

Effects of topical hyaluronic acid on corneal wound healing in dogs: a pilot study

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Abstract

Objective To investigate the efficacy of topical 0.2% hyaluronic acid in canine corneal ulcers *in vivo*.

Procedures Six purpose-bred beagles were randomly assigned into two groups (three dogs/group): group A received experimental product (Optimend™, containing 0.2% hyaluronic acid, KineticVet™); group B received control product (Optimend™ without 0.2% hyaluronic acid and supplemented with carboxymethylcellulose). The clinical scorer was masked to product content and subject assignment. Under sedation and topical anesthesia, 6-mm axial corneal epithelial debridements were performed in the left eye. Wounded corneas received standard ulcer treatment and topical product (group A) or control product (group B) three times a day (TID) until ulcers were healed. Slit-lamp biomicroscopy was performed 6 h after wounding and then every 12 h; findings were graded according to modified McDonald–Shadduck scoring system; extraocular photography was performed after fluorescein stain application at all examination time points. Images were analyzed using NIH IMAGE J software to quantify rate of corneal epithelialization. Gelatin zymography was used to analyze matrix metalloproteinase (MMP) 2 and 9 protein expression in tears collected at set time points during the study period.

Results No statistical differences in clinical ophthalmic examination scores, rate of corneal epithelialization, or MMP2 or MMP9 protein expression were found between groups at any tested time point.

Conclusions The application of 0.2% hyaluronic acid to standard ulcer medical management is well tolerated. Topical addition of the viscoelastic did not accelerate corneal wound healing compared to a topical control with similar viscosity in this study.

Key Words: artificial tears, canine, carboxymethylcellulose, cornea, hyaluronic acid, matrix metalloproteinase

INTRODUCTION

Corneal epithelial injuries are common in both physician and veterinary ophthalmology. Causes of superficial corneal ulceration include trauma, spontaneous chronic corneal epithelial defects (SCCEDs), aberrant hairs, such as distichia or ectopic cilia, select surgical procedures (e.g., burr keratotomy), and keratoconjunctivitis sicca.^{1,2} Regardless of the etiology, corneal epithelial defects heal primarily by epithelial cell migration and mitosis. This cellular proliferation and migration is orchestrated by numerous extracellular molecules, their receptors, and subsequent intracellular signaling cascades. Hyaluronic

acid has been shown to play a role in corneal wound healing.^{3,4}

Hyaluronic acid is a polysaccharide of the repeating disaccharide units β -glucuronic acid and N-acetyl glucosamine which are joined by β -1, 3- and β -1, 4-glycosidic alternating bonds. Hyaluronic acid is abundant in almost all mammalian tissue and was first discovered in bovine vitreous.⁵ Although most naturally occurring hyaluronic acid is found in connective tissue, it is also found in epithelial and neural tissue. Hyaluronic acid may exist in extracellular, pericellular, or intracellular spaces, and at physiologic pH, hyaluronic acid is a polyanion; therefore, the appropriate designation for naturally occurring

hyaluronic acid is hyaluronan.^{5–7} Previous studies have localized hyaluronan in the intact rabbit, rat, and canine cornea and detected this polysaccharide within the intercellular spaces of the epithelium and stroma, within stromal keratocytes, and on the apical surface of the endothelium.^{8–10}

Despite its simple chemical structure, hyaluronic acid possesses unique rheological properties, specifically non-Newtonian or shear thinning behavior.¹¹ As a non-Newtonian fluid, hyaluronic acid is able to exhibit a low viscosity when exposed to high shear stress and yet regain a high viscosity once the shear stress is reduced, allowing hyaluronan to contribute to the structural and physiologic characteristics of the various tissues in which it naturally resides.

Hyaluronic acid serves other important biologic roles, including the ability to promote wound healing. Numerous studies have demonstrated that hyaluronic acid accelerates corneal wound healing both *in vitro* and *in vivo*.^{12–15} Consequently, topical hyaluronic acid is commonly included in the treatment regimen for superficial corneal ulcerations in canine patients. However, past studies have utilized cell culture or small laboratory animals as test subjects and of significance, phosphate-buffered saline (PBS) as control treatment. While PBS lacks hyaluronic acid, it also lacks the viscoelastic properties of hyaluronic acid-containing solutions, a factor which may be contributing to corneal wound healing in addition to any innate hyaluronic acid properties. Therefore, the purpose of this study was to determine whether a commercially available ophthalmic solution containing 0.2% hyaluronic acid would accelerate canine corneal wound healing *in vivo* compared to a control treatment with similar viscosity.

