Topical Cross-Linked HA-Based Hydrogel Accelerates Closure of Corneal Epithelial Defects and Repair of Stromal Ulceration in Companion Animals

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Purpose. The purpose of this study was to determine the safety of topical ocular administration of a cross-linked, modified hyaluronic acid (xCMHA-S) hydrogel, and its effectiveness in accelerating repair and closure of acute and nonhealing corneal ulcers in companion animals as a veterinary treatment and its utility as a model for therapy in human corneal ulceration.

Methods. Two concentrations of xCMHA-S (0.33% and 0.75%) were topically administered to the eyes of rabbits six times daily for 28 days to assess safety. Then, 30 dogs and 30 cats with spontaneous acute corneal ulcers were treated with either xCMHA-S (0.75%) or a non–cross-linked hyaluronic acid (HA) solution ($n = 15$ per group for each species), three times daily until the ulcer had healed. Finally, 25 dogs with persistent nonhealing corneal ulcers were treated with xCMHA-S (0.75%) twice daily until the ulcer had healed.

Results. Both concentrations of the xCMHA-S hydrogel were well tolerated, safe, and nontoxic in the 28-day exaggerated dosing study in healthy rabbits. Topically applied xCMHA-S significantly accelerated closure of acute corneal stromal ulcers in dogs and cats compared with a non–cross-linked HA solution. Further, topical administration of the xCMHA-S aided in closure of nonhealing corneal stromal ulcers in dogs.

Conclusions. Hyaluronic acid has previously been shown to aid in corneal wound repair. This study demonstrates that a cross-linked, modified HA hydrogel provides further benefit by accelerating time to corneal wound closure compared to a non–cross-linked HA solution in companion animals, and therefore may be beneficial in fulfilling an unmet need in humans.

Keywords: corneal repair, crosslinked hydrogel, modified hyaluronic acid, non-healing ulcer

Corneal epithelial and stromal defects with ulceration are common ocular conditions both in humans and in companion animal species, such as dogs and cats. In humans, eye injuries represent up to 18% of emergency room traumas, corneal abrasions account for up to 4% of all US occupational injuries, and approximately 20% of patients with facial burns have ocular injury.[1] Post-traumatic abrasions are a common problem in humans in both developed[2] and Third World countries.[3,4] According to the World Health Organization, it is estimated that corneal opacities, including corneal ulceration, are the fourth leading cause of blindness worldwide.[5] The same is true in canine and feline patients where many corneal ulcerations have a traumatic origin and can lead to corneal blindness.[6] In humans and animals, superficial corneal lesions can heal rapidly and without complication, but stromal ulcers often fail to follow such a benign self-healing course and can become a sight threatening emergency with corneal scarring, ulceration, thinning, and even perforation if not treated promptly. Therefore, a topical treatment that aids in the management and accelerated closure of corneal wounds would help reduce the risk of infections and scarring, and thus improve visual outcomes in all species.

Hyaluronic acid (HA), a high molecular weight glycosaminoglycan, is recognized as having ameliorative effects that can help heal epithelial defects throughout the body, and has been shown to enhance and aid in corneal healing.[7–9] Hyaluronic acid is widely used as a topical ocular tear replacement therapy and is the standard of care for dry eye in Europe and Asia.[10] These HA-containing solutions provide transient, symptomatic relief and have excellent safety profiles. The main limitation with many of these solutions is the rapid clearance of the HA from the eye, requiring that drops be administered at least four times a day and often more frequently. Although HA solutions have been shown to have a longer residence time over other dry eye lubricants, the half-life of the HA on-eye was still on the order of minutes.[11] A subsequent study with fluorescently-tagged HA showed 90% clearance of an HA solution from the surface within 30 minutes.[12] Thus, an HA-based formulation that has increased ocular residence time could lead to decreased dosing frequency, while still having the beneficial properties of the HA, ultimately aiding in the accelerated closure of corneal and ocular surface defects. A cross-linked thiolated carboxymethylated HA (xCMHA-S) hydrogel has previously been shown to be beneficial in managing the

symptoms associated with keratoconjunctivitis sicca in dogs.[13,14]

Given the safety and efficacy in treating dry eye in animals, the current study builds on a previous report demonstrating the beneficial effects of xCMHA-S in corneal wound models in rabbits.[15] In the current study, the safety profile of topical ocular administration of xCMHA-S with exaggerated dosing in rabbits was first demonstrated followed by an efficacy study comparing the time to wound closure of spontaneously occurring corneal stromal ulcers in dogs and cats in a clinical setting using xCMHA-S versus a non–cross-linked HA solution. Finally, the effectiveness of xCMHA-S to aid in the closure of chronic nonhealing corneal ulcers in dogs, again in a clinical setting, is demonstrated.

