

# Aprepitant Restores Corneal Sensitivity and Reduces Pain in DED

Filippo Bonelli<sup>1,2</sup>, Fabiola Campestre<sup>1</sup>, Romina Mayra Lasagni Vitar<sup>1</sup>, Ibrahim Halil Demirsoy<sup>1</sup>, Philippe Fonteyne<sup>1</sup>, and Giulio Ferrari<sup>1,3</sup>

<sup>1</sup> Department of Ophthalmology, Eye Repair Lab, IRCCS San Raffaele Scientific Institute, Milan, Italy

<sup>2</sup> Department of Cell Biology–Inspired Tissue Engineering, MERLN Institute for Technology–Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands

<sup>3</sup> Università Vita-Salute San Raffaele, Milan, Italy

**Correspondence:** Giulio Ferrari, Department of Ophthalmology, Cornea and Ocular Surface Unit, Eye Repair Lab, IRCCS San Raffaele Scientific Institute, Milan, Italy. e-mail: [ferrari.giulio@hsr.it](mailto:ferrari.giulio@hsr.it)

**Received:** September 22, 2023

**Accepted:** January 8, 2024

**Published:** February 12, 2024

**Keywords:** NK1R; pain; inflammation; dry eye disease (DED); aprepitant

**Citation:** Bonelli F, Campestre F, Lasagni Vitar RM, Demirsoy IH, Fonteyne P, Ferrari G. Aprepitant restores corneal sensitivity and reduces pain in DED. *Transl Vis Sci Technol.* 2024;13(2):9. <https://doi.org/10.1167/tvst.13.2.9>

**Purpose:** This study aims to assess the efficacy of two aprepitant formulations (X1 and X2), in a preclinical model of dry eye disease (DED) induced by benzalkonium chloride (BAK).

**Methods:** Two aprepitant formulations were tested on 7 to 8-week-old male mice for their efficacy. In vivo corneal fluorescein staining assessed epithelial damage as the primary end point on days 0, 3, 5, 7, 9, 12, and 14 using slit-lamp microscopy. The DED model was induced with 0.2% BAK twice daily for the first week and once daily for the next week. Mice were randomly assigned to 5 treatment groups: Aprepitant X1 ( $n = 10$ ) and X2 ( $n = 10$ ) formulation, 2 mg/mL dexamethasone ( $n = 10$ ), control vehicle X ( $n = 10$ ), 0.2% hyaluronic acid ( $n = 10$ ), or no treatment ( $n = 10$ ). Eye wiping, phenol red, and Cochet Bonnet tests assessed ocular pain, tear fluid secretion, and nerve function. After 7 days, the mice were euthanized to quantify leukocyte infiltration and corneal nerve density.

**Results:** Topical aprepitant X1 reduced BAK-induced corneal damage and pain compared to gel vehicle X ( $P = 0.007$ ) and dexamethasone ( $P = 0.021$ ). Aprepitant X1 and X2 improved corneal sensitivity versus gel vehicle X and dexamethasone ( $P < 0.001$ ). Aprepitant X1 reduced leukocyte infiltration ( $P < 0.05$ ) and enhanced corneal nerve density ( $P < 0.001$ ). Tear fluid secretion remained statistically unchanged in both the X1 and X2 groups.

**Conclusions:** Aprepitant formulation X1 reduced pain, improved corneal sensitivity and nerve density, ameliorated epitheliopathy, and reduced leukocyte infiltration in male mouse corneas.

**Translational Relevance:** Aprepitant emerges as a safe, promising therapeutic prospect for the amelioration of DED's associated symptoms.

## Introduction

With a prevalence ranging from 5 to 34% of the population, dry eye disease (DED) is one of the most common ocular surface diseases.<sup>1–4</sup> Environmental factors and immune dysregulation are involved in the pathophysiology of DED. Recently, a key role of

neurosensory abnormalities has also been acknowledged.<sup>5–7</sup>

Long-term use of eye drops containing some preservatives is associated with the development of ocular surface disease and DED symptoms.<sup>8,9</sup> Specifically, benzalkonium chloride (BAK) is the most widely used eye drop preservative whose toxic effects on the ocular surface have been extensively reported and

closely resembles DED.<sup>8,10–12</sup> Interestingly, besides its well-known effects in inducing epithelial disease and promoting leukocyte infiltration, disruption of nerve terminals has been described following chronic BAK application.<sup>13–15</sup>

While it is well known that DED is the result of the interaction of immune and environmental factors, the role of neuroinflammation is starting to emerge.<sup>2,16,17</sup> Among the multiple neuropeptides involved in this process, substance P (SP) is a relevant actor as it is secreted in large amounts by sensory terminals and promotes pain, angiogenesis, infiltration, and activation of leukocytes.<sup>18,19</sup> Recent studies have shown that SP secretion in the tear fluid is increased following laser-assisted in situ keratomileusis (LASIK) surgery and is associated with the development of dry eye symptoms.<sup>20–22</sup> Moreover, it has been demonstrated that tear levels of SP are higher in symptomatic contact lenses wearers, and in patients with DED wearing soft contact lens, and, more generally, in patients affected with severe ocular surface inflammation.<sup>23–25</sup> The blockade of SP with a highly potent and selective neurokinin-1 receptor (NK1R) antagonist (fosaprepitant) inhibits pain, inflammation, and angiogenesis in preclinical models of alkali burn- and suture-induced corneal inflammation.<sup>25,26</sup> Therefore, it is tempting to speculate that modulating neuroinflammation by means of topically applied NK1R antagonists could simultaneously treat inflammation and pain in DED.<sup>27–29</sup>

In this study, we aimed to assess the efficacy of topical administration of formulated aprepitant (X1 and X2), the active principle of the prodrug fosaprepitant, in the BAK-induced DED mouse model.

