Modulating Ocular Surface Pain Through Neurokinin-1 Receptor Blockade

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GF and PR are inventors of a patent involving fosaprepitant for the treatment of pain.

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Purpose. The purpose of this study was to test the role of substance P (SP) and its receptor neurokinin 1 (NK1R) on ocular surface pain.

Methods. Eight-week-old C57BL6/N (wild type [WT]) and B6.Cg-Tac1tm1Bbm/J (TAC1-KO) male mice were used. 5 M NaCl was topically applied on the cornea, followed by topical fosaprepitant 2, 10, and 50 mg/mL; 4 mg/mL oxybuprocaine chloride, or 0.1% diclofenac. Th eye wiping test was used to quantify ocular surface pain. SP content was quantified in the tear fluid and trigeminal ganglia (TG), and TAC1 mRNA was assessed in the cornea. Corneas were immunostained for β3-tubulin and NK1R, or CD45, to quantify leukocyte infiltration.

Results. TAC1-KO mice displayed a significant reduction of ocular pain ($P < 0.001$). Similarly, a single dose of 10 or 50 mg/mL fosaprepitant applied topically to WT mice reduced ocular pain as compared to vehicle ($P < 0.001$). Fosaprepitant 2 mg/mL, instead, induced corneal analgesia only when it was administered for 10 days, 6 times/day ($P < 0.05$). Diclofenac or oxybuprocaine reduced corneal nociception when compared to vehicle or fosaprepitant ($P < 0.05$). Fosaprepitant or oxybuprocaine groups showed lower SP content in tear secretions and TG ($P < 0.05$), and reduction in TAC1 mRNA ($P < 0.05$), and leukocyte infiltration ($P < 0.05$) in the cornea. Colocalization of NK1R and β3-tubulin was detected in mouse corneas.

Conclusions. Topical administration of the NK1R antagonist fosaprepitant effectively reduces ocular surface nociception by decreasing SP release in the tear fluid and TG, and corneal leukocyte infiltration. Fosaprepitant repurposing shows promise for the treatment of ocular pain.

Keywords: ocular pain, substance P (SP), neurokinin 1 receptor antagonist

The problem of ocular pain has been largely underestimated for decades. Recently, however, it has been an object of revived interest, and its elevated prevalence has finally been acknowledged.[1] Any ocular surface disease or surgery will induce corneal pain at some level. Ocular pain is a consequence of highly prevalent ocular surface diseases (keratitis, conjunctivitis, blepharitis, corneal edema, and dry eye),[2–5] surgery (refractive surgery, corneal crosslinking, keratoplasty, cataract, and retina surgery),[6–9] and contact lens wearing.[10] Sometimes, corneal pain can be excruciating to the point that affected patients have attempted suicide.[11]

Importantly, it has now become apparent that ocular pain can be not only a consequence of other disorders, but also an autonomous disease entity, where treatment remains suboptimal.[12]

For these reasons, ocular pain is an area of significant and unmet medical need, and a major medical challenge. Current treatments include topical anesthetics (e.g. oxybuprocaine chloride), topical nonsteroidal anti-inflammatory drugs (NSAIDs), and systemic analgesics,[13] which are associated with significant side effects.

Fosaprepitant, a water-soluble drug rapidly converted in vivo to the active molecule aprepitant, is a potent and selective neurokinin-1 receptor (NK1R) antagonist.[14] In the last decade, fosaprepitant has been effectively used against nausea and vomiting in both acute and delayed phases of chemotherapy.[15,16] We recently reported that NK1R antagonists, including fosaprepitant, potently inhibit corneal inflammation and angiogenesis when applied topically on the ocular surface.[17,18] Additionally, we demonstrated that the main endogenous NK1R ligand, substance P (SP), is involved in cornea sensitivity and pain: in fact, mice lacking SP showed a significant reduction of cornea nociception.[19] Interestingly, nociception was restored to normal levels by administration of SP in knock-out animals.[19] These data suggest a role for SP and its receptor NK1R in the transmission of pain from the cornea to the central nervous system. We then hypothesized that pharmacological inhibition of this mechanism could be exploited to treat ocular surface pain. Here, we evaluate the effect of fosaprepitant-induced NK1R blockade on ocular nociception using a published animal model, and we provide a biological mechanism for such effect.

Methods

Mice

Eight-week-old C57BL6/N (Charles-River, Italy) wild type (WT) and B6.Cg-Tac1^{tm1Bbm}/J (Jackson; TAC1-knock-out [KO], which lack SP) male mice were used in all experiments. Carbon dioxide inhalation and subsequent cervical dislocation were applied to euthanize the animals. All experimental protocols were approved by the Animal Care and Use Committee of the IRCCS San Raffaele Scientific Institute, in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Ocular Nociceptive Response

To quantify nociception in mouse eyes, the eye wiping test was used.[20] Briefly, animals were randomized into 4 groups and placed individually in an empty cage for 5 minutes to get acclimatized; 1 drop (10 µL) of 5 M NaCl was put into the right eye of each animal and eye wipings with the ipsilateral forepaw were counted for 30 seconds. After 1 hour, a drop (10 µL) of fosaprepitant (2, 10, and 50 mg/mL in PBS, $n = 20$/group), PBS ($n = 20$), diclofenac (0.1%, dicloftil, Farmingea, $n = 10$), or oxybuprocaine chloride (4 mg/mL, Novesina, Thea, $n = 16$) were topically applied on the right eye for 1 minute. Three minutes after the treatment, the eye was stimulated a second time with 5 M NaCl and wipings were counted for 30 seconds. TAC1-KO animals ($n = 12$) received PBS between NaCl administrations. To confirm that both stimulation with 5 M NaCl were equivalent, we counted the eye-wipes in nontreated animals and we found no significant differences between both stimulation (first trial: 21.0 ± 5.7 eye-wipes; second trial: 16.5 ± 0.7 eye-wipes).