Materials and Methods

Cross-Linked CMHA-S Hydrogel

CMHA-S was synthesized and analyzed as previously described.[13,15] A solution of CMHA-S (0.33% or 0.75% wt/vol) in PBS (pH 7.4) was then filter-sterilized, cross-linked to form a hydrogel (xCMHA-S), and packaged aseptically into sterile 3-mL eye drop bottles as previously described.[13] The xCMHA-S hydrogels are commercially available under the Remend label (Bayer Animal Health, Shawnee, KS, USA).

Safety and Toxicity in Rabbits

Thirty healthy New Zealand White rabbits (15 female, 15 male) were assigned to one of three groups: Group 1 (6 female, 6 male) to receive 0.33% xCMHA-S; Group 2 (6 female, 6 male) to receive 0.75% xCMHA-S; Group 3 (3 female, 3 male) to receive PBS (control). One drop of xCMHA-S (0.33% or 0.75%) or PBS alone was administered six times a day (an exaggerated dosing regimen) via topical application to both eyes of the rabbits for 28 days. Each dose (40 μL) was pipetted with a calibrated pipette and sterile tip for each eye; to minimize runoff, each eye was gently closed for a few seconds after dosing. Blood and urine were collected prior to dosing and prior to necropsy for complete blood count, clinical chemistry, coagulation factor testing, and urinalysis. Gross ocular observations, which consisted of a visual appraisal for swelling, discharge, and/or irritation of the eye, were performed daily. Clinical ophthalmic examinations, using slit-lamp biomicroscopy and indirect ophthalmoscopy, were performed weekly for the study duration. Examinations used a modified McDonald-Shadduck scoring system, and were performed by board-certified veterinary ophthalmologists (R Merideth, E Moeller, Eye Care for Animals, San Diego, CA, USA). Intraocular pressure (IOP) measurements were taken prior to dosing and prior to euthanasia for all animals (Reichert Model 30 pneumotonometer, Depew, NY, USA). One male and one female from Group 3, and two males and two females from both Groups 1 and 2, were observed for an additional 14 days after the final dose was administered.

Following euthanasia, the globes and adnexa were collected, preserved in 10% formalin, and processed for histopathology. The tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The sections were examined under light microscopy, and specific areas that were evaluated included: conjunctiva, cornea, anterior chamber, iris, retina, lenses, choroid, vitreous, clear, and ocular adnexal tissue. For nonneoplastic findings, severity was graded on a five-point scale, where 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, and 5 = severe. Corneal thickness was measured from the surface of the epithelium to the posterior surface of the endothelium in the central cornea of each eye in a masked manner. Three measurements were taken in the central and paracentral cornea using a micrometer eyepiece on the microscope. These measures were directly perpendicular to the surface of the cornea.

This study was approved by the Institutional Animal Care and Use Committee at Absorption Systems (San Diego, CA, USA) and conducted in accordance with the Good Laboratory Practice for Nonclinical Laboratory Studies (Code of Federal Regulations US FDA 21 CFR Part 58) and Authorized Service Interruption Standard Operating Procedures. All animals were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Acute Ulcer Healing in Dogs and Cats

