Short Communication

Lack of effect of a topical regenerative agent on re-epithelialization rate of canine spontaneous chronic corneal epithelial defects: A randomized, double-masked, placebo-controlled study

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Abstract

Spontaneous chronic corneal epithelial defects (SCCEDs) are characteristic ulcers in dogs that are refractory to healing. The aim of the study was to evaluate the use of a topical regenerative agent to promote healing of SCCEDs. Nineteen dogs (20 eyes) were randomized to receive either regenerative agent (10 eyes) or placebo (10 eyes) every 48 h following corneal debridement, which was repeated 1 week later if the SCCED had not yet healed. The mean ± standard deviation time to re-epithelialization was 17.3 ± 12.8 days for the group treated with a topical regenerative agent and 19.3 ± 11.7 days for the group treated with a placebo; the cumulative healing rates were not statistically different ($P > 0.650$). A positive association was found between the initial size of the ulcer and the time to re-epithelialization ($r = 0.555$, $P = 0.011$). Although well tolerated by dogs, there was no therapeutic advantage in using a topical regenerative agent for re-epithelialization of SCCEDs.

Keywords: Canine; Spontaneous chronic corneal epithelial defect; Indolent ulcer; Debridement; Topical regenerating agent

Spontaneous chronic corneal epithelial defects (SCCEDs) are a common ophthalmic disorder in dogs, characterized by ulcers that fail to resolve through normal epithelial wound healing (Bentley, 2005). Although the pathophysiology of SCCEDs is not fully understood, there is mounting evidence that the extracellular matrix subjacent to the epithelial defect is responsible for the failure to heal (Bentley, 2005). Cacicol (Laboratoires Théa), a member of the regenerating agents family, is a chemically engineered polymer that is designed to mimic and replace degraded heparan sulfate in the injured extracellular matrix (Barritault et al., 2017), and might therefore be promising for promoting epithelial healing in SCCEDs. We hypothesized that canine eyes treated with Cacicol would achieve a faster corneal re-epithelialization than placebo-treated eyes.

The study was a prospective, randomized, double-masked, placebo-controlled clinical trial, approved by the Institutional Animal Care and Use Committee of Iowa State University (protocol number 9-16-8356-K; date of approval 29 September 2016). Eligible dogs were randomly assigned (Excel 2016, Microsoft) to receive either Cacicol or placebo. The placebo was preservative-free 0.3% hyaluronan (iDrop Vet Plus, I-Med Animal Health), chosen based on shared properties with Cacicol, i.e. colorless, viscous and preservative-free. The trial drugs were provided by the pharmacist in batches of 1 mL syringes containing 0.15 mL of the drug, and the identity of each batch was masked to both owners and study investigators.

Eligible subjects were dogs who were diagnosed with a SCCED by an ophthalmology clinician based on previously established criteria (Bentley, 2005). Subjects were excluded if they had a Schirmer tear test < 15 mm/min or had previously undergone a stromal altering procedure. All dogs underwent a complete ophthalmic examination, including slit-lamp biomicroscopy

(Kowa SL-17), indirect fundoscopy (Keeler Vantage), Schirmer tear test-1 (Schering-Plough Animal Health), rebound tonometry (TonoVet, Lumic International) and fluorescein staining (Ful-Glo, Akorn).

The study design is described in the supplementary material (see Appendix). A Jameson caliper was used to calculate the area of each ulcer (in mm^2) by multiplying the longest linear dimension by the largest dimension perpendicular to it. The ocular surface was rinsed with 1:50 dilute povidine iodine solution (Betadine, Purdue Frederick Company) and a drop of 0.5% proparacaine (Akorn) was applied. Sterile cotton-tipped applicators (CTAs) were used to remove loose corneal epithelium. The area of each ulcer following the procedure similarly was recorded in mm^2.

