Spontaneous Chronic Corneal Epithelial Defects (SCCED) in Dogs: Clinical Features, Innervation, and Effect of Topical SP, with or without IGF-1

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PURPOSE. To delineate the clinical features and alterations in innervation and substance P (SP) content in spontaneous chronic corneal epithelial defects (SCCED) in dogs and to conduct a preliminary investigation evaluating the efficacy of topical SP, with or without insulin-like growth factor (IGF)-1, in the treatment of this disorder.

METHODS. Complete ophthalmic examinations, including Cochet-Bonnet aesthesiometry, were performed in 45 canine patients that had spontaneous corneal epithelial defects of at least 5 weeks’ duration and with no identifiable cause. Eighteen patients had superficial keratectomies performed, and the corneal nerves were labeled immunohistochemically with antibodies against protein gene product (PGP)-9.5, SP, vasoactive intestinal peptide (VIP), and tyrosine hydroxylase (TH). Relative fiber densities were assessed qualitatively and quantitatively. Corneal epithelial cell and tear SP contents were determined in affected and normal dogs by an enzyme immunoassay. A preliminary open-label treatment trial of topical SP, with and without IGF-1, was conducted in 21 dogs.

RESULTS. The duration of the erosion before admittance into the study was a mean of 9.22 weeks (range, 3–52). The average patient was middle aged (mean, 9.25 ± 1.85 years [SD]); no sex predisposition of the disease was identified. Boxers, golden retrievers, and keeshonds were overrepresented when compared with the normal hospital population. Corneal sensation was normal. Marked alterations in corneal innervation were identified in affected dogs with abnormal increased SP and calcitonin gene-related peptide (cGRP)-immunoreactive nerve plexuses identified surrounding the periphery of the epithelial defect. The SP content of epithelial cells surrounding the defect increased, whereas the tear SP content remained unchanged. Of the canine patients treated with SP, with or without IGF-1, 70% to 75% had complete healing of the defect. The SP content of epithelial cells surrounding the defect was increased in untreated SCCED compared with the normal hospital population. Corneal sensation was normal, with no apparent underlying cause are frequently encountered in dogs in companion-animal veterinary practices, typically in middle aged to older animals averaging 8 to 9 years of age.1–5 Clinically, the defects are characterized by the presence of an epithelial erosion surrounded by a circumferential sheet of loosely adherent or nonadherent epithelial cells (“epithelial lip”). The affected dog displays variable discomfort evidenced by blepharospasm and epiphora. The defects are chronic, with some persisting for longer than 6 months.1–5

These chronic epithelial defects in dogs share numerous clinical features with chronic or recurrent corneal epithelial erosions in humans. Whereas recurrent epithelial erosions in humans may be associated with diverse conditions, including neurotrophic keratitis, epithelial basement membrane dystrophy, anterior stromal dystrophies, and diabetic keratopathy, many continue to be regarded as idiopathic.6–8 The similarity in clinical appearance to neurotrophic keratitis in humans suggests that this disease entity in dogs is a reflection of abnormal corneal innervation. The canine cornea is richly invested with sensory and sympathetic nerves, and the peptidergic innervation of the normal canine cornea has recently been determined.9 Of particular note is a dense innervation with substance P (SP)-containing sensory nerves. SP, both alone and in synergy with other cytoactive compounds, has been shown to exert a wide range of trophic influences, including stimulation of proliferation and promotion of migration.10–12 In conjunction with insulin-like growth factor (IGF)-1, SP has been reported to be a successful topical treatment for chronic corneal erosion in a young child with Riley-Day syndrome.13

Because it has been shown to be extremely efficacious, superficial keratectomy is offered as a treatment option for this disease in dogs.3,14 The clinical material obtained in this procedure provides the opportunity for examination by morphologic and immunohistochemical techniques. In the current issue of IOVS, Bentley et al.13 report alterations in the anterior stroma and in expression of components of the extracellular matrix in dogs with this disorder.