Thirty dogs and 30 cats kept as companion animals in home environments and diagnosed with acute corneal stromal ulceration by the managing veterinary ophthalmologist (DLW) were included in the study. The animals were subject to a full systemic and ophthalmic examination using direct and indirect ophthalmoscopy and slit-lamp biomicroscopy, and those with concurrent ocular or systemic disease were excluded from the study. Ulcer depth was assessed using the slit-lamp biomicroscope and classified as superficial, mid, or deep stromal ulceration. Corneal vascularization and haze were assessed based on the following scale: 0 = none, 1 = mild, 2 = moderate, 3 = severe. Intraocular pressure was measured as previously described. The animals were randomly assigned to receive xCMHA-S (0.75%) or a commercially available non–cross-linked HA solution (0.3%; iDrop Vet Plus; I-MED Animal Health, Dollard-des-Ormeaux, Quebec, Canada) applied topically three times daily, with topical antibiotic, 0.5% chloramphenicol drop, applied concurrently at home by their owners. The investigator was masked as to which group each animal was assigned. Animals were examined clinically by direct and indirect ophthalmoscopy and by slit-lamp biomicroscopy weekly until the ulcer had closed, as demonstrated by failure of the corneal surface to stain with fluorescein dye.

Treatment of Nonhealing Ulcers in Dogs

Dogs with persistent nonhealing corneal epithelial defects and stromal ulceration, that is to say ulceration that had not healed within approximately 2 weeks despite standard antibiotic and tear replacement therapy, were referred to a veterinary ophthalmologist (DLW) and enrolled in this study. Dogs were included if they were not suitable candidates for surgery either because of age, anesthetic risk, or for financial reasons. Informed consent was provided by the owners for their pets to be included in this study. All dogs underwent full clinical and ophthalmic examination using direct and indirect ophthalmoscopy and slit-lamp biomicroscopy. Tear production was measured using the Schirmer tear test and IOP was measured by tonometry using a rebound tonometer (Tonovet; I-Care, Vantaa, Finland). Epithelial size and ulcer width was assessed using a single drop of fluorescein dye (Minims; Chauvan Pharmaceuticals, Watford, UK) flushed away with sterile saline. Ulcer depth was assessed using the slit-lamp biomicroscope and classified as (1) superficial epithelial erosion, (2) superficial stromal ulceration, (3) midstromal ulceration, or (4) deep-stromal ulceration. Animals with descemetoceles or those considered at imminent risk of corneal rupture were excluded from the study and treated surgically. Animals with excessive ocular pain were excluded from the study and treated accordingly medically with systemic and topical pain relief.

Twenty-five dogs referred by first opinion veterinarians with nonhealing corneal ulcers were subsequently treated with

xCMHA-S (0.75%). Dogs appropriate to be included in this study must have had epithelial erosions or stromal ulceration with failure to heal at approximately 2 weeks, and thus considered as chronic lesions unlikely to heal spontaneously. After ophthalmic examination and inclusion in the study, previous treatments were stopped, and animals were topically administered the xCMHA-S twice daily with examinations, thenceforth conducted weekly. Time to corneal ulcer healing on xCMHA-S was noted, as determined by failure of the ocular surface to stain with fluorescein dye.

Both studies were reviewed and accepted by the Ethics and Welfare Committee of the Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom, and all animals were treated in accordance with the welfare guidelines in the Royal College of Veterinary Surgeons Guide to Professional Conduct. The owners provided informed consent.

Statistical Analysis

For the acute ulcer healing study, comparisons between the two groups for each species were determined using an unpaired Student's *t*-test; comparison between corneal haze pre- and posttreatment within a group was determined using a paired Student's *t*-test. The number of dogs and cats included in the study was determined by a power analysis.[16] Using a desired effect size (the difference in mean time to healing between the treatment groups) of 5 days, a type I error of 0.05, a power of 0.8, and an SD between 4 and 5 days, a sample size needed for each treatment group was between 11 and 16. For the nonhealing chronic ulcer study, time to wound closure with xCMHA-S was compared with the time without healing prior to use of xCMHA-S using a χ^2 2×2 contingency table.

Results

Safety and Toxicity in Rabbits

The safety of the xCMHA-S gel was determined using 6× daily topical administration to the eyes of healthy rabbits over 28 days. The xCMHA-S was very well tolerated at both concentrations with only intermittent mild +1 grade conjunctival congestion and mild clinically insignificant swelling of the third eye lid observed in four animals in Group 1, two in Group 2, and three in Group 3 over the course of the study. Following euthanasia, evaluation of the globes and adnexa histopathologically showed no sign of any untoward inflammation or toxicity in any of the eyes in all groups and time points, and there was no significant difference in corneal thickness between the PBS and xCMHA-S groups, indicating the safety of the xCMHA-S gel over at least 28 days.