MATERIALS AND METHODS

Animals

Six healthy female Beagle dogs (9–12 months, 10–15 kg) were purchased from Covance Laboratory (Cumberland, VA, USA) and housed in a research facility at the University of Missouri. Prior to study onset, complete ophthalmic examination of all dogs was performed by a board certified veterinary ophthalmologist (EAG). Examinations included slit-lamp biomicroscopy (SL-15 Kowa Company, Ltd, Tokyo, Japan), indirect ophthalmoscopy (Wireless indirect ophthalmoscope, Keeler Instruments Inc., Broomall, PA, USA and pan retinal 2.2 indirect lens; Volk Optical Inc., Mentor, OH, USA), Schirmer tear test I (Schering-Plough Animal Health, Union, NJ, USA), tonometry (Tono-Pen Vet, Dan Scott and Associates, Westerville, OH, USA), and fluorescein staining (Flu-Glo, Akorn, Inc., Buffalo Grove, IL, USA). All dogs were determined to be free of ocular disease. The dogs received daily socialization and all other husbandry needs including a diurnal 12-h light cycle. All studies were performed

in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the University of Missouri Institutional Animal Care and Use Committee.

Topical test products

Both test products were obtained from KineticVetTM (KineticVet, Lexington, KY, USA) in containers labeled as A or B to mask those administering the medications and the clinical scorer. Product content was not revealed until study conclusion. Test product A was the experimental agent, OptimendTM (KineticVet, Lexington, KY, USA), which contained 0.2% medium weight hyaluronic acid, and test product B was the control agent which was OptimendTM without 0.2% medium weight hyaluronic acid and supplemented with medium weight carboxymethylcellulose to achieve the same viscosity as test product A. At the study's end point, an independent laboratory (Mass Spectrometry Facility, University of Missouri) performed content analysis of the test products and verified that test product A contained hyaluronic acid while test product B did not. Independent analysis of the test products also revealed that both test products A and B had equal viscosities of 1150 cps.

Corneal wounding

Corneal wounding was performed by a board certified veterinary ophthalmologist (EAG). Dogs were sedated with dexmedetomidine (7 mcg/kg, IM; Zoetis Inc., Kalamazoo, MI, USA) and hydromorphone (0.1 mg/kg, IM; Hospira, Inc., Lake Forest, IL, USA). Following sterile placement of an intravenous catheter, propofol (Abbott Animal Health, Abbott Park, IL, USA, 2–4 mg/kg, IV) was administered until the dogs were at an appropriate level of anesthesia for intubation. Intubation was only considered necessary if apnea occurred, and anesthesia was maintained with IV administration of propofol to effect. The left eye was then aseptically prepared, using a 1:20 dilution of betadine solution. Local anesthetic (proparacaine hydrochloride 1%; Bausch & Lomb Inc., Tampa, FL, USA) was applied to the left eye 3 times to numb the corneal surface. Topical mydriatic and cycloplegic agent (atropine sulfate 1%; Bausch & Lomb Inc., Tampa, FL, USA) was applied to the left eye once.

A superficial epithelial debridement was performed in the left eye of all dogs. Briefly, using surgical loupes, a 6-mm area of the axial cornea was outlined with a corneal trephine. The epithelium was mechanically removed with an excimer spatula as previously described.¹⁶ Fluorescein stain was immediately applied to the cornea to verify successful removal of the corneal epithelium as demonstrated in Fig. 1.

Following wounding, the dogs were randomly and equally assigned to one of two groups (three dogs/group): group A (experimental agent: Optimend with 0.2% medium weight hyaluronic acid) or group B (control agent:



Figure 1. Representative extraocular photograph of a study dog in group A immediately following epithelial debridement. The area of fluorescein stain retention confirms a superficial corneal ulcer 6 mm in diameter.

