

# Efficacy of two chondroitin sulfate ophthalmic solutions in the therapy of spontaneous chronic corneal epithelial defects and ulcerative keratitis associated with bullous keratopathy in dogs

Eric C. Ledbetter,\* Robert J. Munger,† Rachel D. Ring† and Janet M. Scarlett‡

\*Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853-6401, USA; †Animal Ophthalmology Clinic, 4444 Trinity Mills Rd., Ste. 201, Dallas, TX 75287, USA; ‡Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853-6401, USA

Address communications to:

Eric C. Ledbetter

Tel.: 607-253-3060

Fax: 607-253-3788

e-mail: ecl32@cornell.edu

## Abstract

**Objective** To determine the efficacy of two antimicrobial-chondroitin sulfate ophthalmic solutions in the therapy of spontaneous chronic corneal epithelial defects (SCCED) and ulcerative keratitis associated with bullous keratopathy in dogs.

**Animals Studied** Eighty dogs with SCCED and 14 dogs with ulcerative keratitis associated with bullous keratopathy.

**Procedure** Following manual debridement of nonadherent epithelium, dogs were treated topically with a chondroitin sulfate ophthalmic solution containing either tobramycin or ciprofloxacin. Patients were re-evaluated at 2-week intervals for 4 weeks.

**Results** After 2 weeks of treatment, 53.6% of eyes with SCCED and 17.6% of eyes with ulcerative keratitis associated with bullous keratopathy had healed. After 4 weeks of treatment, 81.0% of eyes with SCCED and 23.5% of eyes with ulcerative keratitis associated with bullous keratopathy had healed. There were no statistically significant differences in healing percentages between the tobramycin-chondroitin sulfate treatment groups and the ciprofloxacin-chondroitin sulfate treatment groups. Two dogs with SCCED, one treated with the tobramycin-chondroitin sulfate solution and the other treated with the ciprofloxacin-chondroitin sulfate solution, developed sterile corneal stromal abscesses during the study.

**Conclusions** Topical therapy with an antimicrobial-chondroitin sulfate ophthalmic solution combined with manual debridement of nonadherent epithelium compares favorably with other published medical and surgical therapies for SCCED; however, these compounds are only equivocally more effective than therapy with manual debridement alone. These solutions appear to be ineffective in the treatment of ulcerative keratitis associated with bullous keratopathy.

The significance of the two cases of corneal stromal abscessation is unknown at this time and warrants further investigation.

**Key Words:** bullous keratopathy, chondroitin sulfate, cornea, dog, spontaneous chronic corneal epithelial defect

## INTRODUCTION

Superficial corneal ulcers in dogs that fail to heal within a normal time period and are refractory to conventional therapy are a common clinical entity encountered in veterinary practice. Numerous etiologies can result in refractory corneal ulcers including morphologic and neurologic abnormalities of the eyelids, aberrant eyelashes or facial hair,

quantitative or qualitative tear film abnormalities, deficiencies of corneal innervation, foreign bodies, and microbial infection. Spontaneous chronic corneal epithelial defects (SCCED) and ulcerative keratitis associated with bullous keratopathy are two additional categories of chronic corneal ulcers.

Spontaneous chronic corneal epithelial defects are superficial epithelial defects that have no apparent inciting cause, do not involve the corneal stroma, are bordered or partially covered

with nonadherent epithelium, and fail to heal in a normal time period (i.e., 1 week).<sup>1,2</sup> Numerous terms have been used to describe this clinical entity, including recurrent corneal erosion, persistent corneal erosion, refractory epithelial erosion, indolent ulcer, Boxer ulcer, and rodent ulcer. The underlying pathologic mechanisms that result in the occurrence and persistence of these defects have not been fully elucidated; however, histopathologic and immunohistochemical evaluations of affected corneas have revealed numerous abnormalities. Poorly adherent epithelium and epithelial dysmaturation at the periphery of lesions with varying degrees of leukocyte infiltration are present.<sup>3,4</sup> The basement membrane is typically absent or present in discontinuous segments within the lesion.<sup>4</sup> A hyaline acellular zone in the anterior corneal stroma is commonly present in the area of the erosion.<sup>4</sup> Stromal fibroplasia, vascularization, and leukocyte infiltration have been observed in some specimens.<sup>5</sup> Disorganized zones of subepithelial and epithelial hyperinnervation surround the epithelial defect.<sup>6</sup> Matrix metalloproteinase activity is elevated in affected corneas and epithelial-mesenchymal transition, the process in which anchored epithelial cells transform into migrating fibroblast-like cells to re-epithelialize corneal epithelial defects, is abnormal.<sup>7,8</sup>

Bullous keratopathy occurs as a sequela to severe or chronic corneal edema. Endothelial cell dysfunction (either degeneration or dystrophy) and the resultant corneal edema can lead to the formation of intra-epithelial or subepithelial bullae.<sup>3</sup> These fluid-filled pockets predispose to corneal ulceration by structural weakening of the cornea or the bullae may spontaneously rupture, resulting in the formation of superficial corneal ulcers.<sup>9</sup> These corneal ulcers tend to follow a prolonged healing course and recurrence is common.<sup>3</sup>

The purpose of this study was to determine the clinical efficacy of chondroitin sulfate ophthalmic solutions in the therapy of SCCED and ulcerative keratitis associated with bullous keratopathy. Two ophthalmic solutions containing chondroitin sulfate and an antimicrobial (either tobramycin or ciprofloxacin) were evaluated. These solutions were utilized in conjunction with manual debridement of nonadherent corneal epithelium.