In a subgroup of experiments, cornea nociception was tested 24 hours after 10 days of topical treatment (10 µL) with 2 mg/mL fosaprepitant or PBS (6 times/day, every 2 hours, $n = 10$/group). In all experiments, the eye-wipe counting was carried out by the same examiner and in a single-blinded way (the investigator was blinded to the treatment group) in real time. When corresponding, antinociceptive effect was calculated as maximum possible effect (MPE%, 100 × [postdrug wipe count − predrug wipe count]/[0 − predrug wipe count]).

Acute Corneal Nerve Stimulation Model

Mice were subjected to 5 M NaCl stimulus after topical application of PBS ($n = 5$), 10 mg/mL fosaprepitant ($n = 5$), or 4 mg/mL oxybuprocaine chloride (Novesina, Thea; $n = 5$) as described before in the Ocular Nociceptive Response . Five minutes after the NaCl stimulus, eye-washes were performed on each eye using a pipette with 10 µL of PBS and pooled, adding 1% protease inhibitor. The whole procedure was repeated twice with 1-hour interval, during 3 consecutive days. At day 3, mice were euthanized as described in the Mice section and corneas and trigeminal ganglia (TG) were isolated for further analysis.

Substance P Levels

The concentration of SP was determined in eye-washes and TG using an SP competitive Elisa KIT (Cayman, Ann Arbor, MI, USA), following manufacturer instructions. TG was homogenized in 100 uL of PBS containing 1% protease inhibitor with Ultra-Turrax T8 (IKA, Wilmington, NC, USA), and then centrifuged at 800 *g* for 10 minutes at 4°C. Eye-washes were also centrifuged at 800 *g* for 10 minutes at 4°C prior to dilution. The eye-wash and TG samples were diluted 1/2 and 1/50 with ELISA buffer, respectively. Protein content was quantified with Bradford protein assay (Thermo Scientific, Waltham, MA, USA). SP levels were expressed as pg/mg protein.

Analysis of Tachykinin Precursor 1 Transcripts

Corneas were homogenized with Ultra-Turrax T8. Total RNA extraction, DNAse treatment, retrotranscription, and real-time PCR were performed as previously described.[21] We used Taqman Gene Expression Assays (Applied Biosystems, Foster City, CA, USA) for tachykinin precursor 1 (TAC1, Mm01166996_m1) and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Mm99999915_g1) transcripts, that was used as the housekeeping gene. Results are presented as a relative expression (ΔΔCT method).

Leukocyte Infiltration Quantification

Mice were subjected to 5 M NaCl stimulus in the presence of PBS, fosaprepitant 10 mg/mL, or 4 mg/mL oxybuprocaine chloride, as described before in the Acute Corneal Nerve Stimulation Model section. The procedure was repeated 4 times, in 1-hour intervals. One hour after the last stimulus, mice were euthanized and corneas were dissected, washed in PBS, and fixed in acetone at 4°C for 15 minutes. Nonspecific staining was blocked with 2% bovine serum albumin, 5% normal donkey serum followed by immunostaining with goat anti-CD45 (1/200, AF-114; R&D Systems, Minneapolis, MN, USA). After washing with PBS, corneas were incubated with donkey anti-goat Alexa Fluor-546 secondary antibody (1/1000; Invitrogen, Carlsbad, CA, USA) 2 hours at room temperature (RT). Negative control was performed removing the primary antibody. For mounting, Vector Shield mounting medium (Vector Laboratories, Burlingame, CA, USA) was used. Immune cell infiltration was quantified by counting the CD45$^+$ cells per field; 6 peripheral and 3 central fields were taken per cornea (20 ×, 5 µm *z*-stack). Pictures were acquired in a DeltaVision Ultra microscope (GE healthcare, Chicago, IL, USA) and the image analysis was performed using Image J software (National Institutes of Health, Bethesda, MD, USA). Results were expressed as cells/mm^2.