Optimend without 0.2% medium weight hyaluronic acid and supplemented with medium weight carboxymethylcellulose). The left eye of all dogs received standard treatment for corneal ulceration which included topical antibiotic (bacitracin, polymyxin B, gramicidin ophthalmic solution, Paddock Laboratories Inc., Minneapolis, MN, USA, 1 drop OS q 8 hr), and topical mydriatic and cycloplegic (atropine sulfate 1.0%, ophthalmic solution, Akorn Inc., Lake Forest, IL, USA, 1 drop OS to effect); dogs were administered systemic anti-inflammatory medication (carprofen, Zoetis Inc., Kalamazoo, MI, USA, 2.2 mg/kg PO q 12 hr) and systemic analgesic medication (tramadol, Amneal Pharmaceuticals, Bridgewater, NJ, USA, 4 mg/kg, PO q 12 hr); the left eye of the dogs also received either topical hyaluronic acid or control agent (1 drop OS TID). To prevent ocular self-trauma, dogs wore an Elizabethan collar until the study's end point.

Six hours after wounding and then every 12 h, dogs underwent examinations by a veterinary ophthalmologist (EAG) masked to the treatment groups. Ocular health of the left eye was scored according to the modified McDonald-Shadduck (mMS) scoring system (range: 0–40). Examinations were discontinued once the corneal ulcers were healed.

Quantification of corneal wound healing

Photography of the left eye was performed at each examination before and after the application of fluorescein stain using a Nikon D300S camera and AF-S DX NIKKOR 18–200 mm f/3.5–5.6G ED VR II lens (Nikon Inc., Melville, NY, USA). Images were analyzed with IMAGE J software (NIH, Bethesda, MD, USA) to determine the rate of corneal epithelialization. Briefly, the fluorescein stained cornea was outlined and the area of the ulcer bed was calculated for each examination time point until the ulcer

was healed (i.e., the cornea was fluorescein negative). These measurements were used to determine the percent reduction in the area of the corneal ulcer at each time point. For example, at 6 h postwounding the percent reduction in the area of the corneal ulcer was calculated as follows:

$$\left[1 - \left(\frac{\text{Area of ulcer at time 6 hr}}{\text{Area of the ulcer at time 0 hr}}\right)\right] \times 100 \quad (1)$$

The percent reduction in the area of corneal ulceration at each time point is demonstrated in Table 1.

Tear collection and extraction for zymography

Tears were collected from the left eye at baseline (i.e., prior to wounding) and 6, 24, 48, 72, and 96 h postwounding. Baseline samples served as control. Sampling performed at baseline and 24, 48, 72, and 96 h postwounding occurred between 6 and 7 am to avoid diurnal changes in tear volume. To collect tears, a Schirmer tear test strip was placed in the ventral fornix for 1 min. The tear test strip was then placed in an Eppendorf reaction tube (Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) and stored at –80 until further analysis.

Prior to performing zymography, tears were extracted from tear test strips according to methods previously described.¹⁷ Briefly, 200 µL of extraction buffer (0.5 M NaCl, Fisher Scientific, Fair Lawn, NJ, USA and 0.5% Tween-20, Sigma-Aldrich, Saint Louis, MO, USA) was added to each Eppendorf tube and incubated with gentle agitation for 3 h at room temperature. The tear test strip was then transferred to a new 2-mL centrifuge tube, and residual fluid was extracted by pinching the distal end of the strip in the sealed cap and centrifuging the tube at approximately 100 g for 10 s. This liquid was then combined with the stored extraction buffer for that tear test strip, and the strip was discarded. To determine the total protein concentration in the tear fluid, absorption was measured at a wavelength of 595 nm using a nanodrop plate reader (BioTek, Winooski, VA, USA) with Bradford reagent (Protein Assay Kit, BIO-RAD Laboratories, Hercules, CA, USA).