## MATERIALS AND METHODS

### *Case selection*

All dogs diagnosed with SCCED or ulcerative keratitis associated with bullous keratopathy were considered eligible for the study. The diagnosis of SCCED was based on the presence of a superficial epithelial defect that retained fluorescein, nonadherent epithelium adjacent to the lesion periphery, no stromal involvement, and the lack of an identifiable inciting or perpetuating cause (Fig. 1). Additionally, all lesions had been present for  $\geq 10$  days as determined from referring veterinarian records or the observation of compatible clinical signs (e.g., blepharospasm, epiphora, and/or conjunctival hyperemia) in the affected eye by the

client. The duration lesions had been present was determined independently from the duration of treatment prior to referral. Corneal erosions associated with moderate or severe corneal edema were excluded from the SCCED group. The diagnosis of ulcerative keratitis associated with bullous keratopathy was based on the presence of one or more superficial epithelial defects that retained fluorescein, no or minimal stromal involvement by the ulcer, and the identification of corneal bullae and diffuse corneal edema with biomicroscopy (Fig. 2). Corneal lesions from either disease group with identified or suspected microbial infection were excluded from the study. Eyes from either disease group with a Schirmer tear test value  $< 15$  mm/min at initial or recheck examinations were excluded or removed from the study.

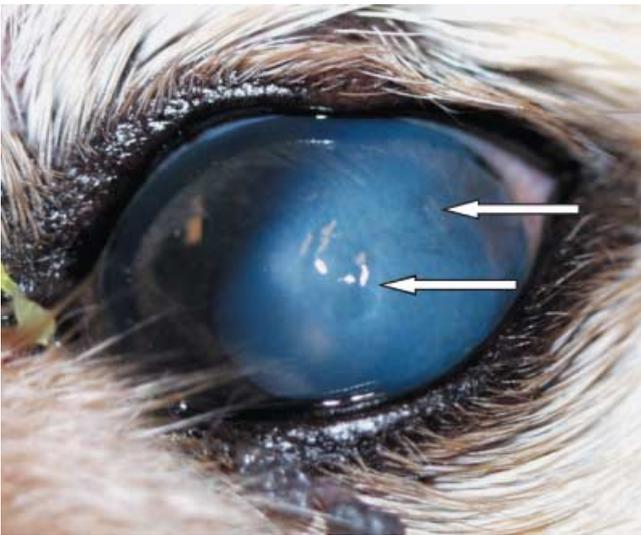
All dogs received a complete ophthalmic examination prior to enrollment in the study, including slit-lamp biomicroscopy, indirect ophthalmoscopy, Schirmer tear testing, applanation tonometry, and fluorescein staining. Participation in the study was voluntary, and informed consent was obtained from all clients prior to the initiation of treatment. Greater than 95% of dogs presented during the study period with SCCED or ulcerative keratitis associated with bullous keratopathy were included in the study. The dogs that were not included in the study as a result of client preference had no identifiable characteristics that would have influenced the outcome of the study.

### *Treatment and follow-up*

Affected eyes were topically anesthetized with one drop of 0.5% proparacaine hydrochloride ophthalmic solution (Allergan, Irvine, CA, USA) 2 min prior to manipulation. Nonadherent corneal epithelium was removed manually with sterile, cotton-tipped swabs. All patients were discharged with one of two products administered topically (one drop, three to four times daily in the affected eye) chosen randomly for each patient: 100 mg/mL chondroitin sulfate and 3 mg/mL tobramycin ophthalmic solution (Tobramax<sup>®</sup>; Labyes S.A., Buenos Aires, Argentina), or 200 mg/mL chondroitin sulfate and 3 mg/mL ciprofloxacin ophthalmic solution (Ciprovet<sup>®</sup>; Labyes S.A., Buenos Aires, Argentina). These two solutions, which differed in chondroitin sulfate concentration and antimicrobial content, were chosen because they were obtainable as commercially manufactured products. Both solutions contained 0.1 mg/mL benzalkonium chloride as a preservative. All dogs enrolled in the study were fitted with Elizabethan collars and clients were instructed to leave the collars on at all times. The clinicians involved in the study were not blinded to the treatment administered following the initial evaluation. A disparity in the numbers of dogs assigned to the tobramycin-chondroitin sulfate solution treatment groups and the ciprofloxacin-chondroitin sulfate solution treatment groups within each disease category resulted from an unequal quantity of each medication received from the pharmaceutical manufacturer. Patients were re-evaluated at 2-week intervals ( $\pm 3$  days) from the start of treatment for a total of 4 weeks.



**Figure 1.** Spontaneous chronic corneal epithelial defect in an 8-year-old, female, spayed Boxer: a superficial erosion is present in the central cornea with fluorescein dye retention, loose epithelial margins, and superficial corneal vascularization.



**Figure 2.** Bullous keratopathy in a 12-year-old, female, spayed mixed-breed dog: corneal edema with two subepithelial bullae (arrows) present in the central and paracentral cornea.

Manual debridement of nonadherent corneal epithelium was not repeated at recheck examinations. To determine treatment efficacy and to monitor for adverse effects, each recheck examination included a complete ophthalmic examination (i.e., slit-lamp biomicroscopy, indirect ophthalmoscopy, Schirmer tear testing, applanation tonometry, and fluorescein staining) and a detailed history was obtained from the client.

Lesions were considered healed when the cornea did not retain fluorescein, the epithelium appeared firmly attached with no folds or breaks evident by biomicroscopy, and

there was no recurrence of the lesion for the subsequent 4 weeks. Cotton-tipped swabs were used to confirm corneal healing by gently brushing the epithelium of all questionably healed cases at the final recheck. Since a control group of dogs treated with debridement alone was not included in the study design, efficacy of the evaluated solutions was determined by comparison to published success rates for healing of these lesions with debridement as the sole therapy.

