV1 activation on stromal fibroblasts resulted in corneal fibrosis and initiation of chronic immune responses in an alkali burn mouse corneal wound healing model [2]. Similarly, TRPV1 and TRPV4 knock-out mice exhibited delayed corneal epithelial wound closure following epithelial debridement, and TRPV4-null cultured ocular fibroblasts showed downregulated myofibroblast differentiation [2]. Additionally, TRPV1 activation has been associated with increased release of pro-inflammatory cytokines and chemokines, while TRPV4 activation has been shown to promote the release of anti-inflammatory factors [5].

Moreover, TRPV1 was found to have a primary role in regulating the immunological responses in the ocular surface. Specifically, TRPV1 signaling in an injured eye was observed to trigger the breakdown of the mucosal tolerogenic response in the contralateral eye, in the context of unilateral eye injuries in mice. Therefore, it appears that the immunological responses of both ocular surfaces are intertwined through a neurogenic inflammatory reflex mediated by TRPV1 [7].

The same Authors later reported that activation of TRPV1 in response to tear hyperosmolarity is involved in impaired ocular mucosal tolerance to a topical surrogate antigen in mice [8].

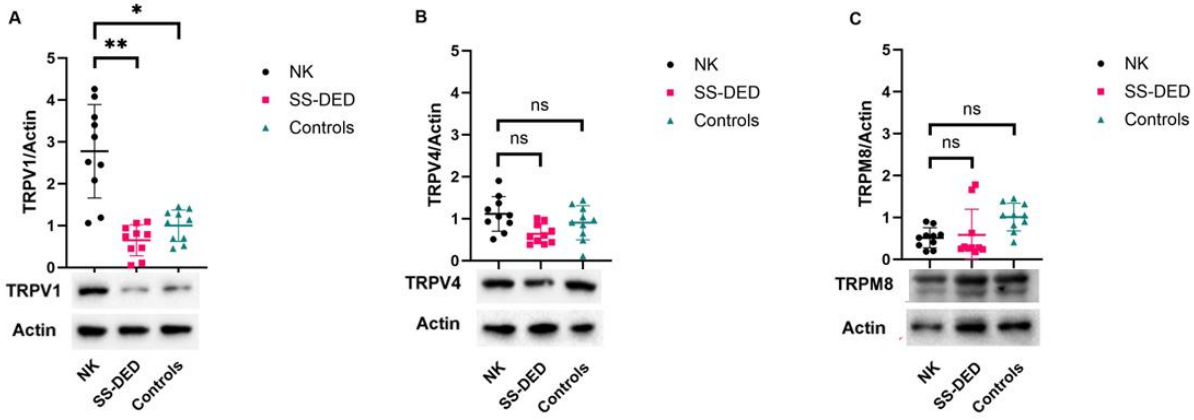
On the other hand, studies have shown that TRPM8 activation on afferent sensory corneal nerves and in different corneal cell types can enhance basal tear flow in response to evaporative cooling and hyperosmolar solutions [6,9]. Also,TRPM8 is a direct stimulator of tear secretion from the lacrimal gland [6,9]. Furthermore, low levels of TRPM8-positive nerve terminals and hypersensitive nerve responses were reported to persist long after corneal injury in mice, suggesting a contribution to the pathogenesis of dry eye-like pain induced by corneal surgery [10].

Interestingly, a functional interaction was demonstrated between TRPV1 and TRPM8 since TRPM8 activation suppressed TRPV1-induced Ca2+ increases [6]. Also, recent studies reported a 67% of TRPM8-positive corneal cells expressing TRPV1 in dry eye, and the increase in heat-evoked responses in corneal cells was associated to a suppression of cool-evoked activity. Specifically, TRPM8 channel desensitization was shown to cause TRPV1 channel sensitization [11].

Our findings, which show a tendency towards reduced expression of TRPM8 in NK compared to SS-DED and controls, align with the aforementioned, and may be attributed to the impairment of neurosensory function in tear regulation that underlie this condition, leading to decreased tear film and hyperosmolarity [1,4].

To conclude, patients with NK demonstrated different expression of TRP channels in the ocular surface compared to SS-DED and controls. Additional prospective studies with larger populations are needed to validate our findings and possibly lead to a better understanding of the mechanisms underlying inflammation, wound healing and dryness in the ocular surface. These findings have the potential to contribute towards the identification of novel biomarkers for ocular surface diseases and offer promising strategies for targeting TRPs as an appealing therapeutic approach to address neuroinflammation in the ocular surface.

Figure 1



TRPV1 (A), TRPV4 (B) and TRPM8 (C) mean levels ± SD in the collected conjunctiva epithelium samples of patients with NK, SS-DED and Controls. Descriptive statistics are reported in the text.

ns*: non-significant, * p<0.05; ** p<0.01; *** p<0.001.*

TRP: transient receptor potential, NK: Neurotrophic Keratitis, SS-DED: Sjogren's Syndrome related Dry Eye Disease.

Disclosures

The authors declare that they do not have any conflicts or potential conflicts of interest

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