## Materials and Methods

### Animals

The efficacy of aprepitant was analyzed on 50 C57BL/6N male mice (Charles River Laboratory, Lecco, Italy). Mice were maintained at a controlled temperature of 22°C, humidity of 50% to 60%, and received light from 07:00 to 19:00. Fresh food and water were freely available. In vivo corneal fluorescein staining was exploited to reveal corneal epithelial disease. The mice were monitored on days  $-7$ ,  $-4$ ,  $-2$ ,  $0$ ,  $2$ ,  $5$ , and  $8$  by in vivo slit-lamp microscopy imaging. On day 11, the mice were euthanized by carbon dioxide inhalation, and following cervical dislocation was used. Supplementary Figure S1 presents a graphical timeline illustrating the sequence of conducted experiments. Corneas were harvested and dissected for

immunofluorescence analyses. All documents related to experiments were approved by the Animal Care and Use Committee of the IRCCS San Raffaele Scientific Institute according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

### Benzalkonium Chloride-Induced DED Model

The induction of the DED model followed the procedures outlined in our prior publication.<sup>30</sup> In brief, the mice received topical applications of 10  $\mu$ L drops containing 0.2% BAK twice daily over the course of 1 week. After the weeklong BAK induction period, the mice were randomly allocated into 5 distinct treatment groups: the vehicle for formulation X ( $n = 10$ ), aprepitant X1 ( $n = 10$ ), aprepitant X2 ( $n = 10$ ), 2 mg/mL dexamethasone ( $n = 10$ ), or 0.2% hyaluronic acid artificial tears ( $n = 10$ ). All groups received a single daily drop of BAK to sustain the model for an additional week (see Supplementary Fig. S1). Corneal sensitivity, phenol red tests, and eye-wiping assessments were conducted to evaluate nerve function, tear secretion, and ocular discomfort.

### Aprepitant Treatment

To evaluate the efficacy of aprepitant (EMEND; Bausch and Lomb Center, Rochester, NY, USA), it was applied topically to the eyes of the mice in different gel formulations: X1 ( $n = 10$ ), X2 ( $n = 10$ ), and vehicle only (X;  $n = 10$ ). The X vehicle group received the formulation with no active compound. Formulation X1 contained aprepitant 0.05%, whereas X2 contained aprepitant 0.5%. The treatment was repeated for 7 days, 3 times a day. All aprepitant X formulations were preservative-free. As a gold standard anti-inflammatory treatment, a group of 10 mice received 2 mg/mL dexamethasone (Luxazone; Allergan). As a further control, a group of 10 mice received 0.2% hyaluronic acid artificial tears.

All the 10  $\mu$ L treatments were applied on both eyes. The mice were held for 20 seconds by the operator following every treatment to allow their absorption on the ocular surface.

### Biomicroscopy Imaging

On days  $-7$ ,  $-4$ ,  $-2$ ,  $0$ ,  $2$ ,  $5$ , and  $8$ , in vivo corneal images were acquired with a slit-lamp microscope SL 990 (C.S.O., Florence, Italy). In vivo corneal fluorescein staining procedure, revealed by irradiation with blue light (with or without a yellow filter) of the slit-lamp microscope, was exploited to evaluate corneal epithelial

damage. Mice received a 2  $\mu$ L drop of 0.1% fluorescein in both eyes. After 30 seconds, the excess solution was gently wiped away. Corneal fluorescein staining was classified according to a grading system, based on the corneal staining area, as described in Suwan-Apichon et al. and Nakamura et al.<sup>31,32</sup> The total area of punctate staining was scored from grade 0 to grade 4, depending on the severity of the damage. Briefly, grade 0 was attributed to corneas with no staining, grade 1 to corneas with equal or less than one-eighth of fluorescein positivity, grade 2 to corneas with equal/less than one-fourth of fluorescein positivity, grade 3 to corneas with equal/less than one half of fluorescein positivity, and grade 4 to corneas with more than half stained with fluorescein.

### Phenol Red Thread Test

To measure tear fluid secretion, on day 8, the phenol red thread test (PRTT) was performed. Briefly, a cotton thread was held with forceps and gently applied on the lower conjunctival fornices in the mice's eyes for 15 seconds. The length of wetted cotton thread (shift from yellow to red) was measured by using a millimeter-scale ruler under incident light. The mice did not receive anesthetics, sedatives, or any other agent during the test.

### Eye Wiping Test

An alternative version of the eye wiping test<sup>33</sup> was used to measure corneal nociception. Mice were topically instilled with 3 drops of 10  $\mu$ L of the drug or gel vehicle in each eye, with a 2-hour interval between each administration. On day 9, an hour following the last administration, the mice were individually placed in an empty cage for 3 minutes to get acclimatized. A 10  $\mu$ L drop of 5 M NaCl was then administered in both eyes. After that, 2 independent observers live-counted the eye wipes for 30 seconds in a single-blinded way (the investigator was blinded to the treatment group), with no video recording.

### Sensitivity Test

Corneal nerve sensitivity was measured in mice using a Cochet-Bonnet esthesiometer (Compagnia Ottica Italiana s.r.l.), with a 0.12-mm diameter nylon filament. Mice were topically administered 3 times with 10  $\mu$ L drops of the drug or vehicle in each eye. Every administration was separately performed with 2 hours intervals. On day 10, an hour following the last administration, corneal sensitivity was assessed by pointing the tip of the filament perpendicularly

to the corneal apex. To determine a positive sensation response, blinking reflexes were counted by two independent operators. Measurements were performed at the length of 60 mm (corresponding to the lowest mechanical threshold), and gradually decreased by 5 mm in each step. For each filament length, five repeat trials were performed. As a positive sensation response (blinking) was observed at least three out of the five times, this value was recorded as the corneal sensitivity threshold.

### Corneal Nerve Quantification

Corneal nerve density was determined by immunostaining excised corneal whole mounts, as previously reported.<sup>34</sup> Corneas were collected on day 11, rinsed with phosphate-buffered saline (PBS) and fixed in acetone for 15 minutes at 4°C. As a primary antibody, rabbit anti- $\beta$ 3 tubulin (Millipore, Burlington, MA, USA) was left on whole-mounts for 16 hours at 4°C, followed by blocking of non-specificity with 2% bovine serum albumin (BSA) and 5% normal donkey serum. Finally, the corneas were incubated with Alexa 488 donkey anti-rabbit secondary antibody (Invitrogen, Carlsbad, CA, USA) for 2 hours at room temperature. The samples were then mounted with Vector Shield mounting medium (Vector Laboratories, Burlingame, CA, USA). Six peripheral and three central fields of the sub-basal nerve plexus (40  $\times$ , 5  $\mu$ m z-stack) of cornea images were taken with a confocal microscope (TCS SP5; Leica Microsystems, Wetzlar, Germany). The percentage of positive epithelial nerves ( $\beta$ 3-tubulin+) was calculated and displayed.