Corneal Epithelial Nerve Expression of NK1 Receptor

To evaluate the expression of NK1R on corneal nerves, corneal cross-sections from untreated wild type mice ($n = 6$) were immunostained as previously described.[21] Mouse corneas were frozen in optimal cutting temperature compound (OCTKillik; Bio-Optica, Milan, Italy), and 8 µm cryosections were performed. After fixation in 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA) for 20 minutes, the sections were blocked with 2% bovine serum albumin, 0.1% Triton X-100 (Sigma-Aldrich), and 10% normal donkey serum for 1 hour at RT. The immunostaining was performed using goat anti-NK1R (1/400, ab61705; Abcam, Cambridge, UK) and rabbit anti-β3-tubulin (1/800, 802001; Biolegend, San Diego, CA, USA) primary antibodies

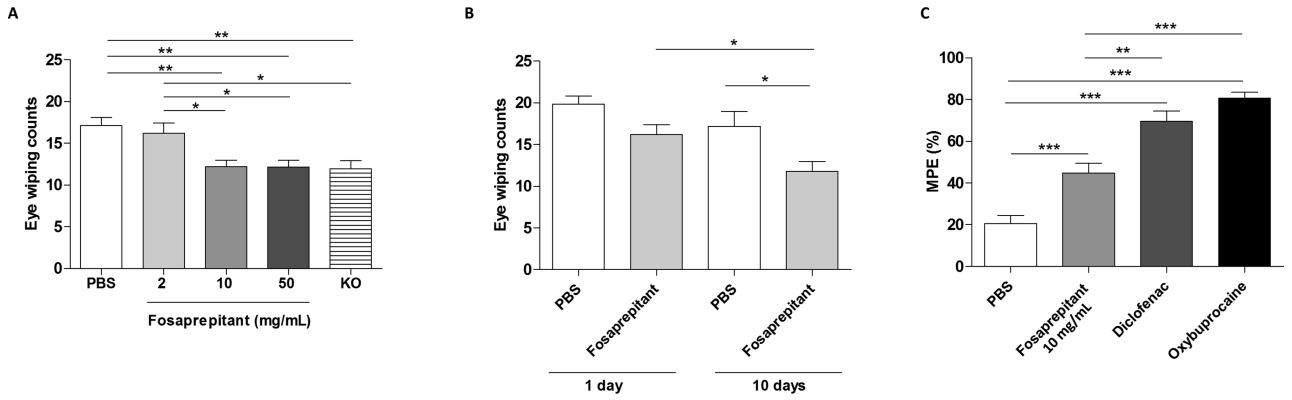

Figure 1. NK1R antagonist fosaprepitant reduces corneal nociception when topically applied in high-dose single administration or in low-dose multiple administrations. (**A**) Eye wipings were counted after a single topical fosaprepitant administration of 2, 10, and 50 mg/mL or PBS into wild type mice ($N = 20$ mice/group) and after PBS in TAC1-KO mice ($N = 12$ mice). (**B**) Eye-wiping counts after 2 mg/mL fosaprepitant administration one shot or 6 times/day for 10 days compared to controls (PBS; $N = 10$ mice/group). (**C**) Maximum possible analgesic effect (MPE) of PBS ($N = 20$ mice), fosaprepitant (10 mg/mL; $N = 20$ mice), diclofenac (0.1%; $N = 10$ mice), and oxybuprocaine (4 mg/mL; $N = 16$ mice), in reducing mouse corneal nociception. Graphs represent mean values ± SEM; statistical analysis by one-way ANOVA followed by Tukey's post hoc test (*$P < 0.05$, **$P < 0.05$, ***$P < 0.001$).

incubated at 4°C overnight. Secondary antibody incubation was assessed for 2 hours at RT with donkey anti-goat Alexa Fluor-546 and donkey anti-rabbit Alexa Fluor-488 (1/1000; Invitrogen Molecular Probes, Paisley, UK). Negative control was performed removing the primary antibodies. The sections were mounted with Vector Shield mounting medium (Vector Laboratories, Burlingame, CA, USA), containing DAPI. Z-stack images (40 ×, 5 µm *z*-stack) were acquired in a DeltaVision Ultra microscope (GE Healthcare, Chicago, IL, USA) and colocalization was assessed with Image J software, using the colocalization plug-in (National Institutes of Health). The percentage of double positive epithelial nerves (β3-tubilin+ and NK1R+) was calculated as the percentage of area of the colocalization points divided by the percentage of area of total β3-tubulin positive nerves located in the epithelium.

Statistics

The statistical software GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA) was used for all analyses. One-way ANOVA followed by Tukey's post hoc test was used to evaluate the difference among experimental groups. A P value < 0.05 was considered to be statistically significant. All data were expressed as mean ± standard error of the mean (SEM).

Results

High-Dose Single Administration or Low-Dose Multiple Administration of Topical NK1R-Antagonist Fosaprepitant Induce Corneal Analgesia

First, we tested a single dose application of three different concentrations of fosaprepitant (2 mg/mL, 10 mg/mL, and 50 mg/mL). As it is shown in Figure 1A, a significant decrease in eye wiping counts in the groups of mice treated with 10 and 50 mg/mL was observed (28.8%, $P < 0.01$ vs. PBS and 29.1%, $P < 0.01$ vs. PBS, respectively), suggesting a reduction in corneal nociception. We found that topical application of 2 mg/mL fosaprepitant one time did not significantly reduce corneal nociception. On the contrary, topical administration of the same dose 6 times/day for 10 days significantly reduced corneal nociception (31.4%, $P < 0.05$ compared to 10 days of PBS instillation; 27.1%, $P < 0.05$ single versus multiple administrations of fosaprepitant 2 mg/mL; Fig. 1B). No significant differences were observed between 10 and 50 mg/mL concentrations. In addition, we quantified corneal nociception in TAC1-KO mice lacking SP, the main ligand of NK1R. We observed that TAC1-KO mice showed a reduction compared to WT mice (30.1%, $P < 0.01$), which resulted to be comparable to the one displayed by fosaprepitant 10 mg/mL or 50 mg/mL groups.