This study was undertaken to characterize the clinical features of SCCED in dogs, to quantify the SP content of the tears and corneal epithelium of normal dogs and of dogs with SCCED, to determine whether there are alterations in the peptidergic innervation of the cornea in dogs that have this disease, and to collect preliminary data on the efficacy of treatment of this disorder by the topical application of SP, with or without IGF-1.

MATERIALS AND METHODS

DOGS

Typically, dogs had been evaluated and treated by local veterinarians, usually by epithelial debridement, without evidence of defect resolution for several weeks to months. The dogs were then referred to the

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ophthalmology service at the Veterinary Medical Teaching Hospital, University of Wisconsin-Madison. A total of 45 dogs (47 eyes) were evaluated in this study. All dogs were privately owned pets. Owners reviewed and signed an informed-consent form before samples were collected. All research was performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and with the institutional animal care and use committee at the university.

Case Criteria for Inclusion
Before inclusion in this study, all dogs underwent a complete ophthalmic examination by a board-certified veterinary ophthalmologist, including slit lamp examination (SL-2 handheld slit lamp; Kowa, Tokyo, Japan), indirect ophthalmoscopy, Schirmer tear test (STT), fluorescein staining, and Cochet-Bonnet (CB) aesthesiometry. Application tonometry (Tono-Pen; Mentor, Norwell, MA) was also performed, with results considered to be within normal limits. Dogs included in the study met the following criteria: presence of a nonhealing corneal epithelial defect in one or both eyes of at least 3 weeks' duration without appreciable progress toward resolution; absence of any identifiable underlying cause of the persistent defect (e.g., normal tear production; absence of lid conformational defects; normal lid function; no clinical evidence of sepsis; and absence of distichiasis, ectopic cilia, and foreign bodies); and absence of any clinical evidence of systemic disease.

Clinical Evaluation
Schirmer Tear Test. As a crude estimate of tear production, both eyes of each dog were evaluated using Schirmer test strips (lot 6011924; Schering-Plough, Kenilworth, NJ). One test strip was placed in each conjunctival sac at the junction between the lateral one third and medial two thirds of the lower lid for 60 seconds, the standard time used for tear evaluation in dogs.16,17 Immediately after the 60-second period, the length of the moistened area of the test strip was measured in millimeters and recorded. Testing was performed before administration of any topical medication.

CB Aesthesiometry. Corneal sensitivity was measured using a CB aesthesiometer (Luneau, Chartres, France). The measurements were performed in both eyes of each dog. Evaluation of corneal sensation was limited to the central cornea. The tip of the aesthesiometer was applied to the central cornea, usually adjacent to the epithelial defect in a perpendicular direction, with enough pressure to produce a slight bending of the monofilament (~4° deflection). The length of the monofilament was reduced until a reaction from the patient was noticed (e.g., squinting, retraction of globe with protrusion of the third eyelid). Repeated measurements were typically made until an identical value was obtained three times. The length of the monofilament at the point of reaction was recorded and expressed in centimeters. Ten dogs, chosen at random from the hospital population and with no evidence of systemic or ocular disease and no history of surgery or drugs administered within 24 hours, were evaluated in an identical fashion.

Measurement and Photography of Erosion. A strip containing fluorescein sodium (Fluor-I-Strip; Ayerst Laboratories, Philadelphia, PA) was wetted with sterile irrigating solution (Eye Wash; Goldline Laboratories, Inc., Fort Lauderdale, FL), and 1 drop of fluorescein added to the affected eye. The epithelial defect of each dog was then marked with the blunt end of a number 15 Bard-Parker blade. The defects were measured with a microfuge tube containing 50 µl of a degradation-inhibiting enzyme cocktail (1 µM thiorphan, 5 µM captopril, 5 µM phosphoramidon, and 14 µM bacitracin; all from Sigma, St. Louis, MO) and sterile saline and stored at −70°C.