Zymography

Gelatin zymography was used to quantify total isoforms of MMP2 and MMP9 proteins. 10% Novex precast SDS polyacrylamide gels (Life Technologies, Novex, Carlsbad, CA, USA) in the presence of 0.1% gelatin were used under nonreducing conditions. A 35-µL zymography sample was prepared with previously harvested protein lysates (4 µg), Tris-glycine-SDS sample buffer (Life Technologies, Novex, Carlsbad, CA, USA) (15 µL), and deionized water (remaining volume). Deionized water was used as a negative control. Zymography standards of active human isoforms of MMP2 (molar mass: 66 000) and MMP9 (molar mass: 67 000) were used as positive standards (EMD Millipore, Darmstadt, Germany). Protein standards were

Dog	Group	Percent reduction of corneal ulcer at study time points								
		0 hr	6 hr	24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr
1	A	0	22.99	58.04	77.00	99.23	99.40	100	100	100
2	A	0	7.57	33.50	48.77	81.01	87.37	97.89	98.57	100
3	A	0	22.46	62.66	75.42	94.89	96.73	98.94	99.76	100
4	B	0	18.93	62.88	69.76	90.53	93.88	99.67	99.74	100
5	B	0	13.30	57.38	73.77	96.81	98.42	99.69	99.93	100
6	B	0	13.19	57.69	83.01	99.37	99.68	100	100	100

Table 1. Percent reduction of corneal ulcer at study time points

run concurrently to approximate molecular weight. Samples were not heated prior to being loaded into the gel. Samples were loaded for SDS-PAGE with 1X Tris-glycine-SDS running buffer and were run at a constant voltage (125 V) for 120 min. Following electrophoresis, the gel was washed once in 1X zymogram renaturing buffer which was then decanted and the gel was next washed twice with 1X zymogram developing buffer. For the second washing, the gel was incubated at 37 °C overnight. For staining, the gel was washed with Simply Blue Safe stain (diluted 1:2 with deionized water) for 60 min. The stain was then decanted, and the gel was washed in deionized water for 24 h. Digital quantification of zymograms was performed using NIH IMAGE J software.

Statistical analyses

Statistical analysis was performed by a biostatistician (RWM). Summary statistics were expressed as a mean $\pm$ standard deviation. To analyze the clinical scores, percent reduction of the area of corneal ulceration and zymography results, a two-factor model was used. The factor of treatment group is a between-subjects factor while the factor of time is a within-subjects factor. Because it is difficult to assess the assumption of normality with the small amount of data available, a nonparametric approach was used. Specifically, a rank transformation was used to test for main effect group differences¹⁸ and an aligned rank transformation was used to test for group by time interactions¹⁹; $P < 0.05$ was considered significant.

RESULTS

Six hours following wounding, the highest scores of each treatment group were recorded as 11 for a dog in group A

and 10.5 for a dog in group B. At this time point, the mean mMS score of group A was 10.67 ± 0.29 and the mean mMS score of group B was 10.50 ± 0 . The mMS scores decreased throughout the study period, and at the study's end point, the mean mMS score of group A was 1.67 ± 0.14 and the mean mMS score of group B was 1.67 ± 0.14 . Regarding clinical scores, the main effect of treatment group was not significant ($P = 0.50$) and the group by time interaction term was not significant ($P = 0.77$). No difference in clinical scores between treatment groups at any time point was detected (see Table 2).

The total time required to achieve corneal re-epithelialization of the ulcer bed was not different between treatment groups; one dog in each group was healed at 72 h after wounding while the remaining four dogs were all healed at 96 h postwounding. Figure 2 is a composite of extraocular photographs documenting the clinical course of re-epithelialization in a representative dog from group A. When analyzing the percent reduction of ulceration, the main effect of treatment group was not significant ($P = 0.55$) and the group by time interaction term was also not significant ($P = 0.13$). No statistical difference between treatment groups in the rate of corneal wound healing was detected at any time point.