### Leukocyte Infiltration Quantification

After 11 days of treatment, corneas were harvested and leukocyte infiltration was quantified. Samples were processed as previously described in section 2.8. Primary immunostaining was performed with goat anti-CD45 (1/200, AF-114; R&D Systems, Minneapolis, MN, USA), followed by incubation with donkey anti-goat Alexa Fluor-546 secondary antibody (1/1000; Invitrogen, Carlsbad, CA, USA) 2 hours at room temperature. Leukocyte infiltration was quantified by counting the CD45+ cells per field. Six peripheral and three central fields were captured per each cornea (20  $\times$ , 5  $\mu$ m z-stack). Images were acquired in a DeltaVision Ultra microscope (GE Healthcare, Chicago, IL, USA). The images were analyzed using Image J software (National Institutes of Health, Bethesda, MD, USA). Results were expressed as cells/fields.

## Statistical Analysis

As a statistical method to analyze the eye wiping test, corneal sensitivity test, and immunohistochemistry, 1-way ANOVA, following Tukey's multiple comparison test, was chosen. A *P* value lower than 0.05 was considered statistically significant. Results are presented as mean ± standard error of the mean (SEM). GraphPad Prism software version 8.0 (GraphPad Software, Inc., San Diego, CA, USA) was used for data processing.

## Results

### Short-Term Aprepitant Administration Ameliorates Epitheliopathy in a DED-Mouse Model

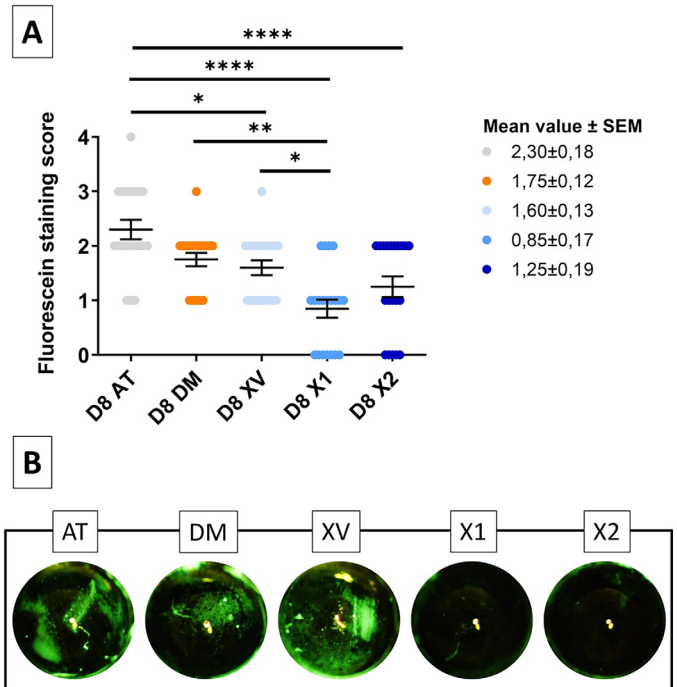
To define whether aprepitant administration affected DED-induced epitheliopathy, *in vivo* biomicroscopy was performed following fluorescein staining on the corneal epithelium (Supplementary Fig. S2). Following 8 days of treatment twice/day, we found that epitheliopathy was significantly reduced in the group treated with formulation X- compared to that receiving artificial tears (AT versus XV *P* < 0.05, AT versus X1 *P* < 0.0001, and AT versus X2 *P* < 0.0001; **Fig. 1A, 1B**). Of note, formulation X1 was more effective than the gold-standard dexamethasone (*P* < 0.01).

### NK1R Blockade Controls DED-Associated Ocular Pain

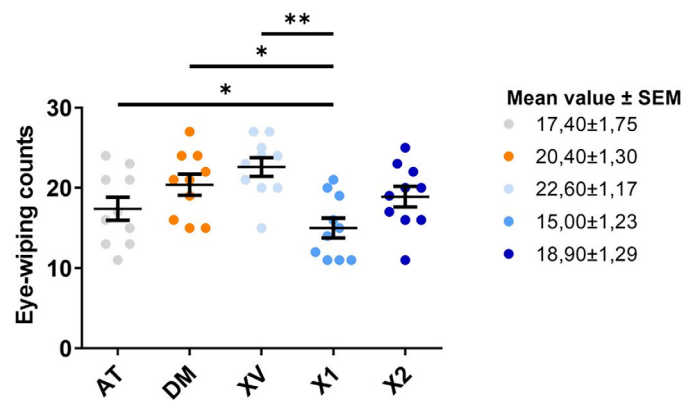
After 9 days of treatment, ocular pain was measured. We found that mice treated with formulation X1 had a reduced pain feeling compared to the vehicle (*P* < 0.01) and to the artificial tears-treated groups (*P* < 0.05; **Fig. 2**). Interestingly, formulation X1 was more effective in reducing nociception compared to the anti-inflammatory dexamethasone (*P* < 0.05). Formulation X2, instead, had an efficacy similar to that of dexamethasone.