Debridement of Epithelial Cells. To assess the level of SP in the epithelium, epithelial cells were obtained by debridement from 13 normal dogs that had been euthanized for reasons other than use in this study. One drop of topical proparacaine (Allergan, Horikawas, Puerto Rico) was applied to the ocular surface to mimic the conditions under which the patient’s epithelial cells were collected, and the debridement was performed with the blunt end of a number 15 Bard-Parker blade. The cells were added to a microfuge tube containing 50 µl of an inhibitor cocktail, frozen on dry ice, and stored at −70°C. Loosely attached epithelial cells were obtained from the affected corneas of 12 canine patients and handled in a similar fashion for SP quantification. The cells were typically loosely attached and constituted a circumferential zone surrounding the exposed stroma.

Treatments. Owners were offered a variety of treatments for their dogs, including a treatment trial with topical SP, SP with IGF-1, superficial keratectomy (described later), anterior stromal puncture (ASP) followed by contact lens placement, topical insulin, or debridement followed by contact lens placement. For this initial phase of our research, no attempt was made to mask treatment groups or to randomize patients. In patients entered into the SP treatment trial, 1 drop of SP was instilled in the affected eyes every 15 minutes for 2 hours, and the regimen was performed twice daily. This regimen was chosen because preliminary in vitro experiments (data not shown) had shown that SP promotion of epithelial cell proliferation persisted after discontinuation of SP if the cells had been exposed to SP for 2 hours. Generally, dogs treated with SP (2.5 ng to 250 µg/ml) or SP (250 µg/ml) and IGF-1 (1 µg/ml) were rechecked at 1 and 2 weeks after initiation of therapy. If no change in the erosion’s size was noted by 2 weeks, therapy was discontinued. If marked improvement was noted, manifested by decrease in the erosion’s size and improved comfort level of the dog, treatment was continued for another 2 weeks. If improvement continued, the treatment was continued for up to 6 weeks.

Superficial Keratectomies. In 18 dogs, a superficial keratectomy was performed to completely excise the region of the cornea containing the epithelial defect. Anesthesia was induced using thiamical sodium, the dog was intubated, and anesthesia was maintained with isoflurane. A corneal trephine was used to outline the region to be removed, a small partial-depth incision was made with a number 64 Beaver blade, and the superficial stroma was separated using a Martinez corneal dissector. Once the superficial lamellae were separated from the underlying stroma (approximately 200 µm of the superficial cornea was removed, as determined by ultrasonic pachymetry; Pach-pen; Mentor). Vannas scissors were then used to excise the superficial corneal button, as outlined by the trephine. Specimens were immediately bisected with a razor blade. Half of each specimen was placed in 2% glutaraldehyde (for morphologic evaluation) and the other half was placed in 4% paraformaldehyde and 0.2% picric acid in phosphate buffer (for immunohistochemical evaluation of corneal nerves).

Immunohistochemical Analyses. The status of corneal innervation in the superficial keratectomy specimens was evaluated by immunohistochemical nerve-staining methods. One half of each superficial keratectomy specimen was cut with a razor blade into four equal, pie-shaped segments. The tissue pieces were then embedded in optimal cutting temperature (OCT) compound (Miles, Inc., Elkhart, IN); sectioned at 30 µm in a cryostat, either parallel or perpendicular to the corneal surface; and collected in serial order in ice-cold 0.1 M phosphate-buffered saline (PBS). Immunohistochemical demonstrations of corneal nerve fibers were performed on free-floating sections incu-
bated in primary antisera directed against one of the following markers: CGRP (1:5000; Amersham, Arlington Heights, IL), SP (1:4000; Peninsula Laboratories, San Carlos, CA), vasoactive intestinal polypeptide (VIP; 1:1000; Peninsula Laboratories), or tyrosine hydroxylase (TH; 1:400; Pel-Freeze Biological; Rogers, AR). A small number of sections from three additional SCCED specimens were incubated in primary antiserum directed against the pan-neuronal marker, protein gene product (PGP)-9.5 (1:5000; Chemicon International, Inc., Temecula, CA). Immunolabeled nerve fibers were visualized by using a kit (Vectastain ABC Elite; Vector Laboratories, Burlingame, CA), with diaminobenzidine (DAB) as the substrate. The specificity of the immunocytochemical procedure was confirmed by incubating randomly selected sections in normal rabbit serum without the appropriate primary antibody.