#### *Statistical analysis*

To determine the similarity of the treatment groups (tobramycin-chondroitin sulfate solution-treated dogs vs. ciprofloxacin-chondroitin sulfate solution-treated dogs) within the two disease categories (SCCED and bullous keratopathy), categorical variables (i.e., gender, neuter status, breed, the presence of other systemic diseases that could affect healing processes, and previous topical corticosteroid treatment) were compared between treatment groups (or between categorical variables) using the chi-square test of independence or Fisher's exact test (where at least one cell value was  $< 5$ ). Continuous variables (i.e., age and length of treatment prior to referral) were compared between groups using the Wilcoxon rank sum test.  $P$  values of  $\leq 0.05$  were considered statistically significant. Length of treatment prior to referral was statistically evaluated because it could be precisely determined from referring veterinarian records, as opposed to the duration lesions had been present, which relied on client observations for some cases.

To determine if differences in gender, breed, age, length of treatment prior to referral, the presence of other systemic diseases that could affect healing processes, or previous topical corticosteroid treatment were present between dogs with lesions that healed vs. dogs with lesions that did not heal the chi-square test of independence or the Fisher's exact test were used. Similarly, the proportions of eyes healing between treatment groups were compared using the chi-square test of independence.  $P$  values of  $\leq 0.05$  were considered statistically significant.

## RESULTS

### *Comparison of treatment groups*

No statistical difference was present between the tobramycin-chondroitin sulfate solution treatment group and the ciprofloxacin-chondroitin sulfate solution treatment group within the SCCED and bullous keratopathy categories for the frequency of the following variables: gender, neuter status, age, breed, the presence of other systemic diseases that could alter healing processes, or previous topical corticosteroid treatment (Tables 1 and 2). Despite randomization, the length of treatment prior to referral was significantly longer for the ciprofloxacin-chondroitin sulfate solution-treated dogs than the tobramycin-chondroitin sulfate solution-treated dogs within the SCCED disease category ( $P = 0.02$ ), but no difference in the length of treatment prior to referral was present for dogs within the bullous keratopathy group.

**Table 1.** Characteristics of dogs participating in the spontaneous chronic corneal epithelial defect treatment groups

| Characteristic            | Tobramycin-chondroitin sulfate solution treatment group (n = 52) |          | Ciprofloxacin-chondroitin sulfate solution treatment group (n = 28) |          | P values |
|---------------------------|--|----------|---|----------|----------|
|                           | No.  | %        | No.   | %        |          |
| Gender                    |  |          |   |          |          |
| Male                      | 21   | 40.4     | 11  | 39.2     | 0.92     |
| Female                    | 31   | 59.6     | 17  | 60.7     |          |
| Neuter status             |  |          |   |          |          |
| Neutered                  | 40   | 76.9     | 24  | 85.7     | 0.35     |
| Intact                    | 12   | 23.1     | 4   | 14.2     |          |
| Breed                     |  |          |   |          |          |
| Boxer                     | 17   | 32.7     | 3   | 10.7     | 0.09     |
| Other purebreds           | 27   | 51.9     | 20  | 71.4     |          |
| Mixed                     | 8  | 15.4     | 5   | 17.9     |          |
| Other systemic disease    |  |          |   |          |          |
| No                        | 42   | 80.8     | 25  | 89.3     | 0.32     |
| Yes                       | 10   | 19.2     | 3   | 10.7     |          |
| Topical corticosteroid    |  |          |   |          |          |
| No                        | 43   | 82.7     | 26  | 92.9     | 0.31     |
| Yes                       | 9  | 17.3     | 2   | 7.1      |          |
|                           | Median   | Range    | Median  | Range    |          |
| Age (years)               | 8.9  | 2.3–15.6 | 9.0   | 3.5–14.8 | 0.53     |
| Previous treatment (days) | 19   | 1–90     | 30  | 1–120    | 0.02     |

**Table 2.** Characteristics of dogs participating in the ulcerative keratitis associated with bullous keratopathy treatment groups

| Characteristic            | Tobramycin-chondroitin sulfate solution treatment group (n = 8) |           | Ciprofloxacin-chondroitin sulfate solution treatment group (n = 6) |          | P values |
|---------------------------|---|-----------|--|----------|----------|
|                           | No.   | %         | No.  | %        |          |
| Gender                    |   |           |  |          |          |
| Male                      | 3   | 37.5      | 3  | 50.0     | 1.00     |
| Female                    | 5   | 62.5      | 3  | 50.0     |          |
| Neuter status             |   |           |  |          |          |
| Neutered                  | 7   | 87.5      | 6  | 100      | 1.00     |
| Intact                    | 1   | 12.5      | 0  | 0.0      |          |
| Breed                     |   |           |  |          |          |
| Boxer                     | 7   | 87.5      | 3  | 50.0     | 0.24     |
| Other purebreds           | 1   | 12.5      | 3  | 50.0     |          |
| Mixed                     | 0   | 0.0       | 0  | 0.0      |          |
| Other systemic disease    |   |           |  |          |          |
| No                        | 7   | 87.5      | 4  | 66.7     | 0.33     |
| Yes                       | 1   | 12.5      | 2  | 33.3     |          |
| Topical corticosteroid    |   |           |  |          |          |
| No                        | 7   | 87.5      | 6  | 100      | 1.00     |
| Yes                       | 1   | 12.5      | 0  | 0.0      |          |
|                           | Median  | Range     | Median   | Range    |          |
| Age (years)               | 12.1  | 11.5–15.8 | 11.2   | 7.6–14.8 | 0.09     |
| Previous treatment (days) | 30  | 1–730     | 82   | 5–120    | 0.56     |

### *Spontaneous chronic corneal epithelial defects*

Ninety-six eyes from 92 dogs with SCCED were enrolled in the study. Of these, 84 eyes from 80 dogs were included in the final results. The 12 dogs that did not complete the study protocol and were not included in the final results included six dogs which were not returned for the 2-week recheck (four dogs from the tobramycin-chondroitin sulfate solution treatment group and two dogs from the ciprofloxacin-chondroitin sulfate solution treatment group), two dogs

that were not returned for the 4-week recheck (both dogs from the ciprofloxacin-chondroitin sulfate solution treatment group), and four clients that elected for early removal from the study (all four dogs from the tobramycin-chondroitin sulfate solution treatment group). Lesions were present unilaterally in 76 dogs and bilaterally in four dogs. Fifty-four eyes were treated with the tobramycin-chondroitin sulfate solution and 30 eyes were treated with the ciprofloxacin-chondroitin sulfate solution.