### Aprepitant Contributes to Restoring Corneal Sensitivity

Functionality of corneal nerves was measured in our model following ten days of aprepitant administration. We observed that the corneal sensitivity of mice treated with formulation X1 was significantly higher than that of mice receiving the vehicle, dexamethasone, or artificial tears (*P* < 0.0001; **Fig. 3**). Similarly,



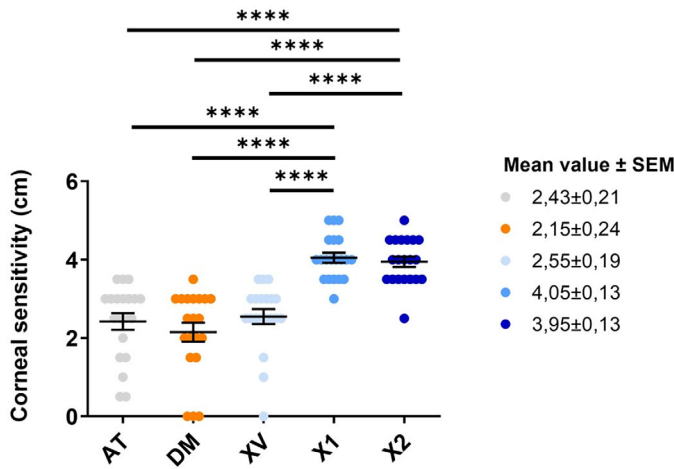
**Figure 1. Aprepitant is beneficial for corneal epitheliopathy.** Quantification of corneal fluorescein staining in artificial tears, dexamethasone and formulation X treatment groups (**A**). Representative pictures of corneal fluorescein staining. Pictures were captured at day 8 (**B**). Graph represents mean values ± SEM, statistical analysis by 1-way ANOVA, following Tukey's test. \**P* < 0.05, \*\**P* < 0.01, \*\*\*\**P* < 0.0001. AT, artificial tears; DM, dexamethasone; XV, formulation X vehicle; X1, formulation X1; X2, formulation X2.



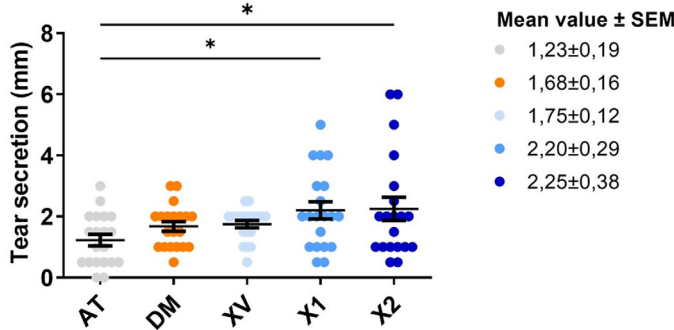
**Figure 2. Aprepitant alleviates ocular pain.** Ocular surface pain quantification in artificial tears, dexamethasone, and formulation X treatment groups. Eye wipes were measured at day 9. Graph represents mean values ± SEM, statistical analysis by 1-way ANOVA, following Tukey's test. \**P* < 0.05, \*\**P* < 0.01. AT, artificial tears; DM, dexamethasone; XV, formulation X vehicle; X1, formulation X1; X2, formulation X2.

formulation X2 induced a recovery in corneal sensitivity if compared to the vehicle, to dexamethasone, and to artificial tears (*P* < 0.0001).





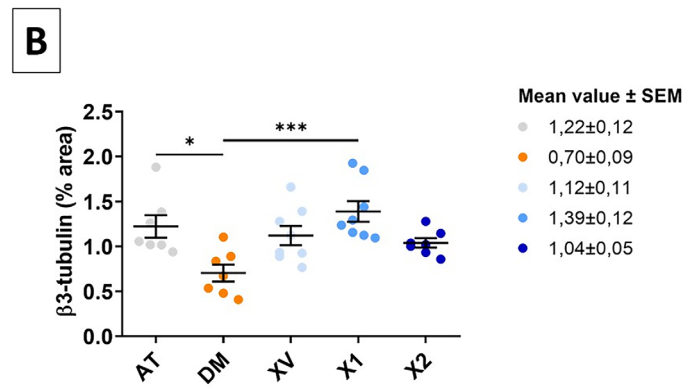
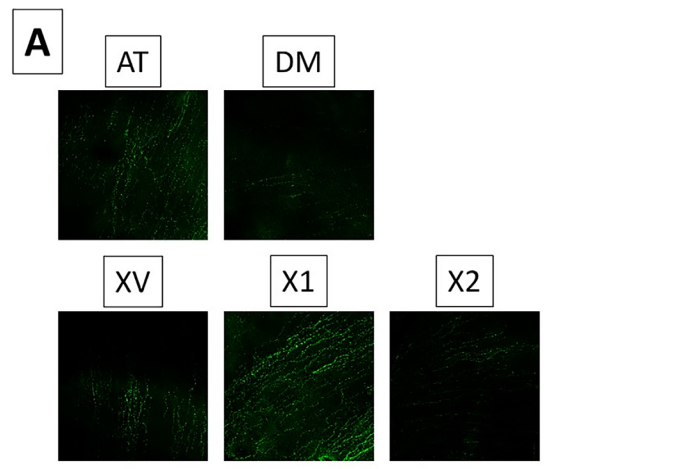
**Figure 3. Corneal sensitivity is not affected following aprépitant administration.** Quantification of corneal sensitivity in artificial tears, dexamethasone, and formulation X treatment groups. Animals underwent esthesiometry at day 10. Graph represents mean values ± SEM, statistical analysis by 1-way ANOVA, following Tukey's test. \*\*\*\* $P < 0.0001$ . AT, artificial tears; DM, dexamethasone; XV, formulation X vehicle; X1, formulation X1; X2, formulation X2.



**Figure 4. Improved tear secretion following aprépitant treatment.** Quantification of tear secretion in artificial tears, dexamethasone, and formulation X treatment groups. Tear production was measured at day 8. Graph represents mean values ± SEM, statistical analysis by 1-way ANOVA, following Tukey's test. \* $P < 0.05$ . YV, formulation Y vehicle; Y1, formulation Y1; Y2, formulation Y2; Y3, formulation Y3; XV, formulation X vehicle; X1, formulation X1; X2, formulation X2.