All sections were critically examined in a light microscope (model BH2; Olympus, Lake Success, NY), and changes in corneal innervation density, nerve distribution pattern, and axonal morphology were compared with control corneas.9 Observations were recorded in a series of line drawings prepared with a drawing tube attached to the microscope, and black-and-white photomicrographs were obtained (T-Max 100 film; Eastman Kodak, Rochester, NY).

The results of the immunohistochemical analyses revealed significant morphologic changes in the corneal innervation of clinical patients compared with control animals (see the Results section). This observation raised the question of whether the observed nerve changes were associated specifically with the pathogenesis of chronic epithelial erosion syndrome or whether they were simply a consequence of a persistent epithelial defect. To answer this question, unilateral 8-mm axial corneal abrasions were created weekly in six normal experimental dogs. Eyes in two dogs were wounded two times, in two dogs three times, and in two dogs four times. One week after creation of the final corneal epithelial defect, the dogs were killed and their corneas were immersion fixed, sectioned, and processed immunohistochemically in a manner identical with that described earlier for the clinical specimens.

**FIGURE 1.** Typical clinical appearance of a canine patient with SCCED. Paraxial nonvascularized defect in a 6-year-old female boxer before (a) and after (b) installation of fluorescein. Intense fluorescein retention was visible in the area of demuded stroma, surrounded by less intense staining in the region where epithelial cells were poorly attached to the substratum (“epithelial lip”).

**FIGURE 2.** Peripheral vascularized defect in a 9-year-old neutered male golden retriever. Approximately 64% of patients had some degree of corneal vascularization present at initial examination, with an increased prevalence of vascularized lesions being associated with more peripheral lesions.

**FIGURE 3.** A 6-year-old spayed female greyhound with a spontaneous chronic corneal epithelial defect. (a) Appearance of defect before initiation of treatment with SP and IGF-1. (b) At 4 weeks after treatment the epithelial defect had healed and all clinical signs had resolved.
Corneal Epithelial Defects in Dogs

**SP Quantification by Enzyme Immunoassay.** The amount of SP was quantitated in samples of corneal epithelium, and tears were collected from normal dogs and patients with SCCED, by using an enzyme immunoassay (EIA) kit (Cayman Chemical Co., Ann Arbor, MI). Corneal epithelial cell samples from 6 to 10 eyes were pooled and sonicated (three times for 10 seconds each, intensity level setting of 30%, using the Sonic Dismembrator 300; Fisher Scientific, Fairlawn, NJ) on ice. The sonicated samples were then purified by solid-phase extraction, as described in the EIA kit protocol. Briefly, the samples were acidified by the addition of 4% acetic acid, and a hot spike of 5000 cpm 3H-SP (1 mCi/ml; Dupont-NEN, Boston, MA) was added. The samples were then applied to C-18 (ODS-5) reversed-phase cartridges (Whatman, Clifton, NJ). After the columns were washed with 4% acetic acid, the SP was eluted by application of ethanol-water-acetic acid (90:10:0.4). The eluted samples were dried by vacuum centrifugation and stored at −20°C until they could be assayed. Before the assay was performed, the purified corneal epithelial samples were reconstituted in EIA buffer. A portion of each reconstituted sample was added to liquid scintillation fluid and counted, with the results used to calculate the recovery of SP from the purification procedure. The amount of SP in each purified corneal epithelial sample was then measured, using the EIA as described by the manufacturer. Tear samples from the eyes of 6 to 10 dogs in each group (normal subjects, patients with SCCED, and patients with nonulcerated eyes) were pooled and used directly in the EIA for the determination of SP content. An aliquot of each corneal epithelial cell sonicate and of each pooled tear sample was collected for protein determination by using a Bradford method-based kit (Bio-Rad, Hercules, CA).

