Effects of Capsaicin on Corneal Wound Healing

Juana Gallar, Miguel A. Pozo, Irene Rebollo, and Carlos Belmonte

This study examined whether the depletion of neuropeptides from sensory nerve terminals induced by capsaicin modifies the healing rate of experimental corneal wounds in adult rabbits. Capsaicin (33 or 3.3 mM solutions) was administered topically and/or by a single retrobulbar injection to one eye while the fellow eye, treated with the vehicle, served as a control. After 1–3 weeks of treatment, an epithelial wound was made in the center of the cornea of both eyes with n-heptanol. Migration rates of epithelial cells surrounding the wound and estimated wound closure times were calculated by measuring the reduction in wound size. Combined treatment with 33 mM retrobulbar and 3.3 mM topical capsaicin for 3 weeks induced a significant delay in epithelial migration rates and in wound closure times ($P < 0.05$). Topical or retrobulbar capsaicin alone for 3 weeks and combined treatment lasting only 1 week were not sufficient to modify wound healing times. The substance P antagonist, spantide (3 mM), applied topically for 1–3 weeks before or immediately after corneal wounding was also ineffective in changing wound closure rates. These findings suggest that the delayed wound healing observed after prolonged treatment with capsaicin could be due to a sustained depletion of neuropeptides from corneal sensory endings, supporting the hypothesis that trophic effects of sensory nerves on corneal epithelium are, at least in part, mediated by neuropeptides contained in peripheral nerve terminals.


Several peptides of the tachykinin family (substance P, neurokinin A, neuropeptide K, and eledoisin) and calcitonin gene-related peptide (CGRP) have been found to coexist in the cell bodies and central and peripheral branches of nociceptive neurons. These neuropeptides are released by the peripheral endings of unmyelinated afferent fibers during tissue injury, contributing to the initiation of a local inflammatory response (neurogenic inflammation).

Administration of the neurotoxin capsaicin produces a depletion of neuropeptides from primary afferent neurons and a suppression of neurogenic inflammation. In small rodents (the mouse, rat, and guinea pig) treated at birth with capsaicin, skin and corneal lesions develop frequently. Furthermore, capsaicin-treated rats show a reduction of fur growth, greater severity of experimentally induced skin ulcers, and decreased survival of musculocutaneous flaps.

Tachykinins have been shown to stimulate proliferation of both epithelial and mesenchymal cells in tissue culture experiments. This information has shed new light on the well-known observation that peripheral nerve lesions induce trophic changes in distal target tissues. Consequently it has been proposed that, in addition to their role in neurogenic inflammation, neuropeptides could also take part in the regulation of growth and repair processes of tissues innervated by capsaicin-sensitive nerve fibers.

Traumatic or surgical lesions of the trigeminal ganglion or of the peripheral branches of the trigeminal nerve produce in humans and in experimental animals a corneal dystrophy known as neuroparalytic keratitis. The cornea is innervated by A-delta and C sensory fibers that terminate as unmyelinated nerve endings within the corneal epithelium and behave functionally like polymodal nociceptors. Some of these sensory afferent fibers contain substance P and CGRP. Capsaicin, administered either topically or by retrobulbar injection, suppresses pain reactions to chemical irritation of the cornea, leaves mechanical sensitivity unimpaired, and reduces markedly substance-P-like immunoreactivity in the cornea. Topical application of capsaicin has also a long-lasting desensitizing action on all corneal C-polymodal nociceptors and on some of the A-delta nociceptors. These effects suggest that capsaicin acts selectively on the population of peptide-containing sensory nerves of the cornea.

We studied whether or not the functional blockade of fine primary afferent fibers with capsaicin alters growth and repair processes in the cornea. We examined the time course of epithelial wound healing in the cornea of rabbits previously treated with topical
and retrobulbar capsaicin or with the substance P-antagonist, spantide. Preliminary observations have been reported elsewhere.35

Materials and Methods

Animal Preparation

Adult albino rabbits of either sex weighing 2.0–2.5 kg were used in this study. The animals were treated according to the ARVO Resolution on the Use of Animals in Research.