### Increased Tear Secretion Following Aprépitant Administration

DED occurs when tears do not adequately lubricate the ocular surface. Therefore, to assess whether aprépitant administration improves tear secretion, we performed the phenol red thread test. We observed that formulation X1-treated mice had a tear production significantly increased with respect to the artificial tears-treated group ( $P < 0.05$ ; Fig. 4). Likewise, formulation X2 had an effect comparable to that of formu-

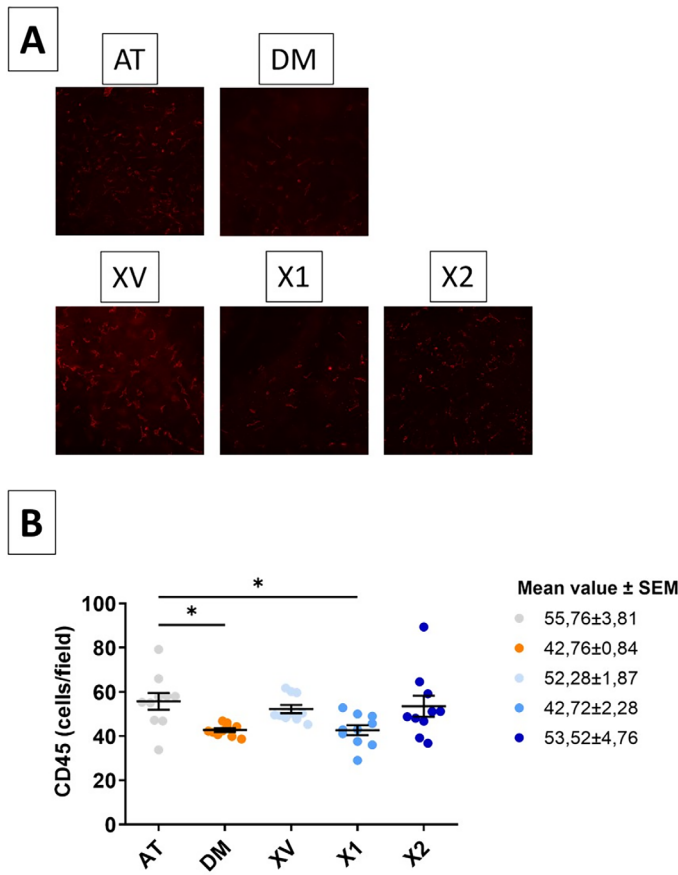


**Figure 5. Aprépitant is non-toxic for corneal nerves.** Representative pictures of  $\beta_3$ -tubulin-stained corneal nerves. Pictures of artificial tears, dexamethasone, and formulation X treatment groups were captured at day 11 (A). Quantification of  $\beta_3$ -tubulin positive nerves by means of % of positive signal per field (B). Graph represents mean values ± SEM, statistical analysis by 1-way ANOVA, following Tukey's test. \* $P < 0.05$ , \*\*\* $P < 0.001$ . DM, dexamethasone; XV, formulation X vehicle; X1, formulation X1; X2, formulation X2.

lation X1, with a tear production significantly higher than that of the artificial tears group ( $P < 0.05$ ).

### NK1R Antagonist Preserves Corneal Nerve Density

To study whether 11 days, 3 times a day of aprépitant administration affects corneal epithelial nerve integrity, immunostaining was performed to detect the expression of the well-known neuronal marker  $\beta_3$ -tubulin. We found that neither formulations X1 and X2, nor their vehicle are neurotoxic if compared to artificial tears. Nevertheless, corneas from animals receiving topical dexamethasone had a significantly lower  $\beta_3$ -tubulin positivity, both compared to the artificial tears-treated group ( $P < 0.05$ ; Fig. 5) and the formulation X1-treated group ( $P < 0.001$ ), meaning



**Figure 6. Aprepitant reduces corneal inflammation.** Representative pictures of CD45+ cells. Pictures of artificial tears, dexamethasone, and formulation X treatment groups were captured at day 11 (A). Quantification of CD45+ cells by means of number of positive cells per field (B). Graph represents mean values ± SEM, statistical analysis by 1-way ANOVA, following Tukey's test. \* $P < 0.05$ . DM, dexamethasone; XV, formulation X vehicle; X1, formulation X1; X2, formulation X2.

that even a short-term dexamethasone administration may cause toxicity.

### Short-Term Aprepitant Administration Reduces Corneal Inflammation

Ocular inflammation is one of the major symptoms of DED. To study whether aprepitant administration can alleviate the inflammatory burst, on day 11, we performed CD45 staining on whole mount corneas. As expected, the gold standard dexamethasone significantly reduced the number of leukocytes in the cornea when compared to the artificial tears-treated group ( $P < 0.05$ ; Fig. 6). Curiously, formulation X1 contributed to reducing inflammation with an intensity similar to that of dexamethasone, being the CD45+ infiltrate signif-

icantly lower than that observed in the artificial tears group ( $P < 0.05$ ). Noteworthy, no differences were observed in the formulation X2 treated group compared to the vehicle or the artificial tears groups.

## Discussion

Among the many pathways involved in the pathophysiology of ocular inflammation, the SP-NK1R axis has been extensively studied.<sup>35–38</sup> Indeed, NK1R is expressed on the surface of lymphocytes and monocytes, whereas nerve-secreted SP can stimulate their activation.<sup>38–40</sup> Activated leukocytes release pro-inflammatory chemokines that are responsible for corneal (including nerves) damage.<sup>41–43</sup> We recently demonstrated that mice lacking SP expression or topical administration of NK1R antagonists ameliorate wound healing and corneal transparency, by inhibiting the mTOR pathway and epithelial cell senescence.<sup>44,45</sup> Here, we show that the administration of aprepitant formulation X rapidly reduced corneal epithelial disease in a DED preclinical model. Noteworthy, formulation X1 improved epitheliopathy more than dexamethasone, which is the most potent and rapid anti-inflammatory medication available for ocular use. These findings also suggest that blockade of neurokinin 1 receptor activity does not induce epithelial toxicity, at least in this model. Interestingly, X vehicle appeared to improve epitheliopathy more than artificial tears, which could be the result of different chemical/physical characteristics of the two formulations.

Our finding that aprepitant formulation X1 reduced ocular pain in the DED model corroborates previous findings on the role of NK1R in pain modulation.<sup>36,46–50</sup> Indeed, we recently demonstrated that administration of fosaprepitant – the prodrug of aprepitant – induces analgesia in a mouse model of trigeminal pain,<sup>51</sup> through a mechanism involving afferent nerve fibers, which express NK1 receptors.<sup>49,52</sup> It should be noted that most of the commonly used topical analgesics/anesthetics also induce corneal anesthesia to a varying degree. Their chronic use is, however, associated with severe side effects, including corneal nerve toxicity, delayed wound healing, and perforation.<sup>53,54</sup> In this vein, it was surprising for us to observe that the aprepitant treated groups showed better corneal sensitivity compared to animals treated with artificial tears, vehicle, or dexamethasone. Indeed, animals treated with aprepitant reached higher corneal nerve density than those

treated with dexamethasone. Indeed, it is well known that corticosteroids induce ocular surface toxicity,<sup>55</sup> their use downregulates nerve growth factor expression in patients with DED<sup>56</sup> and reduces sympathetic nerve sprouting in rats with neuropathic pain.<sup>57</sup> Overall, these data suggest that aprepitant – as opposed to Dexamethasone – is not neurotoxic in this model of DED.