**RESULTS**

**Clinical Evaluation**

A total of 45 dogs (47 eyes) met all inclusion criteria, were examined, and were included in the study. Breeds represented included boxers \((n = 8, 17.7\%)\), golden retrievers \((n = 6, 13.3\%)\), keeshonds \((n = 5, 11.1\%)\), Labrador retrievers \((n = 2, 4.4\%)\), Yorkshire terriers \((n = 2, 4.4\%)\), German shepherds \((n = 2, 4.4\%)\), greyhounds \((n = 2, 4.4\%)\), lhasa apso \((n = 2, 4.4\%)\), toy poodles \((n = 2, 4.4\%)\), West Highland white terriers \((n = 2, 4.4\%)\), and one each \((2.2\%)\) beagle, cairn terrier, bichon frise, cocker spaniel, Jack Russell terrier, malamute, Norwegian elkhound, standard poodle, schnauzer, silky terrier, weimaraner, and Welsh corgi. Boxers, golden retrievers, and keeshonds were overrepresented when compared with the normal hospital population \((1.25\%, 9.12\%,\) and \(0.4\%,\) respectively). There were 10 intact males, 10 neutered males, 2 intact females, and 23 spayed females. The sex distribution is not significantly different from the normal hospital population. The mean age was 9.25 ± 1.85 years \((SD)\) and the median age was 9 years \((range, 6–14)\).

Duration of the erosion before admittance into the study was a mean of 9.22 ± 10.78 weeks \((SD)\), with a median duration of 6 weeks \((range, 3–52)\). The typical epithelial defect was located in the interpalpebral fissure and was characterized by the presence of intense fluorescein dye retention over the area of exposed stroma surrounded by a ring of less intense fluorescein retention. This lighter staining ring represented a zone where corneal epithelial cells were present but were not attached or were poorly attached to the underlying stroma \((Fig. 1)\). Of the 47 erosions, 30 \((63.8\%)\) were vascularized at the initial examination \((Fig. 2); 12 \((25.5\%)\) were located axially, with vascularization occurring in 3; 9 \((19.1\%)\) were located paraxially, with vascularization occurring in 3; and 26 \((55.3\%)\) were located peripherally with 24 \((94\%)\) of those showing vascularization.

**Schirmer Tear Test**

The previously reported normal mean STT values in dogs is 21 ± 4.2 mm wetting/min \((SD)\). In the current study, the mean STT of affected eyes was 21.2 ± 5.1 mm wetting/min, whereas the mean STT of the contralateral unaffected eyes was 17.9 ± 5.6 mm wetting/min. The STT of the affected eyes was significantly greater \((P = 0.0126)\), according to Student’s paired t-test.

**CB Aesthesiometry**

There was no significant difference in corneal sensation between the affected and unaffected eyes in patients with spontaneous epithelial defects \((P = 0.904)\). CB aesthesiometry was 3.24 ± 0.78 cm \((SD)\) in affected eyes and 3.26 ± 0.697 cm in the contralateral unaffected eye. The mean value obtained in 10 normal dogs was 3.7 ± 0.35 cm, which was not significantly different from values in patients with corneal epithelial defects.

![Figure 4. Zones of subepithelial and epithelial hyperinnervation were distributed in patchy, bandlike regions (shaded areas) approximately 0.5 to 2.0 mm peripheral to the ulcer margin (dashed lines). Occasionally, zones of increased nerve density extended centrally as far as the margin of the ulcer.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933590/ on 11/14/2018)
FIGURE 5. SP- and CGRP-IR subepithelial nerve fibers in 30-μm-thick sections from a control cornea (a, c) and from a cornea with SCCED (b, d). The area of the subepithelial nerve plexus surrounding the epithelial lip (dashed lines) in the SCCED specimen comprised a dense network of morphologically abnormal CGRP- and SP-IR nerve fibers. In contrast, the subepithelial stroma located beneath the epithelial defect (*) and extending for approximately 1 mm peripheral to the epithelial lip contained few nerve fibers.
keratectomy. Eyes in all dogs healed with subsequent therapies.

**Superficial Keratectomy**

Superficial keratectomy was performed in eyes of 18 dogs. In eight dogs, surgery was performed after their eyes had not healed with topical SP, with or without IGF-1. In the remaining 10, surgery was elected by the owners as the primary treatment for their pets.