Acute Ulcer Healing in Dogs and Cats

The utility of the xCMHA-S gel in accelerating closure of acute corneal ulcers was determined in companion animals kept in home environments. One cat was lost to follow-up, leaving 15 cats in the xCMHA-S group and 14 in the non–cross-linked HA group. For dogs, there were 15 animals in each of the xCMHA-S and non–cross-linked HA groups. The breed, age, sex, depth and area of ulcer, degree of corneal vascularization, IOP, and corneal haze pre- and posttreatment for each dog and cat is shown in Tables 1 and 2, respectively. There was no significant difference in age between the two groups for each species. Similarly, although not matched for ulcer severity due to randomization in assignment to groups, the treatment groups were not significantly different in their ulcer depth or area, as shown in Tables 1 and 2. Further, there was no significant difference in corneal vascularization, IOP, or corneal haze between the two groups in each species. As shown in Figure 1, canine corneal ulcers receiving xCMHA-S closed significantly faster (14.8 ± 4.1 days) than those receiving the non–cross-linked HA (18.3 ± 4.9 days; $P = 0.04$). Likewise, feline ulcers receiving xCMHA-S closed significantly faster (21.0 ± 11.0 days) than those receiving the non–cross-linked HA (31.8 ± 10.3 days; $P = 0.01$). Although there was no significant change in corneal haze from pre- to posttreatment for either group of the dogs, there was a significant decrease in haze from pre- to posttreatment for both groups of the cats ($P = 0.003$ for the non–cross-linked HA group, $P = 0.007$ for the xCMHA-S group).

Treatment of Chronic Nonhealing Ulcers in Dogs

The ability of xCMHA-S gel to aid closure of chronic nonhealing ulcers was also examined in dogs kept as companion animals in home environments. Results of time to ulcer healing following treatment with xCMHA-S for dogs in this study are shown in Table 3 together with the type of ulcer involved and the time without healing before referral. Where ulcer healing occurred, the resulting stroma was generally opaque (Fig. 2), the epithelium was intact, and the dogs were pain free (as reported by their owners). The average duration of the ulcer before use of xCMHA-S was 25.5 ± 8.3 days while the mean time to ulcer healing when treated with xCMHA-S was 13.4 ± 4.2 days, these times being significantly different ($P = 0.00005$). Dogs with recurrent epithelial erosion associated with defective basal epithelial cell adhesion to the underlying basement membrane (termed spontaneous chronic corneal epithelial defects [SCCEDs])[17,18] and analogous to recurrent epithelial erosions in humans[19,20]) did not heal with xCMHA-S and required epithelial debridement before healing occurred. These ulcers are readily diagnosed as they have a clearly defined devitalized epithelial margin[17,18] and are denoted in Table 3 as those with superficial erosions. One pug with a deep stromal ulcer experienced corneal rupture through self-trauma. These animals were all subsequently removed from the study and treated accordingly. Where chronic nonhealing ulcers did heal, time to healing once placed on the xCMHA-S was similar to that for acute ulcers.

Discussion

Ocular injuries are prevalent in both humans and companion animals, and delayed corneal epithelial healing can lead to subsequent corneal infections with further complications, such as corneal scarring, thinning, ulceration, and even perforation. Therefore, a product that can aid in the healing process and reduce time to corneal epithelial healing would be beneficial in all species.

Solutions of non–cross-linked HA, at 1.5 to 10 mg/mL, have previously been shown to enhance corneal wound healing in debridement or abrasion studies in rats, rabbits, and humans.[7–9] Although a study recently reported that a solution of non–cross-linked HA at 2 mg/mL had no effect on wound closure, compared with controls, in a corneal debridement model in dogs, the study was limited to only three animals per group.[21] It remains unclear exactly what mechanism is responsible for enhanced corneal wound repair, as in vitro and/or ex vivo reports have indicated either an increase in corneal epithelial cell proliferation[22] or cell migration.[23] The differences in these studies, however, may be due to timing, as the first study examined the effect of HA on cells at 15 hours and the second examined the effect at 12 and 16 days. Regardless of the mechanism, HA does appear to provide a benefit to the corneal wound repair process following debridement.