In terms of anti-inflammatory efficacy, our data show that topical aprepitant reduces leukocyte infiltration in the cornea. Of note, the anti-inflammatory efficacy of aprepitant formulation X1 was comparable to dexamethasone. This is in line with previous findings from others and us. For instance, topical administration of lanepitant, a potent NK1 receptor antagonist, reduced corneal inflammation in a corneal neovascularization murine model.<sup>45</sup> Similarly, corneal infiltrating leukocyte counts were reduced in SP-knock-out mice compared to wild-type mice in different murine models of ocular surface damage.<sup>25</sup> More recently, we also showed that following chemical damage of the ocular surface, mice that are genetically unable to express SP showed reduced lymphocyte infiltration and inflammatory response compared to wild-type mice.<sup>58</sup> Finally, we previously observed that topical administration of NK1R antagonist fosaprepitant reduced corneal epithelial disease and inflammation in a murine model of graft-versus-host disease.<sup>59</sup> Others have reported the analgesic potential of NK1 receptor antagonist administration in murine models of acute pancreatitis,<sup>60</sup> interstitial cystitis,<sup>61</sup> and chronic pain.<sup>62</sup>

Our finding that treatment with aprepitant also improved tear secretion was expected. Indeed, the literature suggests that DED is associated with the infiltration of activated leukocytes in the lacrimal glands, and that this mechanistically induces lacrimal gland dysfunction.<sup>63–66</sup> Our data also corroborate our previous finding that NK1R was upregulated in the lacrimal gland of graft-versus-host disease mice, and that NK1R antagonist administration improved tear secretion.<sup>59</sup>

In conclusion, our data have substantial implications for clinical translation. First, pain-associated DED is an area of unmet medical need. Second, drug repurposing of aprepitant – a drug that has been in clinical use for years with an excellent safety profile – could make the regulatory path shorter, especially in light of the fact that it does not seem to induce ocular surface or corneal nerve toxicity. Therefore, we suggest that targeting neuroinflammation by means of topical aprepitant may represent a significant advancement in the treatment of signs and symptoms of DED.

## Acknowledgments

This study has been funded by Bausch & Lomb.

**Author Contributions:** F.B. analyzed the data and wrote the original manuscript draft. I.H.D. was responsible for data collection and analysis. R.L.V. was responsible for data collection and analysis. F.P. was responsible for data collection and analysis. G.F. was responsible for study design and supervision, and writing review and editing.

**Disclosure:** F. Bonelli, None; F. Campestre, None; R.M. Lasagni Vitar, None; I.H. Demirsoy, None; P. Fonteyne, None; G. Ferrari, San Raffaele Hospital (F), filed patent describing the use of neurokinin 1 receptor antagonists for the treatment of ocular pain (P)

## References

1. Stapleton F, Alves M, Bunya VY, et al. TFOS DEWS II Epidemiology Report. *Ocul Surf*. 2017;15(3):334–365.
2. Messmer EM. The pathophysiology, diagnosis, and treatment of dry eye disease. *Dtsch Arztebl Int*. 2015;112(5):71–81; quiz 82.
3. Labetoulle M, Benitez-del-Castillo JM, Barabino S, et al. Artificial tears: biological role of their ingredients in the management of dry eye disease. *Int J Molec Sci*. 2022;23(5):2434.
4. Mittal R, Patel S, Galor A. Alternative therapies for dry eye disease. *Curr Opin Ophthalmol*. 2021;32(4):348–361.
5. Rouen PA, White ML. Dry eye disease: prevalence, assessment, and management. *Home Healthc Now*. 2018;36(2):74–83.
6. Yu L, Yu C, Dong H, et al. Recent developments about the pathogenesis of dry eye disease: based on immune inflammatory mechanisms. *Front Pharmacol*. 2021;12:732887. Accessed April 14, 2023. Available at: <https://www.frontiersin.org/articles/10.3389/fphar.2021.732887>.
7. Perez VL, Stern ME, Pflugfelder SC. Inflammatory basis for dry eye disease flares. *Exp Eye Res*. 2020;201:108294.
8. Lin Z, Liu X, Zhou T, et al. A mouse dry eye model induced by topical administration of benzalkonium chloride. *Mol Vis*. 2011;17:257–264.
9. Pisella PJ, Pouliquen P, Baudouin C. Prevalence of ocular symptoms and signs with preserved and