**Immunohistochemistry for SP, CGRP, VIP, and TH**

Immunohistochemical analyses of the superficial keratectomy specimens from animals with SCCED revealed multiple abnormalities in corneal nerve density, distribution, and morphology, compared with control corneas. The most prominent nerve abnormality consisted of circumscribed areas of hyperinnervation in the subepithelial stroma and epithelium surrounding the epithelial lip (Fig. 4). Within the hyperinnervation zone, the numbers of subepithelial PGP-9.5-, CGRP, and SP-immunoreactive (IR) nerves were greatly increased over control levels, and the normal pattern of superficial stromal nerve architecture was seriously disrupted (Fig. 5). Dichotomously branching stromal axons comparable to those in eyes of control dogs were present; however, they were embedded in a dense, tangled meshwork of morphologically abnormal nerve fibers (Figs. 5c, 5d). The latter axons were randomly oriented and extremely tortuous and demonstrated a variety of atypical morphologies, including prominent axonal blebbing (Figs. 6a–c).

The corneal epithelium overlying the abnormal subepithelial stromal plexus was also hyperinnervated and contained densely packed aggregates of morphologically altered nerve terminals (Fig. 7). The terminals were concentrated in the basal epithelial cell layer and appeared as disorganized clusters of bulbous, club-shaped endings. The intraepithelial endings stained intensely with antiserum against PGP-9.5, but more faintly and inconsistently with antisera against CGRP and SP.

In marked contrast to the prominent zone of hyperinnervation surrounding the epithelial lip, the subepithelial stroma forming the floor of the epithelial defect contained few nerve fibers (Figs. 5c, 5d). Some of the fibers beneath the denuded zone were fragmented and appeared to be degenerating (Fig. 8). Nerve fibers in all other areas of the canine cornea (peripheral cornea, limbus, and corneal stroma deeper than 100 μm beneath the floor of the defect) were of normal number and morphology.

The extent and severity of the nerve abnormalities observed varied moderately from specimen to specimen; however, no positive correlations were noted between the magnitude of nerve alterations and specific clinical features of the disease, such as duration of the ulcer, percentage of corneal area occupied by the ulcer, and the presence or absence of localized neovascularization.

The densities and morphologic appearances of corneal and limbal TH- and VIP-IR nerves were unchanged from control specimens. Corneal nerve architecture in nondiseased animals subjected to repeated mechanical scraping of the central corneal epithelium was of normal appearance and showed no evidence of abnormal nerve sprouting.

**Levels of SP in Corneal Epithelial Cell Samples and Tears**

Sufficient corneal epithelial cell tissue was collected to permit the determination of SP levels in two pooled (n = 7, n = 6) samples obtained from normal dogs and in two pooled samples (n = 6 in both) from the corneas of dogs with
spontaneous chronic corneal epithelial defects. The amount of SP in the two normal samples was 1219 and 1052 pg SP/mg protein (average, 1136 pg SP/mg protein). In contrast, the amount of the peptide in corneal epithelial lip samples from canine patients with spontaneous chronic epithelial defects was 3487 and 4181 pg SP/mg protein (average, 3834 pg SP/mg protein). Thus, the level of SP in the corneal epithelial lip of the affected eyes of canine patients was 3.3 times that found in comparable corneas from normal dogs. In tears pooled from normal dogs, the level of SP was 108.1 ± 20.1 pg/mg protein (SEM; n = 6). The level of the peptide found in tears from eyes with spontaneous corneal defects was not significantly different from that in normal eyes (114.1 ± 13.3 pg SP/mg protein; n = 10, P = 0.2). Similarly, the level of tear SP found in the normal eyes of canine patients was not significantly different from that in either normal control eyes or eyes with spontaneous epithelial defects (97.1 ± 33.7 pg/mg protein; n = 7; P = 0.3).

FIGURE 7. PGP-9.5-IR nerve fibers in the epithelium and anterior stroma of a healthy control cornea (a) and of a cornea with a recurrent epithelial erosion (b). The area illustrated in each panel is indicated by the box in the inset diagram. Dashed line represents the epithelium-stroma interface. The corneal epithelium located between the arrows (b) was hyperinnervated.