MMP2 and MMP9 were detected in baseline tears from the left eye. The expression of MMP2 and MMP9 was increased 6 h after wounding. The expression of both MMP2 and MMP9 in the tears from the left eye continued to increase until 24 h after wounding. Tears collected at 48, 72, and 96 h postwounding showed a dramatic decrease in the MMP2 and MMP9 expression. These trends in MMP2 and MMP9 expression in the tears from the left eye are shown in Figs 3 and 4, respectively. Despite a notable difference between the two treatment

Dog	Group	Modified McDonald-Shadduck Scores (0–40)								
		0 hr	6 hr	24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr
1	A	0	11	9	9	4	4	3	3	1.75
2	A	0	10.5	9	9	4	2.5	1.5	1.5	1.5
3	A	0	10.5	9	9	4	4	2.5	2.5	1.75
4	B	0	10.5	9	9	4	4	2.75	2.75	1.75
5	B	0	10.5	9	9	4	2.5	2.75	2.75	1.5
6	B	0	10.5	9	9	4.5	4.5	2.75	2.75	1.75

Table 2. Modified McDonald-Shadduck clinical scores at study time points



Figure 2. Composite extraocular photographs documenting the clinical course of corneal re-epithelization in a representative study dog from group A. This dog's ulcer was healed in 72 h.

groups in MMP2 and MMP9 expression at 24 h post-wounding with increased expression of MMP2 and MMP9 detected in group A compared with group B, the main effect of treatment group was not significant (MMP2, $P = 0.97$; MMP9, $P = 0.93$) and the group by time interaction term was not significant (MMP2, $P = 0.10$; MMP9 $P = 0.05$). Interestingly, MMP9 was not detected in tears of any dog at 96 h after wounding while MMP2 was found in the tears of two dogs from group B.

DISCUSSION

The addition of hyaluronic acid to standard therapy for canine corneal ulceration is controversial among veterinary ophthalmologists. Results of previous studies performed in other species suggest that hyaluronic acid accelerates epithelial corneal wound healing when compared to saline control.^{12–14,20} The results of this study found no difference in corneal re-epithelialization after superficial ulceration in dogs. Carboxymethylcellulose was utilized as the viscosity-neutral control in the current study, whereas PBS was used as the control in all prior investigations. Hyaluronic acid is a non-Newtonian fluid, similar to the precorneal tear film, and consequently demonstrates shear thinning behavior.^{11,21,22} Thus, hyaluronic acid is able to maintain a high viscosity at a low shear stress resulting in a prolonged precorneal residence time during the inter-blink. During blinking, high shear stress is created across the corneal epithelium and hyaluronic acid becomes less

viscous which allows the shear stress of blinking to be well tolerated.

In addition to its viscoelastic properties, the retention of hyaluronic acid on the cornea may also be the result of biochemical interactions.²³ Hyaluronic acid is a known ligand of CD44, a cell membrane glycoprotein which is normally found on corneal cells.²⁴ The expression of CD44 is upregulated in corneal epithelial wounds, a process which could further enhance the adherence of hyaluronic acid to the cornea.^{3,25} Conversely, PBS lacks these beneficial viscoelastic and biochemical properties. Phosphate-buffered saline acts as a Newtonian fluid, maintaining a constant viscoelastic state, regardless of shear stress and has a significantly shorter precorneal retention time compared to hyaluronic acid and other ocular lubricants.^{11,26} Due to these factors, utilizing PBS as a control in a study evaluating the efficacy of a hyaluronic acid in corneal wound healing is not appropriate in our authors' opinion.

An additional benefit of hyaluronic acid which PBS lacks is its ability to promote precorneal tear film stability.^{27,28} Precorneal tear film is needed for maintenance of a healthy ocular surface as it provides oxygen and various growth factors to the avascular cornea. Healthy precorneal tear film contains epidermal growth factor (EGF) and transforming growth factor-beta (TGF- β), two signaling molecules that play a critical role in corneal epithelial wound healing.^{29,30} The precorneal tear film aids in the mechanical removal of pathogens, foreign bodies, and debris through mechanisms of blinking and reflex tearing. It also contains numerous antimicrobial compounds, including conventional antimicrobial compounds such as lysozyme, lactoferrin, lipocalin, secretory immunoglobulin A, and complement, and also, more recently detected antimicrobial proteins include surfactant protein-D and members of the cationic antimicrobial peptide family.³¹ The superficial lipid layer of the precorneal tear film is thought to possess antimicrobial properties; in a recent study, it was demonstrated that meibomian lipids significantly inhibited bacterial growth and caused extensive damage to bacteria.³² As PBS does not promote precorneal tear film stability, it does not bolster corneal health and defense in a manner similar to hyaluronic acid and thus is further evidence that it is not an appropriate control for this type of study.