**Table 3.** Treatment outcomes for dogs with spontaneous chronic corneal epithelial defects: 84 corneal lesions from 80 dogs treated with antimicrobial-chondroitin sulfate ophthalmic solutions

| Treatment                                  | Total number of eyes treated | Eyes healed at 2 weeks (%) | Eyes healed at 4 weeks (%) |
|--|------------------------------|----------------------------|----------------------------|
| Tobramycin-chondroitin sulfate solution    | 54                           | 30 (55.6)                  | 44 (81.5)                  |
| Ciprofloxacin-chondroitin sulfate solution | 30                           | 15 (50.0)                  | 24 (80.0)                  |
| Total                                      | 84                           | 45 (53.6)                  | 68 (81.0)                  |

The median age of treated dogs was 9.0 years (range: 2.3–14.8 years) with 41 spayed females, 23 castrated males, 9 intact males, and 7 intact females (Table 1). Twenty-six breeds were represented with the most common being Boxer ( $n = 20$ ), mixed breed ( $n = 13$ ), Labrador Retriever ( $n = 5$ ), Cocker Spaniel ( $n = 5$ ), West Highland Terrier ( $n = 4$ ), and Shih Tzu ( $n = 4$ ). Systemic diseases present in the dogs which could potentially alter healing processes, based on historical information gathered from the owner or referring veterinarian, included hypothyroidism ( $n = 8$  within the tobramycin-chondroitin sulfate treatment group and  $n = 2$  within the ciprofloxacin-chondroitin sulfate treatment group), diabetes mellitus ( $n = 1$  within the tobramycin-chondroitin sulfate treatment group and  $n = 1$  within the ciprofloxacin-chondroitin sulfate treatment group), and hyperadrenocorticism ( $n = 1$  within the tobramycin-chondroitin sulfate treatment group).

The corneal lesions had been treated by referring veterinarians for a median of 21 days (range: 1–120 days) prior to enrolment in the study. Medical and surgical treatments administered specifically for the lesions prior to referral included topical antimicrobials ( $n = 57$ ), oral antimicrobials ( $n = 12$ ), topical atropine ( $n = 20$ ), topical corticosteroids ( $n = 11$ ), oral corticosteroids ( $n = 10$ ), oral nonsteroidal anti-inflammatory medications ( $n = 13$ ), artificial tear solutions or ointments ( $n = 5$ ), topical acetylcysteine ( $n = 3$ ), contact lens ( $n = 1$ ), multiple punctate keratotomy ( $n = 1$ ), grid keratotomy ( $n = 1$ ), nictitating membrane flap ( $n = 3$ ), and manual debridement ( $n = 7$ ). Of the 84 eyes included in the study, 16 had received no prior treatment.

After 2 weeks of treatment, 53.6% (30 of 54 eyes treated with the tobramycin-chondroitin sulfate solution and 15 of 30 eyes treated with the ciprofloxacin-chondroitin sulfate solution) of lesions had healed (Table 3). After 4 weeks of treatment, 81.0% (44 of 54 eyes treated with the tobramycin-chondroitin sulfate solution and 24 of 30 eyes treated with the ciprofloxacin-chondroitin sulfate solution) of lesions had healed (Figs 3 and 4). Because 8 dogs and 4 dogs (each with one treated eye) were lost to follow-up by the final 4-week evaluation in the tobramycin-chondroitin sulfate and ciprofloxacin-chondroitin sulfate solution groups, respectively, the best and worst possible response rates were also calculated for each solution. If the affected eye of all eight dogs that were lost had failed to heal, the efficacy of the tobramycin-chondroitin sulfate solution would have been 44/62 (71.0%; 95% confidence interval [CI] 58–82%). If all eight dogs had eyes that healed, the proportion healed would have been 52/62 (83.9%; 95% CI 72–92%). Similarly, if the four dogs lost to follow-up in the ciprofloxacin-chondroitin

sulfate solution group had eyes that failed to heal, the efficacy would have been 24/34 (70.6%; 95% CI 52–86%), and if all four eyes had healed, the proportion healed would have been 28/34 (82.4%; 95% CI 64–94%). No significant difference in healing percentages was present between the two treatment groups (i.e., tobramycin-chondroitin sulfate solution vs. ciprofloxacin-chondroitin sulfate solution) using the observed healing estimates ( $P = 0.87$ ). Additionally, when the worst-case healing estimate for each group was compared to the best-case estimate in the other group, there were still no significant differences ( $P > 0.12$ ) between treatments. There were also no statistically significant differences in gender, breed, age, length of treatment prior to referral, the presence of other systemic diseases that could affect healing processes, or previous topical corticosteroid treatment between dogs with lesions that healed vs. dogs with lesions that did not heal.