FIGURE 8. CGRP-IR subepithelial nerve fibers (arrows) in the central stroma beneath a recurrent epithelial erosion. The axons were heavily segmented and appeared to be degenerating.
The clinical findings verify and extend information contained in previous reports concerning spontaneous chronic corneal epithelial defects in dogs. The average canine patient is middle aged to elderly, and no clear sex predilection is evident. Several breeds appear to be predisposed to the development of this disorder with boxers, golden retrievers, and keeshonds being overrepresented in our patient population. No predisposing factor could be identified for the development or persistence of the epithelial defect. Tear production is increased in affected eyes. Similar to human patients with recurrent epithelial erosions, a history of trauma is sometimes identified as an initiating event, although usually an inciting event could not be identified. The typical location of the defect is in the interpalpebral space. The defect is equally distributed between axial–paraxial and peripheral locations. The interpalpebral space is subject to the most stress, which probably plays a contributing role to the persistence of the defect.

Vascularization occurred in 56% of the patients. Most (80%) of the defects that became vascularized were located peripherally. The correlation of an increased vascular response with increased proximity to the limbus suggests that the elaboration of vasculogenic factors from peripheral wound beds are more likely to reach the perilimbal vasculature at a critical concentration that stimulates neovascularization. Although SP and CGRP have been reported to promote proliferation of vascular endothelial cells, corneal SP and CGRP content in this study is not clearly associated with the presence of vascularization, in that the profile of expression is similar in patients, regardless of vascularization status. In future studies, it would be of interest to examine other factors from vascularized and nonvascularized corneas that are known to promote vascularization in the cornea, such as VEGF and FGF.

Corneal sensation was typically normal in affected corneas. Our findings in CB aesthesiometry (3.7 cm) in normal dogs differs from that in a previous report in dogs and also from that in humans. In a previous report by Barrett et al. a mean CB value of 1.55 cm was reported for normal dogs. The reasons for the increase in corneal sensitivity recorded in our study are not readily apparent. We think it likely that confounding variables associated with restraint and performance of CB aesthesiometry are more apt to result in an artificial lowering in the recorded sensitivity than in an increase. Normal humans are reported to have a CB value of more than 5 cm, suggesting that the human cornea is inherently more sensitive than the canine cornea. Corneal sensation was determined for the central cornea, typically in an epithelialized region adjacent to the defect. This region correlates to the area in which an increase in SP-containing nerve fibers was identified. This finding suggests that the increase in SP-containing fibers is not associated with an increase in corneal sensitivity. Sensation in the region of bared stroma was not determined.

This disease entity in dogs has features in common with both neurotrophic ulcers and recurrent epithelial erosions in humans. Some of the clinical findings in dogs (paracentral location, geographic outline, presence of an epithelial lip) and general chronicity, rather than recurrence, are features shared with neurotrophic ulcers in humans. The presence of normal corneal sensation in all the affected canine patients, however, demonstrates this disease entity in dogs to be distinct from typical cases of neurotrophic or neuroanesthetic keratitis in humans. In humans these keratitides are typically associated with a hypoesthetic or anesthetic cornea that can occur subsequent to neural damage associated with herpes zoster and with damage to the sensory division of cranial nerve V associated with surgical procedures or direct trauma. The normal corneal sensation and idiopathic nature of this spontaneous canine disease, more closely approximates the clinical picture observed in the majority of recurrent epithelial erosions in humans.

The presence of SP in the tear film has been reported in humans and guinea pigs and increases in cases of allergic and viral keratoconjunctivitis. Data we have generated document SP and CGRP to be heavily invested in the cornea and lacrimal tissue of the normal dog (Marfurt et al., unpublished observations, 2001) and SP to be present in canine tears. SP quantification in epithelial cells and tears of dogs was determined to elucidate the physiologic sources of SP available to the cornea. The increase of SP in epithelial cells appears to be correlated with the dense abnormal plexus of SP-IR fibers around the periphery of the erosion. The absence of alteration of SP levels in tears suggests that epithelial nerves are not a major source of SP in tears and that lacrimal secretion of SP is not affected. A limitation of the methods used for quantification of SP in tears is the lack of accurate data on the volume of tears collected from each animal. Because the SP levels remained unchanged relative to the overall protein content even though an increased level of tearing was documented, the total exposure of the surface to SP would be increased. A possible confounding variable is that the tear total protein content could be modulated with the presence of an epithelial defect. Tear protein content has been shown, however, to be remarkably stable over a large range of tear flow rates.