Capsaicin (Sigma, St. Louis, MO and Merck, Rahway, NJ) was dissolved in a saline solution of 1.5% ethanol and 8.5% Tween 80 to final 1% (33 mM) or 0.1% (3.3 mM) solutions. Spantide (kindly provided by Allergan Spain) was dissolved in 0.05% bovine serum albumin in saline to final concentrations of 3 mM.

For retrobulbar injection of capsaicin, rabbits were anesthetized by slow intravenous administration of sodium pentobarbital (40 mg/kg). As described by previous workers36,37 a volume of 0.5 ml of a 33 mM capsaicin solution was injected into the retrobulbar space with a 25-G needle. The contralateral eye received the same volume of the vehicle. Topical capsaicin was applied as a drop (30 μl) of 3.3 mM capsaicin in the conjunctival sac of one eye while the contralateral eye received the vehicle.36 Number of wipes, ie, displacements of the ipsilateral paw to rub the treated eye, were counted.

The rabbits were divided into four groups according to the different treatments used: (1) ten rabbits were treated with topical 33 mM capsaicin (n = 5) or 3.3 mM capsaicin (n = 5) one drop every 2 days for 3 weeks; (2) nine animals received a single retrobulbar injection of 33 mM capsaicin; (3) 18 rabbits received a retrobulbar injection of 33 mM capsaicin and topical 3.3 mM capsaicin one drop every 2 days for 1 (n = 11) to 3 (n = 7) weeks; and (4) nine animals were treated with topical spantide (30 μl of a 3 mM solution). Three animals received two doses daily (at 9:00 AM and 9:00 PM) for 1 week; two other rabbits were treated with a single dose per day for 3 weeks. Finally, in the last group of four animals, spantide was administered just before corneal wounding and every 4 hr throughout the healing period. The contralateral eye received 30 μl of the vehicle.

Wounding of the Corneal Epithelium

Corneal wounds were made in both eyes at various times after the onset of the capsaicin or spantide treatment. The rabbits were lightly anesthetized with thiopental (25 mg/kg). After topical anesthesia with 0.4% oxybuprocaine, a corneal wound was produced in the epithelia of both eyes by applying a 3-mm circle piece of Whatman 1 paper embedded in n-heptanol to the center of the cornea for 30 sec.38,39 The eye was repeatedly washed afterwards with isotonic saline. Every 4 hr (with a pause between midnight and 10:00 AM) the wounds, stained with 2% fluorescein, were photographed and also measured in situ with a calibrated eyepiece from a dissecting microscope (8× magnification) until complete healing was observed. Measurements were repeated from enlarged photographs of the wounds.

Analysis of Data

To model the nonlinear decrease in wound area during epithelial healing, the constant velocity method of Crosson et al40 and the kinematic analysis of Kwok and Madigan41 were used.

After Crosson et al,40 migration rates were determined by linear regression of the decrease in wound radius during the healing phase (10–34 hr) and were given by the slope of the regression line, expressed as μm/hr. The time required for total closure of the corneal wound was calculated by extrapolation of the best fit of regression lines during the healing phase (10–34 hr) to 100% closure for each eye tested. Migration rates (estimated migration rate, EMR) and the estimated time for wound closure (estimated time of healing, ETH) in treated and control eyes were compared using paired t-tests. Average values were expressed as mean ± standard error of the mean. The level of significance for the differences was P < 0.05.

When the method of Kwok and Madigan41 was used, planar wound areas were corrected for corneal curvature and the equivalent wound radius calculated. After confirmation that the nonlinear time course was well fitted by a second-order (quadratic) function (coefficients of determination, r² > 0.95), the instantaneous velocity on the curved surface was calculated. The difference between paired results was tested for significance with the Wilcoxon signed rank test.