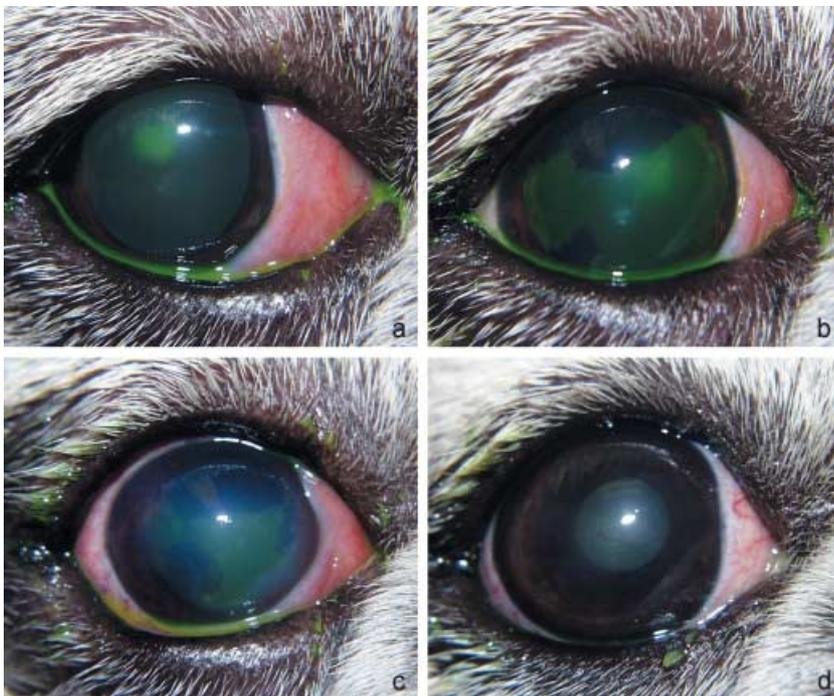
The 16 lesions that failed to heal after 4 weeks of therapy were treated with a soft contact lens and either a multiple punctate keratotomy ( $n = 14$ ) or a grid keratotomy ( $n = 2$ ). Topical therapy with the chondroitin sulfate ophthalmic solutions was continued after the keratotomy procedures. Following this treatment, 100% (16/16) of lesions healed within 14 days.

#### *Ulcerative keratitis associated with bullous keratopathy*

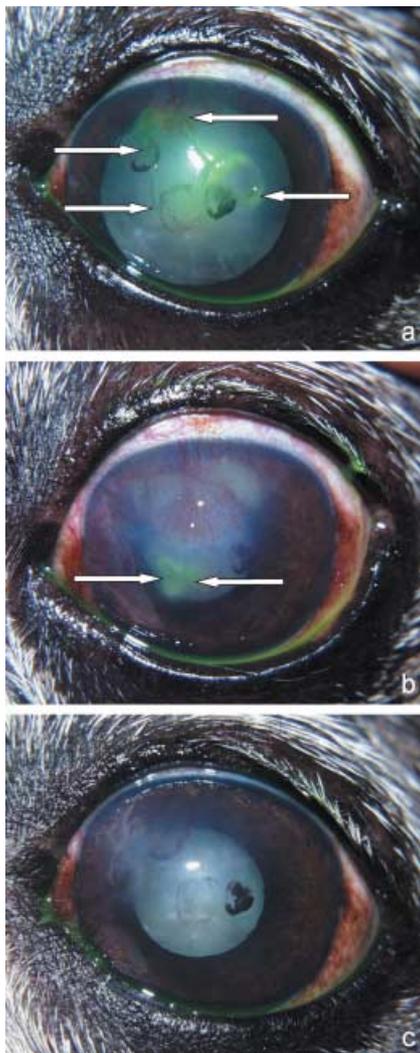
Seventeen eyes from 14 dogs with ulcerative keratitis associated with bullous keratopathy were enrolled in the study. All dogs enrolled met the study protocol and were included in the final results. Corneal lesions were present unilaterally in 11 dogs and bilaterally in three dogs. Eleven eyes were treated with the tobramycin-chondroitin sulfate solution and six eyes were treated with the ciprofloxacin-chondroitin sulfate solution.

The median age of treated dogs was 12.0 years (range: 7.6–15.8 years) with 7 spayed females, 6 castrated males, and 1 intact female (Table 2). Eleven breeds were represented with mixed breed ( $n = 3$ ) and Golden Retriever ( $n = 2$ ) being the only breeds with more than one individual included in the study. Systemic diseases present in the dogs that could potentially alter healing processes, based on historical information gathered from the owner or referring veterinarian, included hypothyroidism ( $n = 1$  within the tobramycin-chondroitin sulfate treatment group and  $n = 2$  within the ciprofloxacin-chondroitin sulfate treatment group), and hypoadrenocorticism ( $n = 1$  within the ciprofloxacin-chondroitin sulfate treatment group).

The corneal lesions had been treated by referring veterinarians for a median of 30 days (range: 1–730 days) prior to enrollment in the study. Treatments administered specifically for the ophthalmic disease prior to referral included



**Figure 3.** Spontaneous chronic corneal epithelial defect in a 7-year-old, intact female, English Bulldog: (a) Fluorescein dye-stained eye prior to debridement of nonadherent epithelium. (b) Eye immediately postdebridement of nonadherent epithelium with a dramatic enlargement of the area of fluorescein dye retention. (c) Eye following 2 weeks of treatment with a tobramycin-chondroitin sulfate ophthalmic solution. A decrease in the area of fluorescein dye retention is evident. (d) Healed corneal epithelial defect, with mild superficial corneal vascularization and scarring, after 4 weeks of treatment with a tobramycin-chondroitin sulfate ophthalmic solution.