The MPE represents the analgesic efficacy of a drug. When measured following topical application of 10 mg/mL fosaprepitant in a single administration, it was significantly higher than the MPE of control treatment with PBS (44.5% vs. 20.6%, $P < 0.001$; Fig. 1C). Topical application of 0.1% diclofenac or 4 mg/mL oxybuprocaine resulted to be the most effective treatments in reducing corneal nociception (MPE = 69.7% and 80.6%, $P < 0.01$ and $P < 0.001$, respectively, versus 10 mg/mL fosaprepitant; see Fig. 1C).

Fosaprepitant Application Induces Immediate Reduction of SP Levels in the Tear Fluid. TAC1 Gene Expression in the Cornea and SP Levels in the Trigeminal Ganglion Are Reduced

In order to elucidate the mechanism by which topical fosaprepitant reduces corneal nociception, the levels of SP were measured in the eye-washes immediately after acute corneal nerve stimulation. When compared to the PBS group, the SP content significantly decreased in the tear fluid of mice pretreated with fosaprepitant or oxybuprocaine (73%, $P < 0.05$, and 87%, $P < 0.01$, respectively; Fig. 2A). In addition, we found that after 3 days there was a decrease in TAC1 mRNA in the cornea in fosaprepitant and oxybuprocaine groups (68% and 85%, $P < 0.05$, respectively) compared to the PBS group (Fig. 2B). Of note, corneal nerve density was not affected by NaCl stimulus or treatments (see Supplementary Fig. S1 included in the Supplementary Material).

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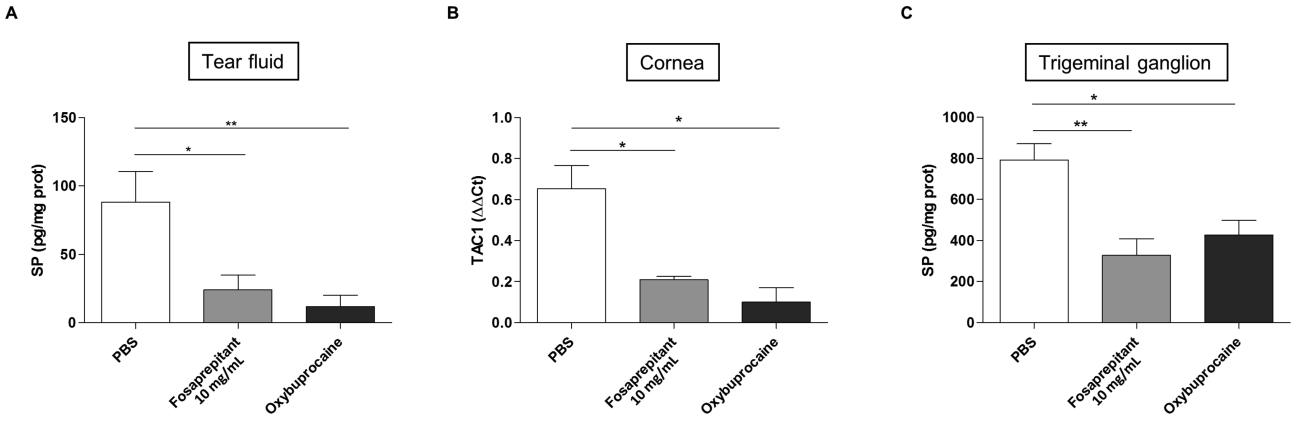

Figure 2. NK1R antagonist fosaprepitant reduces corneal nociception through inhibition of substance P (SP) release into the tear fluid and the trigeminal ganglion. (**A**) SP content in the tear fluid immediately after acute corneal nerve stimulation in wild type mice treated with PBS, 10 mg/mL fosaprepitant, or 4 mg/mL oxybuprocaine ($N = 5$ mice/group). (**B**) TAC1 mRNA levels in the cornea after 3 days of nerve stimulation in wild type mice treated with PBS, 10 mg/mL fosaprepitant, or 4 mg/mL oxybuprocaine ($N = 5$ mice/group). (**C**) Levels of SP in the trigeminal ganglion after 3 days of nerve stimulation in wild type mice treated with PBS, 10 mg/mL fosaprepitant, or 4 mg/mL oxybuprocaine ($N = 5$ mice/group). Graphs represent mean values ± SEM; statistical analysis by one-way ANOVA followed by Tukey's post hoc test (*$P < 0.05$ and **$P < 0.01$).

We then investigated if corneal nerve stimulation could be transmitted to the TG, because corneal sensory nerves derive from TG neurons. When the levels of SP were evaluated in the TG, a reduction in SP content was observed in both the fosaprepitant and oxybuprocaine groups (59%, $P < 0.01$, and 46%, $P < 0.05$, respectively; Fig. 2C), whereas no significant differences were found when TAC1 mRNA levels were assessed (see Supplementary Fig. S2 included in the Supplementary Material). Other markers related to pain (CalcB, TRPV1, and TRPM8) and inflammation (IL-1β and CD45) were evaluated at a gene level in the TG, but no significant differences were found among groups (see Supplementary Fig. S2 included in the Supplementary Material). These results suggest that topical administration of NK1R antagonist fosaprepitant or oxybuprocaine, a well-recognized anesthetic drug, early prevents the release of SP into the tear fluid after acute nerve stimulation followed by a downregulation of TAC1 gene expression in the cornea and SP levels in the TG.