Results

Behavioral Effects of Capsaicin

Topical application of capsaicin to the eyes of control rabbits produced blinking, blepharospasm, and wiping movements. The number of wipes caused by a first application of 3.3 mM capsaicin did not vary significantly when the instillation of the drug was repeated on alternate days for 3 weeks; the same was true when 33 mM capsaicin was used. Furthermore, the mean number of wipes per topical application was similar with both concentrations of capsaicin (3.3...
mM capsaicin: 9.6 ± 8.1 wipes/instillation, n = 51; 33 mM capsaicin: 11.8 ± 7.9 wipes/instillation, n = 17). Retrobulbar capsaicin produced an acute miotic response that subsided in 24–48 hr. After retrobulbar injection of capsaicin, topical application of the neurotoxin elicited a reduced wiping response (3.7 ± 3.4 wipes/instillation, n = 62).

Wound Healing Rates After Capsaicin

*Topical capsaicin:* Ten rabbits were treated with topical capsaicin applied to one eye every 2 days for 3 weeks before wounding the cornea; the contralateral eye received the vehicle. In five animals 3.3 mM capsaicin was applied; in the remaining five, a 33 mM solution of capsaicin was used. Neither treatment affected the reduction in wound radius with time (Fig. 1A) or the values of ETH and EMR (Table 1).

*Retrobulbar capsaicin:* In a group of nine rabbits, a retrobulbar injection of 33 mM capsaicin was delivered to one eye; the contralateral eye, injected with the vehicle, served as the control. A bilateral corneal wound was made 3 weeks later. In one of the animals, signs of inflammation appeared in the control eye a few hours after wounding. Therefore, data from this eye were not included for statistical comparison with the treated side. The treatment had no significant effect on the reduction of wound radius with time, ETH, or EMR (Fig. 1B and Table 1).

*Topical plus retrobulbar capsaicin:* Eleven rabbits received a retrobulbar injection of 33 mM capsaicin in one eye 1 week before corneal wounding; topical 3.3 mM capsaicin was also applied to the same eye once every 2 days throughout this time. Figure 2A shows similar values of wound radius versus time were observed in treated and control eyes. The values of EMR and ETH are shown in Table 1. No significant differences between these parameters were found in capsaicin-treated versus vehicle-treated eyes.

Seven rabbits received in one eye a single retrobulbar injection of 33 mM capsaicin and subsequently one drop of the drug at a concentration of 3.3 mM once every 2 days; the contralateral eye, injected ret-

### Table 1. Effect of various capsaicin treatments on the estimated time of healing (ETH) and epithelial migration rate (EMR) of corneal wounds

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ETH (hr) Vehicle</th>
<th>ETH (hr) Capsaicin</th>
<th>EMR (μm/hr) Vehicle</th>
<th>EMR (μm/hr) Capsaicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3 mM topical 3 wk, n = 5</td>
<td>37.5 ± 2.8</td>
<td>38.8 ± 4.8</td>
<td>68.5 ± 14.1</td>
<td>69.1 ± 11.5</td>
</tr>
<tr>
<td>33 mM topical 3 wk, n = 5</td>
<td>31.9 ± 1.5</td>
<td>34.5 ± 0.6</td>
<td>56.5 ± 5.1</td>
<td>60.5 ± 2.7</td>
</tr>
<tr>
<td>33 mM retrobulbar 3 wk, n = 9</td>
<td>39.0 ± 2.5</td>
<td>46.3 ± 5.7</td>
<td>60.0 ± 3.6</td>
<td>54.5 ± 5.7</td>
</tr>
<tr>
<td>33 mM retrobulbar + 3.3 mM topical 1 wk, n = 11</td>
<td>34.6 ± 1.4</td>
<td>35.9 ± 1.7</td>
<td>74.1 ± 12.7</td>
<td>66.3 ± 7.0</td>
</tr>
<tr>
<td>33 mM retrobulbar + 3.3 mM topical 3 wk, n = 7</td>
<td>34.0 ± 1.1</td>
<td>42.6 ± 4.0*</td>
<td>63.8 ± 3.0</td>
<td>49.3 ± 6.0*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *P < 0.05. n = number of animals.
Fig. 2. Effects of topical plus retrobulbar capsaicin on corneal wound healing. (A)Decrease in corneal wound radius with time in 11 rabbits treated in one eye with a single retrobulbar injection of 33 mM capsaicin 1 week before corneal wounding and also with topical 3.3 mM capsaicin (open circles) once every 2 days during that week. The contralateral eye (filled circles) was treated in a similar fashion but with the vehicle. (B) Data from rabbits that received a single retrobulbar injection of 33 mM capsaicin and a drop of topical 3.3 mM capsaicin once every 2 days (n = 7) during the 3 weeks. Note that in these animals the radii of wounds at 34 hours in capsaicin-treated and control eyes were significantly different (P < 0.05). Insets, regression lines obtained with the values of wound radii measured between 10 and 34 hr after wounding. Values are mean ± SEM.