**Figure 5.** Superficial corneal stromal abscess (arrow) in a 7-year-old, female, spayed Australian Shepherd following 2 weeks of treatment with a ciprofloxacin-chondroitin sulfate ophthalmic solution for a spontaneous chronic corneal epithelial defect.

**Figure 4.** Spontaneous chronic corneal epithelial defect in a 13-year-old, female, spayed Boston Terrier: (a) Fluorescein dye-stained eye prior to debridement of nonadherent epithelium, with the epithelial borders marked with arrows (endothelial pigmentation unrelated to the erosion is present). (b) Eye following 2 weeks of treatment with a ciprofloxacin-chondroitin sulfate ophthalmic solution, with the epithelial borders marked with arrows. A decrease in the area of fluorescein dye retention is evident. (c) Healed corneal epithelial defect, with mild superficial corneal vascularization and scarring, after 4 weeks of treatment with a ciprofloxacin-chondroitin sulfate ophthalmic solution.

**Table 4.** Treatment outcomes for dogs with ulcerative keratitis associated with bullous keratopathy: 17 corneal lesions from 14 dogs treated with antimicrobial-chondroitin sulfate ophthalmic solutions

| Treatment                                  | Total number of eyes treated | Eyes healed at 2 weeks (%) | Eyes healed at 4 weeks (%) |
|--|------------------------------|----------------------------|----------------------------|
| Tobramycin-chondroitin sulfate solution    | 11                           | 2 (18.2)                   | 2 (18.2)                   |
| Ciprofloxacin-chondroitin sulfate solution | 6                            | 1 (16.7)                   | 2 (33.3)                   |
| Total                                      | 17                           | 3 (17.6)                   | 4 (23.5)                   |

topical antimicrobials ( $n = 12$ ), oral antimicrobials ( $n = 2$ ), topical atropine ( $n = 8$ ), topical corticosteroids ( $n = 1$ ), oral corticosteroids ( $n = 1$ ), oral nonsteroidal anti-inflammatory medications ( $n = 3$ ), sodium chloride ointment ( $n = 3$ ), and artificial tear solutions or ointments ( $n = 1$ ). Three eyes had received no prior treatment for the ophthalmic condition.

After 2 weeks of treatment, 17.6% (2 of 11 eyes treated with the tobramycin-chondroitin sulfate solution and 1 of 6 eyes treated with the ciprofloxacin-chondroitin sulfate solution) of lesions had healed (Table 4). After 4 weeks of treatment, 23.5% (2 of 11 eyes treated with the tobramycin-chondroitin sulfate solution and 2 of 6 eyes treated with the ciprofloxacin-chondroitin sulfate solution) of lesions had healed. No significant difference in healing percentages was present between the two treatment groups. No statistically significant differences in gender, breed, age, length of treatment prior to referral, the presence of other systemic diseases that could affect healing processes, or previous topical corticosteroid treatment were present between dogs with lesions that healed vs. dogs with lesions that did not heal.

Of the 13 lesions that failed to heal after 4 weeks of therapy, four were treated with thermokeratoplasty, two clients elected to continue the tobramycin-chondroitin sulfate solution therapy, and the remaining seven clients elected various other medical therapies. Topical therapy with the chondroitin sulfate ophthalmic solutions was continued for patients treated with thermokeratoplasty. Following thermokeratoplasty, 100% (4/4) of lesions healed within 21 days. The two eyes in which tobramycin-chondroitin sulfate solution therapy was continued past the 4-week study protocol healed after 7 and 8 weeks, respectively.

#### *Adverse effects*

Mild ocular irritation manifested as blepharospasm and attempted rubbing of the treated eye, was reported by approximately 10% of clients after administration of the ciprofloxacin-chondroitin sulfate solution. This irritation was typically transient and persisted for less than 5 min after administration. No ocular irritation was reported by clients administering the tobramycin-chondroitin sulfate ophthalmic solution. One dog with SCCED treated with the tobramycin-chondroitin sulfate solution and another dog with SCCED treated with the ciprofloxacin-chondroitin sulfate solution developed superficial corneal stromal abscesses after 4 and 2 weeks of treatment, respectively (Fig. 5). Cytological evaluation of corneal scrapings from both eyes revealed nondegenerate neutrophils with no organisms identified. Fungal and aerobic bacterial culture results of corneal scrapings from both eyes were negative. These apparently

sterile abscesses resolved after superficial debridement of the overlying epithelium, discontinuation of the chondroitin sulfate solutions, and treatment with 0.5% chloramphenicol ophthalmic solution (Allergan, Irvine, CA, USA). No dogs were removed from the study for developing reduced Schirmer tear test values (i.e.  $< 15$  mm/min) during recheck examinations.

#### DISCUSSION

The results of this study suggest that chondroitin sulfate ophthalmic solutions combined with manual debridement of nonadherent corneal epithelium may be beneficial in the therapy of SCCED but are not effective in the treatment of ulcerative keratitis associated with bullous keratopathy. There was no statistically significant difference in the percentage of SCCED or bullous keratopathy lesions that healed between the two chondroitin sulfate solutions evaluated. Ideally, solutions containing equal concentrations of chondroitin sulfate would have been compared; however, we intended to evaluate commercially manufactured solutions and no such products were available. The groups of dogs treated with the ciprofloxacin-chondroitin sulfate solution were statistically similar to the groups of dogs treated with the tobramycin-chondroitin sulfate solution for the evaluated variables within each disease category, with the exception of a longer treatment period prior to referral for the ciprofloxacin-chondroitin sulfate solution-treated dogs in the SCCED disease category. Despite this difference, healing of lesions within both disease categories was statistically independent of the length of treatment prior to referral.