Topical Fosaprepitant Reduces Leukocyte Infiltration in the Cornea Hours After Acute Nerve Stimulation

To analyze the inflammatory response triggered by acute nerve stimulation, we evaluated $CD45^+$ leukocytes infiltrating the cornea (Fig. 3A). When compared to PBS administration, a reduced number of total $CD45^+$ cells was observed after 10 mg/mL fosaprepitant ($P < 0.05$) and 4 mg/mL oxybuprocaine treatments ($P < 0.05$; Fig. 3B). This reduction was observed in both the peripheral and central cornea, as it is shown in Figure 3C.

Corneal Sub-Basal Nerves Express NK1 Receptor

β3-tubulin and NK1R double staining of WT corneal cross-section showed colocalization of the two proteins (Fig. 4A, arrowheads), indicating that corneal sub-basal nerves express NK1R, the main receptor for SP. Moreover, we found that 44.7 ± 7.6% of corneal β3-tubulin positive epithelial nerves also co-express NK1R (Fig. 4B).

Discussion

Ocular pain represents a significant clinical problem and an area of current unmet medical need. Topical anesthetics, such as oxybuprocaine, are highly effective, but they can be administered only for a limited time. In fact, they are associated with significant side effects, including toxic keratopathy, corneal melting, and perforation, which make their safety profile unacceptable for many.[22–25] Moreover, their use does not seem to be beneficial in the treatment of pain associated with corneal abrasions,[26] a common cause of ocular pain. In this vein, a recent meta-analysis did not find any significant improvement in symptoms and pain in patients treated with topical anesthetics versus placebo.[27]

Topical NSAIDs, such as diclofenac, have also been proposed for the treatment of corneal pain. Although some analgesic efficacy was demonstrated, the limited sample size of the studies makes it difficult to draw definitive conclusions.[26] Additionally, NSAIDs are generally less effective than topical anesthetics, and a recent Cochrane meta-analysis failed to provide strong evidence supporting them in corneal abrasions.[28] Importantly, the safety profile of long-term NSAIDs treatment is low, as it is associated with delayed wound healing, corneal melting, and perforation.[29–32]

Finally, systemic analgesics can successfully control ocular pain, although their use comes with significant side effects (e.g. reduced alertness, hallucinations, gastrointestinal, liver, and kidney toxicity).[33] In addition, when pain is limited to the ocular surface, there is no rationale for using systemic pain control if topical alternatives are available.

It is well-known that the release of SP and activation of NK1R are implicated in pain transmission.[34–36] The effect of NK1R antagonists on corneal pain has not been reported before; however, their role in nonocular pain has been extensively debated. Although preclinical evidence definitely demonstrated the analgesic effect of NK1R antagonists in peripheral and central pain,[37–39] clinical trials showed controversial effects. Beneficial outcomes were observed