Table 1. Information on Dogs With Corneal Stromal Ulcers Treated With Cross-Linked CMHA-S Hydrogel (xCMHA-S) or Non–Cross-Linked HA Solution

Dog ID#	Breed	Age, y	Sex	Ulcer Type	Degree of Corneal Vascularization	IOP, mm Hg	Estimated Ulcer Area, mm^2	Time to Healing	Corneal Haze Pre tx	Corneal Haze Post tx
HA Solution										
901	JRT	6	Fn	Mid	1	12	6.5	18	2	3
904	Shih Tzu	7	Fn	Mid	2	10	7	7	3	2
905	X-bred	9	Fn	Superficial	1	14	5	15	2	1
909	Golden Ret	10	Mn	Mid	1	13	7	20	3	2
910	CKCS	8	Mn	Mid	2	10	5	22	3	2
913	Lhasa Apso	4	Me	Mid	1	12	6.5	18	3	3
914	WHWT	3	Fe	Superficial	0	16	7	12	1	1
919	Lhasa Apso	8	Mn	Mid	1	13	7	14	2	3
920	Dandie Din	6	Mn	Superficial	0	15	7.5	19	1	2
922	GSD	8	Me	Mid	1	11	6.5	18	3	2
923	X-bred	8	Fn	Mid	2	10	7	22	3	3
924	Yorkshire T	6	Fn	Mid	1	13	6.5	26	2	2
925	Border T	8	Me	Mid	1	12	7	20	3	2
929	X-bred	9	Me	Mid	0	15	7.5	25	2	1
930	St. Bernard	6	Fe	Superficial	1	14	6.5	18	1	1
Mean ± SD		7.1 ± 1.9			1.0 ± 0.7	12.7 ± 1.9	6.6 ± 0.7	18.3 ± 4.9	2.3 ± 0.8	2.0 ± 0.8
xCMHA-S										
902	X-bred	7	Mn	Mid	1	12	7	14	2	1
903	Lab	5	Mn	Mid	1	14	8	18	2	2
906	CKCS	7	Fn	Superficial	0	17	7.5	23	1	1
907	WHWT	8	Fn	Mid	2	12	8	22	3	2
908	X-bred	9	Fn	Mid	2	10	6.5	14	3	2
911	X-bred	7	Me	Mid	1	14	7	17	3	3
912	Boston T	8	Fn	Superficial	0	16	6	9	2	1
915	Lab	6	Fn	Superficial	0	17	7	14	1	1
916	Dalmatian	7	Fe	Mid	1	12	6	10	2	2
917	Briard	8	Fn	Superficial	1	13	7	17	1	1
918	Golden Ret	6	Fn	Mid	2	10	6.5	15	2	1
921	X-bred	7	Mn	Superficial	1	12	7	12	1	1
926	Lhasa apso	6	Fe	Superficial	0	14	7	10	1	2
927	Lakeland T	9	Mn	Superficial	1	13	7.5	13	2	2
928	Lab	10	Fn	Mid	1	10	7	14	3	2
Mean ± SD		7.3 ± 1.3			0.9 ± 0.7	13.1 ± 2.3	7.0 ± 0.6	14.8 ± 4.1	1.9 ± 0.8	1.6 ± 0.6

JRT, Jack Russell terrier; Ret, retriever; CKCS, Cavalier King Charles spaniel; WHWT, West Highland white terrier; Din, dinmont; GSD, German Shepherd dog; T, terrier; Lab, Labrador retriever; Fn, neutered female; Fe, unaltered female; Mn, neutered male; Me, unaltered male; tx, treatment; 0, none; 1,mild, 2, moderate; 3, severe.