This study relied on client compliance for the administration of the medications. Based on requests for medications to be refilled and client communications, we perceive that compliance was high; however, both clients compliance and effectiveness in medication administration may have been variable between cases. No client refused to have Elizabethan collars placed on their dogs or informed us of removing the collars; however, this is an additional variable that may have affected treatment outcomes. Corneal vascularization was present in approximately 50% of the eyes included in the study, with the majority graded as mild or moderate. Although it was not statistically evaluated, the presence and degree of corneal vascularization did not subjectively influence treatment outcome.

Although ophthalmic solutions containing both chondroitin sulfate and antimicrobials were utilized in this study, it is unlikely that either tobramycin or ciprofloxacin directly contributed to the healing of the lesions. To the contrary,

most topical antimicrobials inhibit epithelial migration and delay corneal healing.<sup>10</sup> *In vitro* models have determined that ciprofloxacin induces alterations of canine corneal epithelial cell morphology and slows cellular migration; and tobramycin induces acute corneal epithelial cell membrane damage.<sup>10,11</sup> Antimicrobials are used in these conditions to prevent the potentially devastating sequelae of bacterial infection, not to treat existing sepsis. The ideal antimicrobial to combine with chondroitin sulfate for the treatment of these conditions would result in minimal inhibition of corneal healing, provide broad-spectrum bacterial infection prophylaxis, and not contribute to bacterial antimicrobial resistance of drugs normally reserved for severe infections. Ciprofloxacin- and tobramycin-containing solutions were used because they were the only commercially available products at the time of the study. The preservative included in both of the evaluated solutions (i.e., benzalkonium chloride) has also been demonstrated to induce morphologic changes in canine corneal epithelial cells and delay cellular migration in a concentration and time-dependent manner *in vitro*; however, no adverse effects were observed in a separate *in vivo* canine study evaluating benzalkonium chloride's toxicity on intact corneal epithelium when administered alone and in combination with a viscosity-enhancing agent.<sup>12,13</sup>

In general, both chondroitin sulfate ophthalmic solutions used in this study were well tolerated by patients. The frequency of mild, transient ocular irritation observed by our clients after the administration of the ciprofloxacin-chondroitin sulfate solution is similar to the incidence of ocular discomfort reported by human patients treated with ciprofloxacin ophthalmic solution.<sup>14</sup> The significance of the two dogs that developed corneal stromal abscesses and the possible contributing effects of the evaluated medication are unknown at this time. Although no infectious agents were identified on cytological and microbiological evaluation of corneal scrapings, an infectious etiology could not be definitely ruled out. No other potential etiologies for the corneal stromal abscesses were identified on clinical examination. To the knowledge of the authors, sterile corneal stromal abscesses have not been previously reported as a complication of SCCED in dogs or as a result of topical ophthalmic chondroitin sulfate administration in dogs or humans.

Numerous surgical and medical therapeutic strategies have been reported for the treatment of SCCED. Mechanical debridement, chemical cauterization, epidermal growth factor, contact lenses, collagen shields, cyanoacrylate tissue adhesive, aprotinin, polysulfated glycosaminoglycans, substance P, insulin-like growth factor, and tetracyclines have been described.<sup>1,6,15-22</sup> Surgical procedures previously evaluated include multiple punctate keratotomy, grid keratotomy, superficial keratectomy, thermokeratoplasty, nictitating membrane flaps, and conjunctival grafts.<sup>21-26</sup> Treatments that have been reported for bullous keratopathy include topical hyperosmotics (e.g., 5% NaCl solution or ointment), thermokeratoplasty, conjunctival grafts, and penetrating keratoplasty.<sup>27-29</sup> The success of these procedures

has varied widely between method, and in some instances, between published reports.

The success of manual debridement of loose epithelium, with or without a topical antimicrobial solution, to result in the healing of SCCED and corneal ulcers associated with bullous keratopathy has been very inconsistent between reports.<sup>16,21,22</sup> If manual debridement is to be used as a standard to evaluate the success of other treatments, including the results of this study, interpretation of these previous reports must be undertaken cautiously. Small population sizes and the fact that some investigators separated these two distinct clinical entities while others grouped them together as nonhealing corneal erosions make direct comparisons problematic. Reports describing successful healing of nonhealing corneal erosions (including studies with both SCCED and bullous keratopathy-associated lesions grouped together and studies in which the specific lesion type was not specified) with manual debridement as the sole therapy, with or without topical antimicrobials, include 20% in a group of 10 dogs (healing times not reported), 40% in a group of 25 dogs (with a mean healing time of 24.4 days), and 84% in a group of 19 dogs (with a mean healing time of 23.4 days).<sup>16,21,22</sup> Successful healing of corneal ulcers associated with bullous keratopathy by manual debridement alone has been reported to be 50% in a group of six dogs (with a healing time range of 18-97 days).<sup>22</sup>

The highly variable success rates published for the resolution of nonhealing corneal erosions with manual debridement of loose epithelium as the sole therapy makes interpretation of this study's results difficult. Our data suggests that healing percentages associated with these tobramycin-chondroitin sulfate and ciprofloxacin-chondroitin sulfate solutions are highly likely to exceed 58 and 52%, respectively (considering the 95% confidence intervals on the worst-case healing percentage estimates), within a 4-week treatment period. The healing percentages reported in this study for SCCED (both observed and worst-case estimates) were significantly higher than those reported in two of the previous reports utilizing only manual debridement; however, a similar healing percentage was achieved with manual debridement alone in one study.<sup>16,21,22</sup> In this previous study, manual debridement was repeated at 10 days intervals for all corneal erosions that had persistent loose epithelial edges without a decrease in the area of fluorescein retention and dogs were monitored for healing up to 2 months after the initiation of treatment.<sup>21</sup> After a single manual debridement, only 63% of corneal erosions were healed with the remainder of erosions healing after two or more debridements (with an overall healing percentage of 84% reported).<sup>21</sup> This is in contrast to our study in which manual debridement was not repeated after the initial examination and the study period was restricted to 4 weeks. Despite these protocol differences, the similar success rates in this previous study and the lack of a control group for direct statistical comparison in our study renders chondroitin sulfate solutions combined with manual debridement only speculatively more effective than manual

debridement alone to result in the healing of SCCED. To definitively determine if chondroitin sulfate ophthalmic solutions combined with manual debridement are superior to manual debridement alone to result in the healing of SCCED, a masked, randomized controlled clinical trial should be performed.