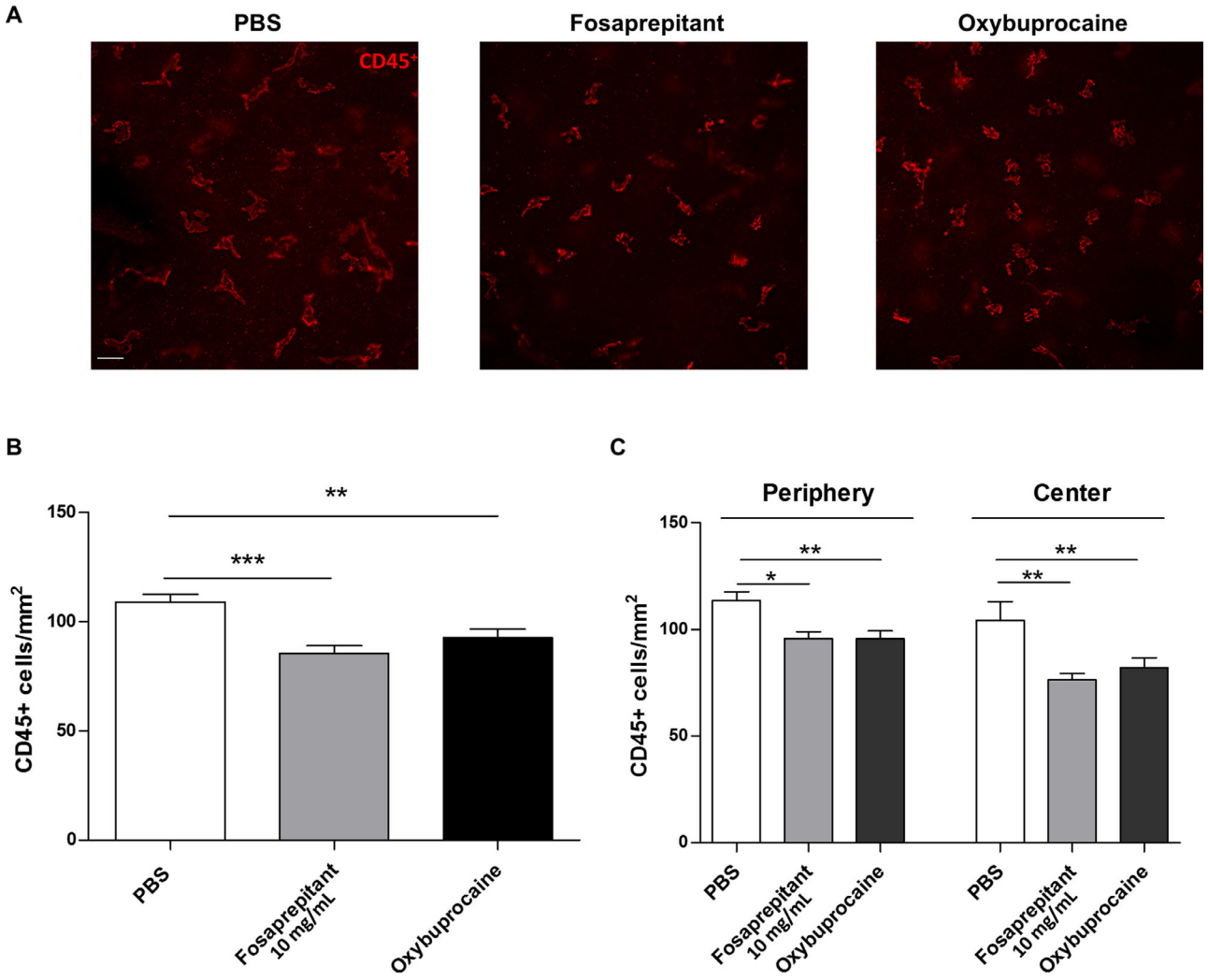

Figure 3. NK1R antagonist fosaprepitant and oxybuprocaine reduce corneal inflammation after acute corneal nerve stimulation. (**A**) Representative fluorescence images of CD45+ cells in the cornea after 10 mg/mL fosaprepitant or 4 mg/mL oxybuprocaine treatments (20 times; $N = 10$ mice/group). (**B**) Cell quantification showed a significant reduction of CD45+ cells after 10 mg/mL fosaprepitant and 4 mg/mL oxybuprocaine treatments. (**C**) Localized quantification of CD45+ cells showed a significant decrease in both peripheral and central cornea after treatment with 10 mg/mL fosaprepitant and 4 mg/mL oxybuprocaine. Graphs represent mean values ± SEM. Statistical analysis was performed by one-way ANOVA followed by Tukey's post hoc test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Scale bar = 25 µm.

in postoperative dental pain,[40] whereas no effects were observed in other types of neuropathic and nociceptive pain.[41,42] This discrepancy could result from different doses of NK1R antagonists, which would affect the receptor occupancy in key target sites, such as the brain and the spinal cord. Importantly, all the published clinical trials have addressed NK1R analgesic efficacy via systemic administration, whereas we have used topical administration. In this vein, different distributions of SP and NK1R density in various tissues, and/or higher local concentration of the drug could explain our findings.

In this paper, we showed that topical inhibition of NK1R by means of fosaprepitant is effective in reducing ocular pain in a well-characterized animal model. We observed that – among tested dilutions – 10 mg/mL fosaprepitant was the lowest capable of inducing analgesia after a single administration. Higher concentrations did not significantly decrease corneal nociception, possibly as a consequence of saturation of NK1R binding sites. Interestingly, fosaprepitant has many advantages when compared to other potent and selective NK1R antagonists, such as befetupitant and lanepitant. First, fosaprepitant is not toxic to the ocular surface or the corneal nerves when it is applied for 10 days at a similar concentration,[18] differently from befetupitant that induces epithelial damage and leukocyte infiltration.[17] Although Lanepitant is not toxic to the ocular surface, it is less effective in inhibiting cornel inflammation, probably as a consequence of poor tissue penetration, which was in fact demonstrated.[43] Second, fosaprepitant can be easily formulated as eye drops because it is water soluble (differently from befetupitant). Finally, it is approved for clinical use (differently from lanepitant), which makes drug repurposing a viable option. Therefore, inhibition of SP activity by means of topical NK1R antagonist fosaprepitant represents an attractive and safe option to treat corneal pain.