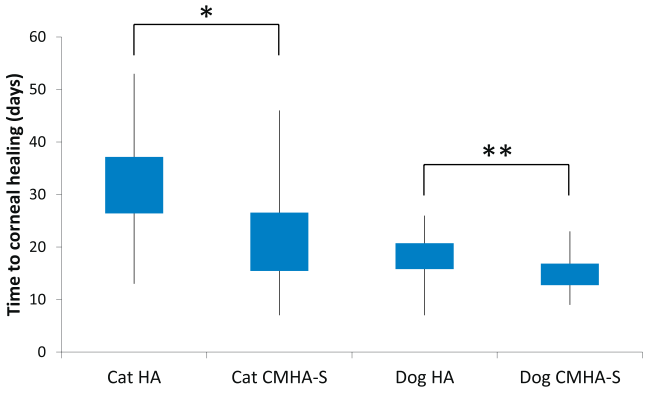

Figure 1. Corneal ulcer healing time in cats and dogs. *Bars* represent 95% confidence intervals of the means; *lines* represent range of values obtained. $^{*}P = 0.01$; $^{**}P = 0.04$.

Although the non–cross-linked HA solution used in the rat debridement model enhanced wound healing, it did not provide the same acceleration of healing in an alkali burn model in rats[7]. However, a cross-linked HA gel, similar to the hydrogel used in the current study, was shown to accelerate corneal wound healing in both a debridement and a burn model in rabbits.[15] Thus, with the deeper wound created by the alkali burn, a cross-linked HA may provide more benefit, potentially due to a longer residence time on the ocular surface. Despite the successful use of both non–cross-linked and cross-linked HA in aiding corneal wound repair in wound models, the current study appears to be first one to report the use of cross-linked HA in companion animals with spontaneous corneal ulcers.

Here, in dogs and cats kept as companion animals in home environments, we have shown that a cross-linked gel formulation, xCMHA-S, based on a modified HA, was able to accelerate time to epithelial closure both in acute and chronic corneal stromal defects. The xCMHA-S hydrogel was shown to be well tolerated, safe, and nontoxic in a GLP 28-day rabbit study. Subsequently, topical administration of the xCMHA-S led to significantly faster closure of acute corneal stromal ulcers in

Table 2. Information on Cats with Corneal Stromal Ulcers Treated With xCMHA-S or Non–Cross-Linked HA Solution

Cat ID#	Breed	Age, y	Sex	Ulcer Type	Degree of Corneal Vascularization	IOP, mm Hg	Estimated Ulcer Area, mm^2	Time to Healing	Corneal Haze Pre tx	Corneal Haze Post tx
HA Solution										
801	Dsh	8.2	Mn	Mid	0	14	6	13	2	1
805	Dsh	8.2	Mn	Mid	0	16	7	22	2	2
806	Dsh	8.7	Mn	Mid	1	12	7.5	27	2	1
807	Dsh	10.1	Mn	Mid	1	13	7	28	2	2
810	Dsh	13.1	Fn	Mid	0	16	6.5	28	1	1
813	Dsh	4.7	Fe	Mid	1	18	7	29	2	1
814	Mau	12.0	Fn	Mid	1	15	7	29	3	2
817	Dsh	10.1	Mn	Mid	2	16	7.5	30	3	3
820	Dsh	7.8	Fn	Mid	1	14	8	31	2	2
823	Dsh	6.2	Fn	Mid	0	12	7	33	2	2
824	Dsh	8.2	Fn	Mid	0	14	7.5	34	3	2
825	Sia	9.2	Fn	Mid	1	17	4	37	2	1
827	Bur	8.2	Fn	Deep	2	12	8.5	51	3	3
830	Dsh	5.0	Fe	Mid	0	15	7	53	3	2
Mean ± SD		8.6 ± 2.4			0.7 ± 0.7	14.6 ± 1.9	6.9 ± 1.0	30.6 ± 10.9	2.3 ± 0.6	1.8 ± 0.7
xCMHA-S										
803	Dsh	7.5	Mn	Mid	1	14	6.5	7	3	2
804	Per	10.3	Mn	Mid	1	16	7	10	3	2
808	Dsh	3.4	Mn	Superficial	0	15	5.5	12	1	2
809	Dlh	8.2	Mn	Mid	0	15	7	12	2	1
811	Dsh	14.9	Fn	Deep	2	12	7	14	2	1
812	Dsh	10.8	Fn	Mid	1	14	7	14	2	2
815	Dsh	8.2	Mn	Mid	0	16	7.5	16	3	1
816	Dsh	14.2	Fn	Mid	0	17	6.5	17	2	1
818	Dsh	4.7	Me	Deep	1	13	7	19	2	2
819	Dsh	6.3	Fn	Deep	2	11	7	27	3	2
821	Brsh	6.5	Me	Superficial	0	15	7.5	27	1	1
822	Dsh	7.1	Fn	Mid	1	14	7	28	2	1
826	Dsh	14.5	Fn	Deep	2	13	7	29	3	2
828	Dsh	9.8	Mn	Mid	1	17	8	37	2	2
829	Dsh	13.5	Fn	Superficial	0	19	8.5	46	1	1
Mean ± SD		9.3 ± 3.7			0.8 ± 0.8	14.7 ± 2.1	7.1 ± 0.7	21.0 ± 10.9	2.1 ± 0.7	1.5 ± 0.5