The results of this study have demonstrated significant alterations in corneal innervation of animals with chronic recurrent epithelial erosions. The tangle, disorganized zones of subepithelial and epithelial hyperinnervation surrounding the epithelial lip stand in marked contrast to the more regular nerve patterns observed in the peripheral cornea of these specimens and in healthy corneas of control dogs. It is of interest to note that hyperinnervation and corneal nerve abnormalities are also seen in human patients with band keratopathy, bullous keratopathy, opaque corneal grafts, herpetic keratitis, and advanced age and after ocular inflammation or trauma. Similarly, wound-associated stromal nerve sprouts develop in rabbit and cat corneas after excimer laser photokeratectomy, cryodamage, perilimbal incision, and penetrating autografts.

The signal responsible for initiating the nerve-sprouting response seen in the current study is unknown. Physical loss of corneal epithelial cells alone is apparently not an adequate stimulus, because repeated mechanical scraping of the corneal epithelium in control canine eyes failed to induce a sprouting response. Alternatively, it is tempting to speculate that neuronotrophic factors released from dying, regenerating, or metabolically compromised epithelial cells surrounding the wound margin may play a role. Corneal epithelial cells produce neuronotrophic factors that stimulate neurite formation and after corneal injury in rabbits, wound-oriented neurites arrange themselves perpendicular to the wound margin as if elongating in response to a chemotactic gradient. Data from the present study suggest that neuronotrophic factors are released preferentially by epithelial cells surrounding the wound margin, because nerve sprouting is restricted to this location.

Of interest, the nerve sprouting in the present study occurred preferentially in CGRP- and SP-IR nerves. In contrast, corneal TH-IR nerves failed to elaborate sprouts, suggesting that chemically defined subpopulations of canine corneal axons may respond differently to epithelium-derived neuronotrophic signals. The physiologic implications, if any, of the corneal nerve-sprouting response seen in the present study remain to be determined. Nerve sprouting may represent an attempt by the peripheral nervous system to deliver increased quantities of neuropeptides and other trophic factors to the wound site and thereby promote wound healing. Work in other animal models of wound healing has shown that CGRP-IR nerve sprouting is a reversible process and that after several weeks of progressive
healing. CGRP-IR nerve density gradually returns to normal levels. It would be of interest to learn whether resolution of the corneal epithelial defects in the canine patients examined in this study was accompanied by a restoration of normal nerve density and architectural pattern.

SP, with or without IGF-1, was effective as a topical therapeutic agent in 70% to 75% of the dogs treated. SP has been shown to have a variety of actions, both alone and in concert with other cytoactive compounds, that promote wound healing. A mitogenic effect for SP has been documented in a variety of cell types. SP has been documented to stimulate DNA synthesis in ocular cell lines at picomolar concentrations and to act synergistically with insulin. Although SP has been effective in vitro at picomolar concentrations, a number of groups have reported the need for micromolar concentrations in vivo for an optimal biological response. This is probably due to a combination of factors, including the rapid dilution and washout of applied medications and the rapid breakdown of SP by endogenous proteases. In vitro, SP has been shown to synergize with IGF-1 in stimulating proliferation of corneal epithelial cells although synergism for proliferation was not observed in a rabbit corneal block model. SP has also been shown to stimulate DNA synthesis in a variety of nonocular cells.

A child with Riley-Day syndrome has been reported in whom persistent corneal epithelial defects developed that were successfully treated with a combination of SP and IGF-1, and topical SP and IGF-1 was used in the successful treatment of two human patients with chronic corneal epithelial defects. Finally, in our study, 70% to 75% of dogs with chronic corneal epithelial defects had complete resolution when treated with topical SP, with or without IGF-1. Taken together, these data clearly point to an important role for SP in the pathogenesis of chronic or recurrent corneal epithelial defects.

References