Different cellular mechanisms have been described with regard to NK1R-induced analgesia. For instance, inhibition of nerve depolarization has been extensively reported.[39]

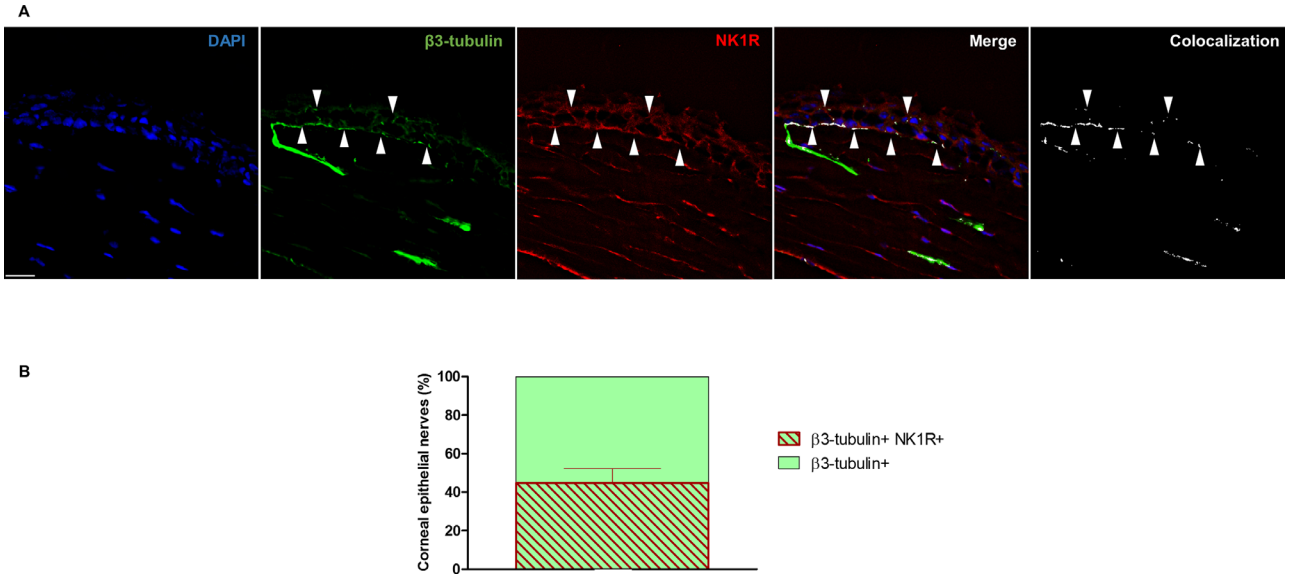

Figure 4. NK1R is expressed in mouse corneal nerves. (**A**) Representative images of β3-tubulin and NK1R co-staining in mouse corneal cross-sections (40 times; $N = 6$ mice). Colocalization points are shown with *arrowheads* and in the last image. Scale bar = 25 µm. (**B**) Quantification of β3-tubulin and NK1R positive epithelial nerves as a percentage of total β3-tubulin positive epithelial nerves.