Brsh, British shorthair; Bur, Burmese; Dsh, domestic shorthair; Dlh, domestic longhair; Per, Persian; Sia, Siamese; tx, treatment.

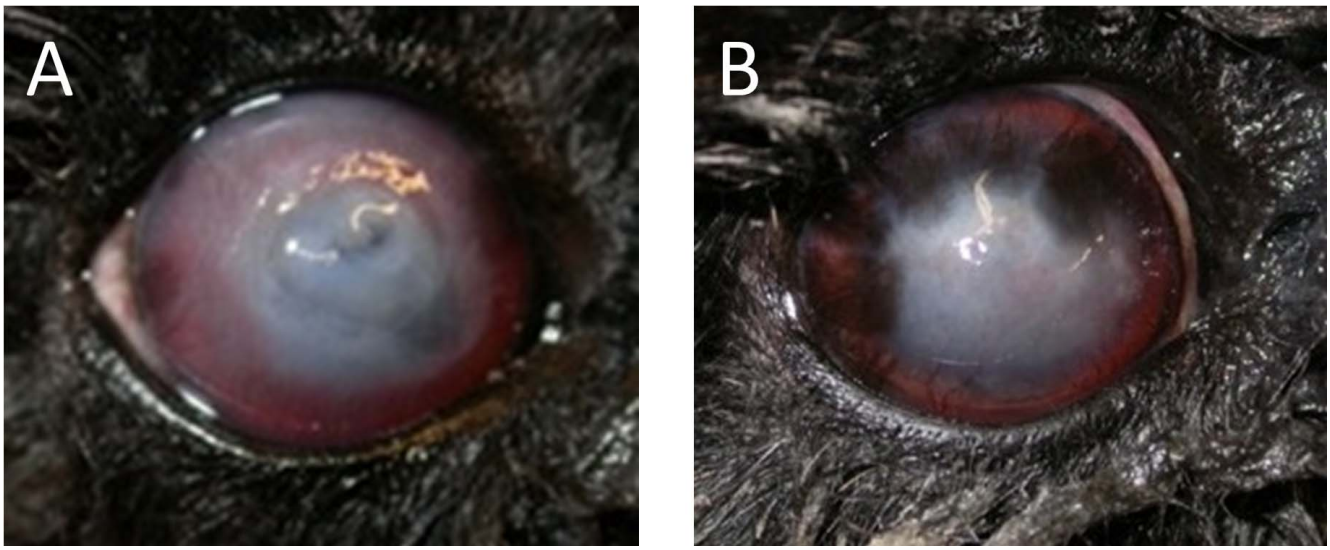

Figure 2. Healing of a corneal ulcer on a 7-year-old mixed-breed dog. The dog is case #4 in Table 3. (**A**) Day 21 of the nonhealing ulcer; (**B**) the healed ulcer after 14 days of treatment with xCMHA-S.

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Table 3. Non-Masked Clinical Study in Dogs With Nonhealing Corneal Ulcers