Chondroitin sulfate may be beneficial in promoting the healing of SCCED by physiologic or purely mechanical mechanisms. Chondroitin sulfate is a glycosaminoglycan that is a widespread constituent of the extracellular matrix of mammalian cells. It is found in high concentrations in the corneal stroma, where it contributes to corneal stability, rigidity, and transparency.<sup>30–32</sup> After corneal wounding, the relative abundance of chondroitin sulfate acutely increases within the provisional extracellular matrix of the healing corneal stroma and the tear film.<sup>33,34</sup> Chondroitin sulfate levels then slowly decrease as wound repair and remodelling progress, suggesting an important role in corneal wound healing.<sup>35</sup> *In vitro*, chondroitin sulfate promotes the migration of fibroblasts into the corneal stromal matrix.<sup>36</sup> It has been speculated that chondroitin sulfate facilitates the entry of fibroblasts into the matrix by increasing the space between collagen fibrils.<sup>36</sup> In addition, chondroitin sulfate is involved in numerous other aspects of cell adhesion, cell migration, axonal guidance, and wound healing.<sup>36–39</sup> Chondroitin sulfate inhibits proteolytic enzymes (e.g., collagenase and elastase) and protects cell membranes from oxygen reactive species.<sup>40</sup> Chondroitin sulfate also possesses anti-inflammatory and immunomodulating properties that include the inhibition of leukocyte directional chemotaxis, phagocytosis, and lysozyme release.<sup>40</sup> One or a combination of these physiologic activities may assist in the healing of SCCED, but definitive conclusions cannot be made until the underlying pathophysiologic mechanisms involved with these lesions are elucidated and a more thorough understanding of chondroitin sulfate's physiologic activities is achieved.

Chondroitin sulfate's antiproteolytic properties have been demonstrated in cartilage and synovial fluid.<sup>40–42</sup> Matrix metalloproteinase (MMP) activity is suppressed *in vivo* and *in vitro* by chondroitin sulfate in osteoarthritis.<sup>40–42</sup> This suppression is primarily a result of reduced MMP-9 activity, and may be caused by both pretranslational and translational regulation of MMP expression.<sup>41–43</sup> Matrix metalloproteinase-9 production by corneal epithelial and stromal cells is induced following corneal wounding.<sup>44</sup> Elevated tear proteolytic activity and elevated corneal MMP-9 activity has been documented in dogs with SCCED.<sup>7,45</sup> In contrast, fluoroquinolone antimicrobials (e.g., ciprofloxacin) have been demonstrated to induce MMP expression in a variety of tissues including the cornea.<sup>46,47</sup> The induction of MMP expression by fluoroquinolones has been speculated to contribute to the increased incidence of corneal perforations observed in human patients treated with topical fluoroquinolones.<sup>48,49</sup> Considering these seemingly opposing influences on MMP expression, it is currently unknown how an ophthalmic product

containing both a fluoroquinolone and chondroitin sulfate will affect corneal MMP activity.

The second theoretical mechanism where chondroitin sulfate may assist in SCCED healing involves its mechanical protective qualities. When in solution, chondroitin sulfate is a Newtonian fluid with a high affinity for the anterior corneal surface.<sup>50</sup> This affinity for the anterior corneal surface may be partially due to muco-adhesive properties which result in prolonged contact between the chondroitin sulfate solution and the corneal surface after administration.<sup>51</sup> Newtonian fluids maintain a relatively constant viscosity at all shear rates. This is in contrast to pseudoplastic fluids (e.g., sodium hyaluronate and methylcellulose), which exhibit higher viscosity when they are under low shear rates.<sup>50</sup>

During blinking, low shear forces are created by eyelid and nictitating membrane movement over the ocular surface.<sup>52</sup> These low shear forces are transmitted to the corneal epithelium as a drag force and can induce corneal epithelial shedding and apoptosis.<sup>52</sup> The fraction of this shear force transmitted to the corneal epithelium as a drag force increases as the viscosity of the fluid separating them increases.<sup>53</sup> Under these conditions, Newtonian fluids provide exceptional corneal lubrication because they minimize the transmission of shear force to the corneal epithelium.<sup>53</sup> This inherent mechanical property of chondroitin sulfate solutions may serve as a pharmacologic bandage to the damaged cornea and protect the underlying migrating epithelium. Chondroitin sulfate ophthalmic solutions also exert a positive effect on tear film stability, resulting in a rapid and prolonged recovery of stability after the tear film is disrupted.<sup>54</sup> By increasing tear film stability, shear forces transferred to the corneal epithelium may be further reduced.

Topical therapy with an antimicrobial chondroitin sulfate ophthalmic solution compares favorably with other published medical and surgical therapies for SCCED; however, these compounds are only equivocally more effective than therapy with manual debridement alone. These solutions appear to be ineffective in the treatment of ulcerative keratitis associated with bullous keratopathy. Topical therapy with antimicrobial chondroitin sulfate ophthalmic solutions may also be of benefit in conjunction with keratotomy procedures for SCCED. The significance of the two cases of corneal stromal abscessation is unknown at this time and warrants further investigation.

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