Due to structural, physiologic, and biologic differences between hyaluronic acid and PBS, carboxymethylcellulose was selected as control for this study. Carboxymethylcellulose is a negatively charged, cellulose-derived polysaccharide which demonstrates shear thinning behavior and subsequent prolonged precorneal retention times and increases precorneal tear film stability.^{33–36} Despite possessing similar characteristics to hyaluronic acid, previous studies comparing the efficacy of carboxymethylcellulose and hyaluronic acid in various ocular diseases produced conflicting results. Moreira *et al.* found that hyaluronic acid accelerates corneal epithelial wound healing in rabbits

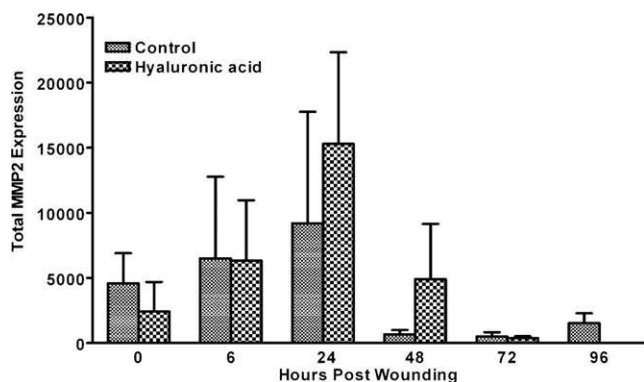


Figure 3. Changes in total MMP2 expression of the precorneal tear film of dogs being treated with control treatment (small checkered box) or hyaluronic acid (large checkered box). No significant difference was noted between treatment groups at any time point.

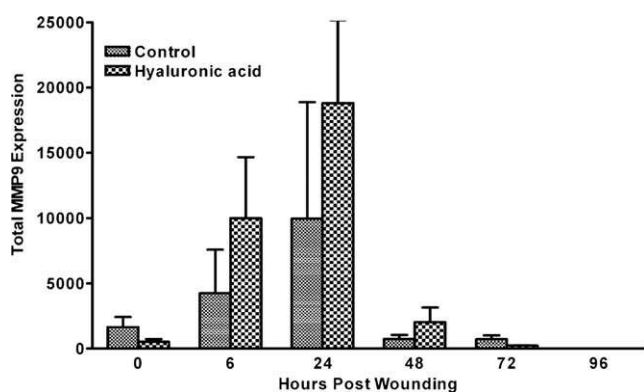


Figure 4. Changes in total MMP9 expression of the precorneal tear film of dogs being treated with control treatment (small checkered box) or hyaluronic acid (large checkered box). No significant difference was noted between treatment groups at any time point.

compared to carboxymethylcellulose while Lee *et al.* showed that both hyaluronic acid and carboxymethylcellulose significantly improved corneal and conjunctival vital staining, tear film break up time, and dry eye symptom scores but did not find a statistical difference between the two treatment groups.^{37,38} As carboxymethylcellulose is an anionic polysaccharide, with non-Newtonian rheological properties and prolonged precorneal residence times, it was considered a reasonable and appropriate control for the current study.

While neither hyaluronic acid nor carboxymethylcellulose altered the rate of corneal wound healing, administration of modified forms of these polysaccharides may promote rapid re-epithelialization of corneal ulceration. Specifically, a recently developed form of hyaluronic acid, known as cross-linked hyaluronic acid, has shown promise as a corneal wound healing accelerant and superior tear substitute.^{12,39} However, the previous studies assessing the efficacy of cross-linked hyaluronic acid in corneal wound healing used PBS as a control. Therefore, future studies should compare the efficacy of cross-linked

and non-cross-linked hyaluronic acid in corneal wound healing.