Case	Breed	Age	Sex	Ulcer Type	Days Unhealed	Ulcer Size, mm	Days to Healing
1	CKCS	8.2	Mn	Mid stromal	25	5	14
2	Corgi	10.1	Mn	Mid stromal	28	6	14
3	Lhasa Apso	14	Mn	Deep stromal	21	3	7
4	X-bred	7	Fn	Mid stromal	21	4	14
5	Pug	10	Fn	Deep stromal	25	5	14
6	Pug	5	Mn	Deep stromal	28	4	12
7	X-bred	13	Mn	Mid stromal	42	3	14
8	Boxer	9	Fn	Superficial stromal	32	6	21
9	Boxer	8	Fn	Superficial stromal	32	5	18
10	JRT	9	Fn	Mid stromal	21	3	21
11	Shi Tzu	11	Fn	Mid stromal	42	4	14
12	Newfoundland	2	Fn	Superficial stromal	20	6	7
13	Labrador	7	Fn	Superficial stromal	18	5	7
14	CKCS	6	Me	Deep stromal	22	2	10
15	Boxer	8	Fn	Superficial stromal	24	4	14
16	Pug	2	Me	Deep stromal	24	3	14
17	JRT	8	Fn	Superficial stromal	24	6	14
18	X-bred	13	Me	Deep stromal	21	3	Nh (required cpg)
19	Boxer	8	Me	Superficial erosion	42	7	Nh (required edgk)
20	St Bernard	4	Fe	Superficial stromal with entropion	14	8	Nh (required correction of entropion)
21	Corgi	12	Fe	Superficial erosion	32	5	Nh (required edgk)
22	Pug	3	Me	Deep stromal	12	2	Nh (ruptured)
23	Boxer	8	Fn	Superficial erosion	14	5	Nh (required edgk)
24	ECS	9	Mn	Superficial erosion	21	4	Nh (required edgk)
25	Corgi	12	Fe	Superficial erosion	32	4	NH (required EDGK)
Mean ± SD					25.5 ± 8.3	4.5 ± 1.5	13.4 ± 4.2

ECS, English cocker spaniel; NH, not healed on xCMHA-S; CPG, conjunctival pedicle graft; EDGK, epithelial debridement and grid keratotomy. Days unhealed indicates the number of days the animal had the ulcer and was treated with SOC prior to referral and beginning treatment with xCMHA-S.

dogs and cats compared with a non–cross-linked HA solution. Further, topical administration of the xCMHA-S aided in closure of nonhealing corneal stromal ulcers in dogs. The product did not improve healing in animals with chronic nonhealing superficial erosions where defective epithelial cell adhesion to the underlying basement membrane (recurrent erosions, or SCCEDs) was present and where epithelial debridement was required prior to healing.

While the portion of our study that examined the effect of the xCMHA-S on acute corneal wound healing in cats and dogs was masked and randomized, the portion using the xCMHA-S for nonhealing ulcers was not. Although it could be argued that simply removing the animals from their previous tear replacement would lead to the ultimate wound closure, and not the use of the xCMHA-S, we were reluctant to potentially extend the period of nonhealing by first removing them from the tear replacement for a period of time, and then beginning use of the xCMHA-S. Further, as there was no appropriate alternative to use for treating the nonhealing ulcers in a manner that could be masked, the study could not be masked or randomized. This aspect of removing the animals from their previous treatment regimen also has the potential that the ulcers, from the point of removal of the other treatment, could then be considered "acute" ulcers, and may account for the similar healing times for dogs administered xCMHA-S in the two portions of the study.

The average healing time for acute corneal ulcers was slower in cats than dogs in this study, which was unexpected. Although there have been previously reported studies on healing of experimentally induced corneal ulcers in dogs,[21,24] there have been no equivalent studies published on corneal ulcer healing in cats, nor any studies directly comparing the two species. The feline ulcers in this study were slightly larger in width and on average more severe in depth than the canine lesions, and thus could have resulted in some difference in healing time. However, in both cats and dogs, some more severe stromal ulcers (in terms of depth) healed faster than more superficial lesions. This may be due to the fact that these are clinical cases, companion animals living in homes rather than laboratory animals in more controlled experimental environments. As such, variations in temperature and humidity, degree of self-trauma, presence of other animals or aspects of the home environment that may affect the eye, and owner compliance are all confounding variables that have the potential to influence healing rates.

It should be stressed that complicated cases of nonhealing ulcers, such as those having a devitalized nonadherent epithelium or having a concurrent condition, should be treated accordingly and may require surgical intervention both in humans and in animals. Nevertheless, with the benefits shown for accelerating corneal epithelial and stromal ulcer closure in companion animals, this xCMHA-S gel formulation also is likely to be useful in aiding corneal epithelial defect closure in humans.

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