- preservative free glaucoma medication. *Br J Ophthalmol*. 2002;86(4):418–423.
10. Datta S, Baudouin C, Brignole-Baudouin F, Denoyer A, Cortopassi GA. The eye drop preservative benzalkonium chloride potently induces mitochondrial dysfunction and preferentially affects LHON mutant cells. *Invest Ophthalmol Vis Sci*. 2017;58(4):2406–2412.
  11. Aguayo Bonniard A, Yeung JY, Chan CC, Birt CM. Ocular surface toxicity from glaucoma topical medications and associated preservatives such as benzalkonium chloride (BAK). *Expert Opin Drug Metab Toxicol*. 2016;12(11):1279–1289.
  12. Clouzeau C, Godefroy D, Riancho L, Rostène W, Baudouin C, Brignole-Baudouin F. Hyperosmolarity potentiates toxic effects of benzalkonium chloride on conjunctival epithelial cells in vitro. *Mol Vis*. 2012;18:851–863.
  13. Sarkar J, Chaudhary S, Namavari A, et al. Corneal neurotoxicity due to topical benzalkonium chloride. *Invest Ophthalmol Vis Sci*. 2012;53(4):1792–1802.
  14. Chen W, Zhang Z, Hu J, et al. Changes in rabbit corneal innervation induced by the topical application of benzalkonium chloride. *Cornea*. 2013;32(12):1599.
  15. Ivakhnitskaia E, Souboch V, Dallacasagrande V, et al. Benzalkonium chloride, a common ophthalmic preservative, compromises rat corneal cold sensitive nerve activity. *Ocul Surf*. 2022;26:88–96.
  16. Fakh D, Baudouin C, Réaux-Le Goazigo A, Mélik Parsadaniantz S. TRPM8: a therapeutic target for neuroinflammatory symptoms induced by severe dry eye disease. *Int J Molec Sci*. 2020;21(22):8756.
  17. Fakh D, Zhao Z, Nicolle P, et al. Chronic dry eye induced corneal hypersensitivity, neuroinflammatory responses, and synaptic plasticity in the mouse trigeminal brainstem. *J Neuroinflammation*. 2019;16(1):268.
  18. Demirsoy IH, Ferrari G. The NK-1 receptor signaling: distribution and functional relevance in the eye. *Receptors*. 2022;1(1):98–111.
  19. Lasagni Vitar RM, Rama P, Ferrari G. The two-faced effects of nerves and neuropeptides in corneal diseases. *Prog Retin Eye Res*. 2022;86:100974.
  20. Chao C, Tajbakhsh Z, Stapleton F, et al. Corneal epithelial dendritic cells, tear neuropeptides and corneal nerves continue to be affected more than 12 months after LASIK. *Acta Ophthalmol*. 2023;101(3):e302–e314.
  21. He J, Pham TL, Kakazu AH, Bazan HEP. Remodeling of substance P sensory nerves and transient receptor potential melastatin 8 (TRPM8) cold receptors after corneal experimental surgery. *Invest Ophthalmol Vis Sci*. 2019;60(7):2449–2460.
  22. Chao C, Golebiowski B, Zhao X, Chen S, Zhou S, Stapleton F. Long-term effects of LASIK on corneal innervation and tear neuropeptides and the associations with dry eye. *J Refract Surg*. 2016;32(8):518–524.
  23. Yang T, Ma B, Xie J, et al. Evaluation of ocular surface characteristics in dry eye disease with and without soft contact lens wear: a comparative study. *Eye Contact Lens*. 2022;48(9):377–383.
  24. López-de la Rosa A, García-Vázquez C, Fernández I, Arroyo-del Arroyo C, Enríquez-de-Salamanca A, González-García MJ. Substance P level in tears as a potential biomarker for contact lens discomfort. *Ocul Immunol Inflamm*. 2021;29(1):43–56.
  25. Barbariga M, Fonteyne P, Ostadreza M, Bignami F, Rama P, Ferrari G. Substance P modulation of human and murine corneal neovascularization. *Invest Ophthalmol Vis Sci*. 2018;59(3):1305–1312.
  26. Bignami F, Lorusso A, Rama P, Ferrari G. Growth inhibition of formed corneal neovascularization following fosaprepitant treatment. *Acta Ophthalmol*. 2017;95(7):e641–e648.
  27. Yang Y, Zhou W, Xu X, et al. Aprepitant inhibits JNK and p38/MAPK to attenuate inflammation and suppresses inflammatory pain. *Front Pharmacol*. 2021;12:811584.
  28. Zhao XN, Bai ZZ, Li CH, Sheng CL, Li HY. The NK-1R antagonist aprepitant prevents LPS-induced oxidative stress and inflammation in RAW264.7 macrophages. *Drug Des Devel Ther*. 2020;14:1943–1952.
  29. Patel L, Lindley C. Aprepitant—a novel NK1-receptor antagonist. *Expert Opin Pharmacother*. 2003;4(12):2279–2296.
  30. Suanno G, Fonteyne P, Ferrari G. Neurosensory abnormalities and stability of a mouse model of dry eye disease. *Exp Eye Res*. 2023;232:109516.
  31. Suwan-apichon O, Rizen M, Rangsin R, et al. Botulinum toxin B-induced mouse model of keratoconjunctivitis sicca. *Invest Ophthalmol Vis Sci*. 2006;47(1):133–139.
  32. Nakamura S, Shibuya M, Nakashima H, Imagawa T, Uehara M, Tsubota K. D-beta-hydroxybutyrate protects against corneal epithelial disorders in a rat dry eye model with jogging board. *Invest Ophthalmol Vis Sci*. 2005;46(7):2379–2387.
  33. Farazifard R, Safarpour F, Sheibani V, Javan M. Eye-wiping test: a sensitive animal model for acute