robulbarly with the vehicle, served as the control. A bilateral corneal wound was made 3 weeks after the capsaicin injection. As shown in Figure 2B, slowing of the wound healing rate began to appear in the treated eye approximately 20 hr after wounding, thus giving significantly larger radii of the wound in these corneas at 34 hr (P < 0.05). At 42 hr healing was complete in the control eye, but the wound persisted in the treated side. This delay in wound healing was also reflected in the values of ETH and EMR; these were significantly different (P < 0.05) in the treated eyes compared with the controls (Table 1). In this group of animals, the curved surface velocity of the wound edge was calculated at 34 hr. Values of 55 ± 25 μm/hr were found for the control eyes versus 33 ± 12 μm/hr for the treated eyes, the difference being significant (Wilcoxon signed rank test, P = 0.10 two-tailed).

Wound Healing Rates After Spantide

Spantide (3 mM) was applied topically to one eye in three groups of rabbits. The first group of three animals was treated daily with two doses (30 μl) of spantide during the week preceding wounding. A second group of two rabbits received one dose given 3 weeks before the day when corneal lesions were produced. Finally, another group of four rabbits received the same dose of spantide applied immediately before wounding and repeated every 4 hr throughout the healing period.

Neither treatment modified the time course of wound healing or the values of EMR and ETH (Figs. 3A-B, Table 2).

Fig. 3. Effects of topical spantide on average wound radius reduction as a function of time. Filled triangles = vehicle-treated eyes. Open triangles = eyes treated with spantide. (A) Topical spantide (30 μl, 3 mM) applied for 3 weeks (n = 2). (B) Topical spantide (30 μl, 3 mM) applied immediately before wounding and throughout the healing period (n = 4). Inset, regression lines of wound radii measured between 10 and 34 hr after wounding. Values are mean ± SEM.
Topical Effect of various spantide treatments on the estimated time of healing (ETH) and epithelial migration rate (EMR) of corneal wounds

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ETH (hr)</th>
<th>EMR (μm/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 wk, n = 3</td>
<td>35.8 ± 2.9</td>
<td>36.0 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>63.8 ± 4.6</td>
<td>65.3 ± 5.6</td>
</tr>
<tr>
<td>Topical</td>
<td>35.4 ± 1.7</td>
<td>35.6 ± 1.9</td>
</tr>
<tr>
<td>3 wk, n = 2</td>
<td>66.9 ± 1.9</td>
<td>68.0 ± 2.9</td>
</tr>
<tr>
<td>After wounding</td>
<td>34.3 ± 0.6</td>
<td>34.8 ± 0.3</td>
</tr>
<tr>
<td>n = 4</td>
<td>78.3 ± 2.4</td>
<td>74.6 ± 3.4</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. n = number of animals.