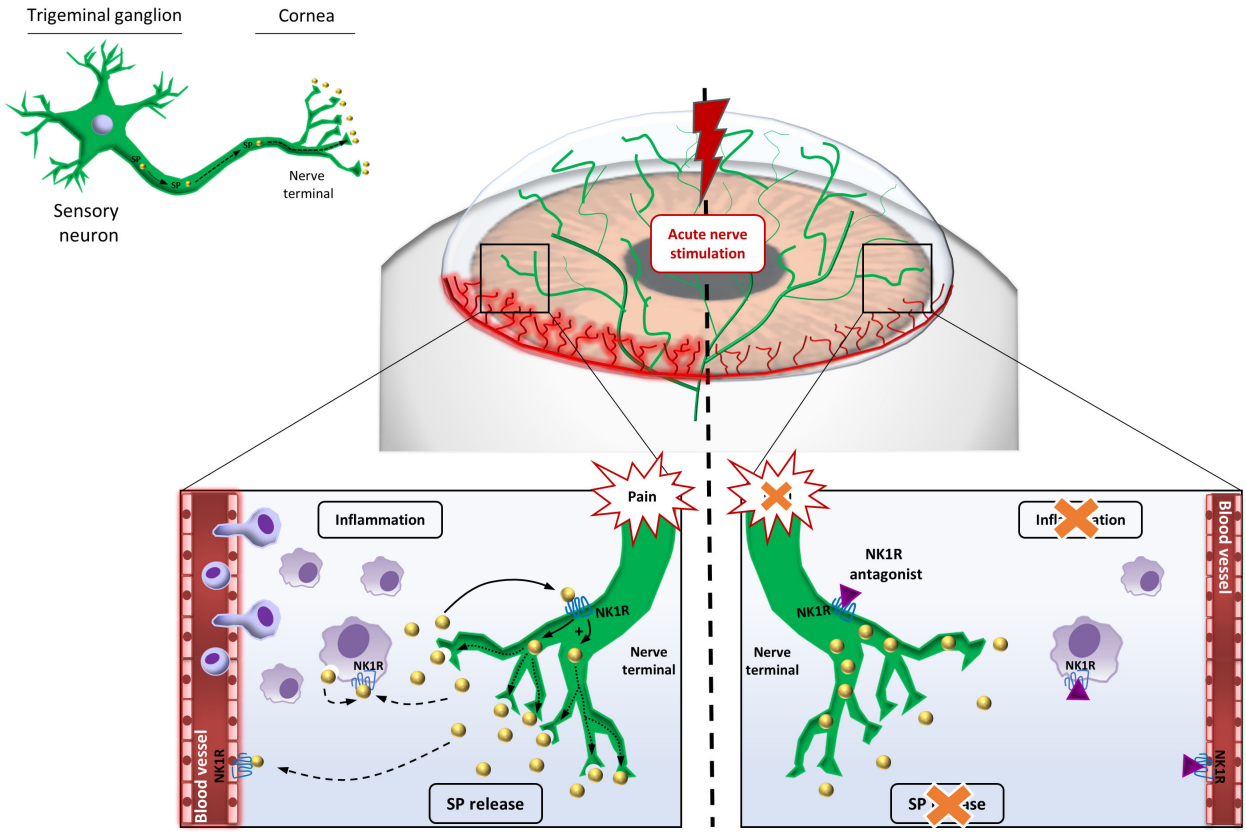

Figure 5. Schematic representation of the mechanism of action of substance P in corneal pain. Corneal nerve stimulation induces local release of substance P (SP). Binding of SP to nerve-expressed neurokinin-1 receptor (NK1R) results in increased nociception and tissue infiltration of leukocytes. NK1R antagonists inhibit SP-induced nociception and inflammation.

The fact that fosaprepitant decreased nociception minutes after administration suggests that NK1R blockade is acting through a local mechanism, because systemic absorption is minimal and reaching therapeutically active systemic concentration would require much longer times. Interestingly, corneal nerve terminals contain large amounts of SP,

which is stored in secretory vesicles rapidly secreted upon nerve stimulation.[44] In this vein, we observed an increase of SP levels in the tear fluid immediately (5 minutes) after corneal nerve stimulation that was prevented by fosaprepitant treatment. This suggests that SP is locally modulating the nociceptive response by binding to NK1R. In favor of this hypothesis, we found that corneal nerves widely express NK1R. In summary, our data support the existence of a positive feedback mechanism linking nerve-released SP and nerve-expressed NK1R, which eventually leads to pain initiation (Fig. 5). Our hypothesis is supported by previous literature, which shows that SP release is promoted by nerve injury through an autocrine loop both peripherally[45] and in the TG.[46] Therefore, NK1R antagonist not only blocks the activity of secreted SP, but also inhibits SP release from nerves.

In line with these observations, we observed reduced corneal pain in SP knockout mice. Importantly, we did not observe changes in corneal nerve density between WT and TAC1-KO mice,[19] suggesting that analgesia is not a consequence of denervation. Because SP is the primary ligand of the NK1R, we conclude that either pharmacological inhibition of NK1R or congenital absence of its ligand SP are able to reduce corneal pain.

Besides peripheral effects observed in the cornea, fosaprepitant also had an impact on the TG. Indeed, previous literature suggests that application of a hyperosmolar solution to the cornea rapidly triggers neural activity in the TG.[47] Interestingly, we observed that the expression of SP was increased in the TG after nerve stimulation. On the other hand, we did not observe changes in the expression of well-known pain-associated genes after 3 days of nerve stimulation (Supplementary Material). A possible explanation is that our model of mild nerve stimulation is not strong enough to induce upregulation of TG pain-related genes. In fact, upregulation of these genes was observed only in alkali burn or disepithelization animal models, which permanently damage corneal nerves.[48,49] In any case, the fact that SP levels in the tear fluid and TG are increased supports the existence of a corneatrigeminal axis,[48] which is activated even by mild nerve stimulation. NK1R blockade avoids SP/NK1R pathway activation, inhibiting the corneal-trigeminal axis and, ultimately, the SP synthesis by sensory neurons located in the TG (see Fig. 5).

In addition to the direct effect on nerves, we also observed that NK1R blockade significantly reduced infiltration of leukocytes into the cornea. The role of SP in promoting inflammation has been extensively reviewed as most leukocytes also express NK1R and are a relevant source of SP.[50,51] Moreover, the existence of a positive feedback between inflammatory cytokines and SP release has been reported.[52] Additionally, the role of leukocyte infiltration in pain induction and maintenance has been well-characterized.[53–56] Leukocytes recruited to the site of nerve injury contribute to pain generation by releasing pain-promoting factors (chemokines and cytokines) that promote nerve depolarization.[56] Moreover, the inhibition of leukocyte infiltration and hence, the pro-inflammatory response, have been associated with decreased hyperalgesia.[55] In this context, it is reasonable to hypothesize that topical application of fosaprepitant will also block NK1R expressed by leukocytes. Therefore, fosaprepitant can promote analgesia by synergic inhibition of leukocyte infiltration and blockade of nerve depolarization (see Fig. 5). Further studies are needed in order to better characterize the immune response phenotype.

Interestingly, treatment with oxybuprocaine, a well-known anesthetic drug, resulted in a similar response (reduced leukocyte infiltration and SP release in tear fluid and TG). This confirms the specificity of the animal model that we used, because oxybuprocaine selectively binds to sodium channels on peripheral nerves, eventually blocking the generation of the action potential.

On a broader perspective, our data show that stimulation of corneal nerves simultaneously initiates pain and promotes ocular inflammation through the release of neuropeptide SP. Several cellular mechanisms synergically contribute to the generation of corneal pain, including many membrane receptors and ion channels.[1] Our work illuminates a novel mechanism contributing to ocular pain, and provides evidence that SP modulation can be exploited therapeutically. Our study – in conjunction with preliminary data supporting safety (Bignami et al., 2017) – suggests that topical use of fosaprepitant, or other NK1R antagonists, is an attractive option to treat the myriad ocular conditions and surgeries causing pain, which affect millions of people worldwide.

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