- trigeminal pain studies. *Brain Res Brain Res Protoc.* 2005;16(1-3):44–49.
34. Ferrari G, Bignami F, Giacomini C, Franchini S, Rama P. Safety and efficacy of topical infliximab in a mouse model of ocular surface scarring. *Invest Ophthalmol Vis Sci.* 2013;54(3):1680–1688.
  35. Suvas S. Role of substance P neuropeptide in inflammation, wound healing, and tissue homeostasis. *J Immunol.* 2017;199(5):1543–1552.
  36. Singh RB, Naderi A, Cho W, et al. Modulating the tachykinin: role of substance P and neurokinin receptor expression in ocular surface disorders. *Ocul Surf.* 2022;25:142–153.
  37. Lasagni Vitar RM, Barbariga M, Fonteyne P, Bignami F, Rama P, Ferrari G. Modulating ocular surface pain through neurokinin-1 receptor blockade. *Invest Ophthalmol Vis Sci.* 2021;62(3):26.
  38. Mashaghi A, Marmalidou A, Tehrani M, Grace PM, Pothoulakis C, Dana R. Neuropeptide substance P and the immune response. *Cell Mol Life Sci.* 2016;73(22):4249–4264.
  39. Laurenzi MA, Persson MA, Dalsgaard CJ, Haegerstrand A. The neuropeptide substance P stimulates production of interleukin 1 in human blood monocytes: activated cells are preferentially influenced by the neuropeptide. *Scand J Immunol.* 1990;31(4):529–533.
  40. O'Connor TM, O'Connell J, O'Brien DI, Goode T, Bredin CP, Shanahan F. The role of substance P in inflammatory disease. *J Cell Physiol.* 2004;201(2):167–180.
  41. Elgebaly SA, Gillies C, Forouhar F, et al. An in vitro model of leukocyte mediated injury to the corneal epithelium. *Curr Eye Res.* 1985;4(1):31–41.
  42. Chin AC, Parkos CA. Pathobiology of neutrophil transepithelial migration: implications in mediating epithelial injury. *Ann Rev Pathol: Mech Dis.* 2007;2(1):111–143.
  43. Sumioka T, Iwanishi H, Okada Y, et al. Impairment of corneal epithelial wound healing is association with increased neutrophil infiltration and reactive oxygen species activation in tenascin X-deficient mice. *Lab Invest.* 2021;101(6):690–700.
  44. Lasagni Vitar R, Triani F, Barbariga M, Fonteyne P, Rama P, Ferrari G. Substance P/neurokinin-1 receptor pathway blockade ameliorates limbal stem cell deficiency by modulating mTOR pathway and preventing cell senescence. *Stem Cell Reports.* 2022;17(4):849–863.
  45. Bignami F, Giacomini C, Lorusso A, Aramini A, Rama P, Ferrari G. NK1 receptor antagonists as a new treatment for corneal neovascularization. *Invest Ophthalmol Vis Sci.* 2014;55(10):6783–6794.
  46. Taketani Y, Naderi A, Wang S, et al. Neurokinin-1 receptor antagonism ameliorates ocular pain and immune responses in dry eye disease. *Invest Ophthalmol Vis Sci.* 2021;62(8):1286.
  47. Jensen DD, Lieu T, Halls ML, et al. Neurokinin 1 receptor signaling in endosomes mediates sustained nociception and is a viable therapeutic target for prolonged pain relief. *Sci Transl Med.* 2017;9(392):eaal3447.
  48. Mai QN, Shenoy P, Quach T, et al. A lipid-anchored neurokinin 1 receptor antagonist prolongs pain relief by a three-pronged mechanism of action targeting the receptor at the plasma membrane and in endosomes. *J Biol Chem.* 2021;296:100345.
  49. Zieglgänsberger W. Substance P and pain chronicity. *Cell Tissue Res.* 2019;375(1):227–241.
  50. Bonelli F, Demirsoy I, Lasagni Vitar RM, Fonteyne P, Ferrari G. Topical formulations of aprepitant are safe and effective in relieving pain and inflammation, and drive neural regeneration. *Ocul Surf.* 2023;30:92–103.
  51. Lasagni Vitar RM, Barbariga M, Fonteyne P, Bignami F, Rama P, Ferrari G. Modulating ocular surface pain through neurokinin-1 receptor blockade. *Invest Ophthalmol Vis Sci.* 2021;62(3):26.
  52. Cao YQ, Mantyh PW, Carlson EJ, Gillespie AM, Epstein CJ, Basbaum AI. Primary afferent tachykinins are required to experience moderate to intense pain. *Nature.* 1998;392(6674):390–394.
  53. Mastropasqua L, Massaro-Giordano G, Nubile M, Sacchetti M. Understanding the pathogenesis of neurotrophic keratitis: the role of corneal nerves. *J Cell Physiol.* 2017;232(4):717–724.
  54. Dua HS, Said DG, Messmer EM, et al. Neurotrophic keratopathy. *Prog Retinal Eye Res.* 2018;66:107–131.
  55. McGhee CNJ, Dean S, Danesh-Meyer H. Locally administered ocular corticosteroids. *Drug Safety.* 2002;25(1):33–55.
  56. Lee HK, Ryu IH, Seo KY, Hong S, Kim HC, Kim EK. Topical 0.1% prednisolone lowers nerve growth factor expression in keratoconjunctivitis sicca patients. *Ophthalmology.* 2006;113(2):198–205.
  57. Li H, Xie W, Strong JA, Zhang JM. Systemic antiinflammatory corticosteroid reduces mechanical pain behavior, sympathetic sprouting, and elevation of proinflammatory cytokines in a rat model of neuropathic pain. *Anesthesiology.* 2007;107(3):469–477.

58. Lasagni Vitar RM, Fonteyne P, Chaabane L, Rama P, Ferrari G. A hypothalamic-controlled neural reflex promotes corneal inflammation. *Invest Ophthalmol Vis Sci.* 2021;62(13):21.
59. Lasagni Vitar RM, Bonelli F, Atay A, et al. Topical neurokinin-1 receptor antagonist fosaprepitant ameliorates ocular graft-versus-host disease in a preclinical mouse model. *Exp Eye Res.* 2021;212:108825.
60. Han C, Du D, Wen Y, et al. Chaiqin chengqi decoction ameliorates acute pancreatitis in mice via inhibition of neuron activation-mediated acinar cell SP/NK1R signaling pathways. *J Ethnopharmacol.* 2021;274:114029.
61. Rudick CN, Schaeffer AJ, Klumpp DJ. Pharmacologic attenuation of pelvic pain in a murine model of interstitial cystitis. *BMC Urol.* 2009;9(1):16.
62. Latorre R, Ramírez-García PD, Hegron A, et al. Sustained endosomal release of a neurokinin-1 receptor antagonist from nanostars provides long-lasting relief of chronic pain. *Biomaterials.* 2022;285:121536.
63. Stern ME, Gao J, Siemasko KF, Beuerman RW, Pflugfelder SC. The role of the lacrimal functional unit in the pathophysiology of dry eye. *Exp Eye Res.* 2004;78(3):409–416.
64. Conrady CD, Joos ZP, Patel BCK. Review: the lacrimal gland and its role in dry eye. *J Ophthalmol.* 2016;2016:e7542929.
65. Uchino Y, Kawakita T, Ishii T, Ishii N, Tsubota K. A new mouse model of dry eye disease: oxidative stress affects functional decline in the lacrimal gland. *Cornea.* 2012;31(Suppl 1):S63–S67.
66. Ji YW, Lee JH, Choi EY, et al. HIF1 $\alpha$ -mediated TRAIL expression regulates lacrimal gland inflammation in dry eye disease. *Invest Ophthalmol Vis Sci.* 2020;61(1):3.