Discussion

On the basis of the existing data concerning the effects of capsaicin applied locally to sensory nerve trunks, retrobulbar capsaicin is expected to produce a vigorous excitation of unmyelinated and thinly myelinated afferent fibers in the ciliary nerves, followed by a selective blockade of nervous conduction in these fibers. The strong reflex responses observed in the rabbits at the moment of injection indicate that massive excitation of nociceptive fibers also occurred in our experiments. Gross mechanical sensitivity and pupillary reflexes were apparently unaffected after retrobulbar capsaicin, supporting the contention that most of the A-delta sensory afferents and the autonomic C fibers are insensitive to the action of the neurotoxin. Likewise, excitatory effects followed by inactivation of the corneal C afferents have been described upon topical application of capsaicin. This explains the relative insensitivity noticed after retrobulbar capsaicin in our study and in previous reports.

A delayed corneal wound healing rate resulted from the administration of capsaicin in our experiments. This effect became evident 3 weeks after the onset of the treatment with capsaicin and required both topical and retrobulbar application of the drug. Bynke showed that the capacity of the rabbit’s eye to respond to different inflammatory stimuli (infrared irradiation of the iris and administration of prostaglandin E2) was impaired for several weeks after retrobulbar injection of 1% capsaicin. A delay in wound repair was also reported after selective trigeminal denervation. However, it is doubtful that in our experiments, delayed healing was produced by the degeneration of part of the sensory nerve fibers. Degeneration of corneal nerves and its trophic consequences on the corneal epithelium are fully established 1 week after denervation, in contrast with the absence of trophic effects of capsaicin after the same time interval. Furthermore, Lynn and Shakhaneh demonstrated that direct application of 1% capsaicin to the saphenous nerve of rabbits did not produce structural changes on C fibers suggestive of nerve degeneration. However, they observed a large drop in the levels of substance P in the skin of the treated rabbits to values close to those obtained with nerve section.

Capsaicin, applied to the sciatic nerve of rabbits, inhibits the axonal transport of substance P and somatostatin, decreasing the content of this, and presumably other, neuropeptides in the periphery. Retrobulbar injection of capsaicin in this species also prevented inflammatory responses of the eye that seemed to be mediated by release of neuropeptides. Neonatal injection or local application of capsaicin to the cornea of the rat causes a marked decrease in substance P. It seems likely, therefore, that the effects of capsaicin on corneal wound healing are not due to the nonspecific reduction of a neurogenic process secondary to nerve degeneration but rather to the peptide-depleting action of the neurotoxin.

Retrobulbar capsaicin blocks specifically the axonal flow of substance P, and topical capsaicin partially depletes the deposits of neuropeptides at their peripheral stores. According to our data, it would appear that both procedures need to be combined for prolonged periods of time to produce a noticeable wound-healing delay. This is not surprising considering that unlike the rat, guinea pig, or mouse, capsaicin does not completely block substance P transport in the rabbit. Thus, it is tempting to speculate that neuropeptides transported along the peripheral axon of primary sensory neurons and stored at the nerve endings are released to maintain the corneal epithelium trophically. Topical and retrobulbar capsaicin would slowly decrease the peripheral levels of neuropeptides to values low enough to reduce their stimulatory role on the growth and repair processes that follow wounding.

The lack of effect of spantide on corneal wound closure dispute the idea that substance P is the peptide mediating the trophic influences of corneal sensory terminals; however, this result should be interpreted with caution. Spantide possesses also agonistic actions that could mask its antagonistic role. Nevertheless, other neuropeptides that are present in corneal nerve endings and are also released by capsaicin could be responsible for the modulation of the wound repair process by the sensory innervation.

Key words: capsaicin, nociceptors, trophic effects, sensory neurons, substance P, corneal wound healing
Acknowledgments

The authors thank Dr. Stephen Kwok for his help with data analysis. The expert technical assistance of Simón Moya and Alfonso Pérez is greatly appreciated.

References

36. Camras PCB and Bito LZ: The pathophysiological effects of


47. Lynn B and Shakhanbeh J: Substance P content of the skin, neurogenic inflammation and numbers of C-fibres following capsaicin application to a cutaneous nerve in the rabbit. Neuroscience 24:769, 1988.