The current study not only evaluated the rate of wound healing but also the expression of MMP2 and MMP9. MMP2 and MMP9 are integral components of the natural wound healing process as these proteinases and their inhibitory proteins (tissue inhibitor of metalloproteinase) orchestrate the degradation and remodeling of ulcerated cornea.³⁹ A previous immunohistochemistry study investigating the presence of MMP2 and MMP9 in normal and ulcerated canine corneas demonstrated that MMP2 appears to be constitutively expressed in normal cornea and is upregulated in ulcerated corneas, while MMP9 is only expressed in ulcerated cornea.¹⁰ While intimately associated with the cornea, the precorneal tear film does not directly reflect the MMP content of the cornea. Wang *et al.* used zymography to demonstrate that MMP2 and MMP9 are found in the lacrimal fluid of normal canine eyes and that the expression of these MMPs increases in *Pseudomonasa aeruginosa*-associated keratitis.⁴⁰ In the current study, gelatin zymography was used to evaluate the expression of MMP2 and MMP9 in the precorneal tear film and results were consistent with the aforementioned study, as both MMP2 and MMP9 were detected in the lacrimal fluid of the left eye prior to epithelial debridement and their expression increased following corneal wounding. One difference between the current study and that by Wang *et al.* was MMP9 expression in the precorneal tear film of healed corneas. MMP9 was undetectable in the tears collected after corneal epithelialization was complete in the current study while Wang *et al.* found that MMP9 continued to be expressed in the lacrimal fluid of healed corneas. A possible explanation for this difference is the lack of exogenous proteinases in this study. *Pseudomonasa aeruginosa* produces alkaline protease and elastase and promotes the expression of endogenous proteases specifically MMP2 and MMP9.^{41–43} Here, ulcerated canine corneas were not infected by bacterial or fungal pathogens, and therefore without the influence of exogenous proteinase, production of MMP9 is likely not promoted.

Hyaluronic acid may have an effect on MMP expression. While the expression of MMP2 and MMP9 was not significant between treatment groups in this study, the MMP2 and MMP9 expressions of those dogs receiving hyaluronic acid were notably increased not only from baseline but also in comparison with those dogs receiving the control treatment, most notably at the 24-h time point. Hyaluronic acid can increase MMP2 and MMP9 expression and promote the conversion of these MMPs from their latent to their active isoform in various ocular tissues, including corneal epithelial cells *in vitro* and debrided corneas *in vivo*.^{44–46} In the current study, expression of total MMPs was measured. Hyaluronic acid may accelerate epithelial cell migration by increasing the expression of MMPs which help remodel the extracellular matrix of wounded corneas.

The authors acknowledge the small sample size used in the present study. Prior to study initiation, a power analysis was performed and it was determined that 12 animals per group would be required to detect a clinically significant difference in healing rates of 12 h with a power of 0.8 and $\alpha = 0.05$. A study size of 24 dogs was deemed impractical given concerns of animal resources for this pilot study.

Another potential limitation of this study was the age of the canine subjects. The dogs used in this study were approximately 1 year old and considerably younger than many clinical canine patients who present with nontraumatic, superficial ulcerative keratitis.^{47,48} With age, the diameter of corneal epithelial cells enlarges and keratocytes decrease in number.⁴⁹ Additionally, SCCED patients, with a mean age of 9 years,⁴⁷ develop an atypical hyaline membrane in the anterior stroma. These various, age-related changes to the epithelial cells and underlying extracellular matrix could alter the manner in which viscoelastic agents interact with the surrounding ocular environment and affect corneal wound healing.

A third possible limitation was our choice of control. The ideal control would only contain vehicle; however, if topical vehicle was utilized, it would have lacked essential rheological properties and been similar to PBS. Although carboxymethylcellulose shares rheological properties similar to those of hyaluronic acid, previous studies suggest that it too may promote wound healing.⁵⁰ Future studies could utilize not only hyaluronic acid and carboxymethylcellulose but also other topical tear substitutes to assess the effect of different viscoelastic substances in corneal wound healing.

CONCLUSION

In summary, the results of this study indicated that while a topical ophthalmic agent containing hyaluronic acid is well tolerated, the addition of this viscoelastic polysaccharide does not accelerate corneal wound healing when compared to a control with similar viscosity and biochemical properties.

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