A drop of 1% atropine ophthalmic solution (Bausch & Lomb) was instilled for cycloplegia, followed 10 min later by the entire content of the masked syringe (0.15 mL). At home, dogs were administered the topical trial drug (Cacicol or placebo; 0.15 mL every 48 h) and oxytetracycline-polymyxin B (1/4" strip every 8 h; Terramycin, Pfizer Animal Health), separated in time by at least 30 min. Dogs also received either carprofen (2.2 mg/kg perorally every 12 h; Rimadyl, Pfizer Animal Health) or meloxicam (0.1 mg/kg perorally every 24 h; Metacam, Boehringer Ingelheim) to control for discomfort and reflex uveitis, and an Elizabethan-collar was placed.

Ophthalmic examination and application of the fluorescein dye were repeated approximately 1 week after the initial debridement. If the ulcer was still present, a CTA debridement was repeated as described above, and the same therapy was repeated. An independent

staff member ensured the dog received the same trial drug as for the first week, while both investigator and owner remained masked. If the ulcer was still present at the second visit (week 2), the trial drug was discontinued and a procedure other than CTA debridement was performed. Such dogs underwent diamond burr debridement, grid keratotomy, or a combination of both (Bentley, 2005; Gosling et al., 2013; Wooff and Norman, 2015), and post-procedure therapy was left to the clinician's discretion. No bandage contact lens was placed in any dog during the study. Statistical analysis was performed using SigmaPlot version 13.0 (SPSS) and values of $P \leq 0.05$ were considered to be significant.

A total of 19 dogs (20 eyes) were enrolled in the study, supported by a power calculation aimed to detect a mean difference in healing time of 11 days, a standard deviation of 5 days, a power of 80% and an α value of 0.05, assuming that Cacicol would be as effective as grid keratotomy (Stanley et al., 1998; Wooff and Norman, 2015). Various breeds were represented, including Boxer (n = 4), Shih Tzu (n = 3), Chihuahua (n = 2), and one each of Cairn terrier, Yorkshire terrier, Labrador retriever, Golden retriever, Golden Retriever-Poodle cross, English bulldog, American bulldog, English Springer spaniel, Boston terrier and Jack Russell terrier. One dog was randomly assigned to Cacicol treatment in the left eye, then received placebo in the right eye when it developed another SCCED 10 months later.

Median (mean ± standard deviation; range) time to ulcer re-epithelialization was 12 days (17.3 ± 12.8; 7-42 days) for dogs treated with Cacicol and 19 days (19.3 ± 11.7; 7-42 days) for the placebo-treated group; this difference in healing time was not statistically significant (Mann-Whitney test; P = 0.701; Fig. 1). A moderate positive association was found between the initial

size of ulceration (pre-debridement) and the time to ulcer re-epithelialization (Spearman's correlation test; $r = 0.555$, $P = 0.011$; Fig. 2). The cumulative proportion of SCCEDs that had healed at each visit is represented in Fig. 3. At the first recheck (mean 7 days, range 6-9 days), 50% of eyes treated with Cacicol and 30% of placebo-treated eyes healed. The percentage of healed SCCEDs increased to 60% and 50% at the second recheck (mean 14 days, range 14-15 days), 80% and 90% at the third recheck (mean 24 days, range 20-28 days), and 100% at the last recheck (mean 38 days, range 35-42 days) for Cacicol and placebo groups, respectively. Differences in cumulative healing rates at each recheck were not statistically significant (Fisher's exact test; $P \geq 0.650$). There were no statistical differences between Cacicol-treated and placebo-treated groups with respect to right versus left eye affected, Boxer breed and sex (Fisher's exact test; $P \geq 0.58$), age, body weight, Schirmer tear test-1 values, duration of ulceration prior to referral, size of the ulcer pre-debridement and size of the ulcer post-debridement (Mann-Whitney test; $P \geq 0.198$) (see Appendix: Supplementary material).

Cacicol is a bioengineered compound that replaces degraded heparan sulfate in the damaged cornea and which has been shown to bind to extracellular matrix proteins, protecting them from proteolysis, enabling growth factors and cytokines to act on the injured site, and restoring a microenvironment conducive to tissue repair (Barritault et al., 2017). Cacicol has been used with good success as an adjunct therapy for several ocular surface diseases in human patients, including persistent epithelial defects (Chebbi et al, 2008; Kymionis et al., 2014), neurotrophic ulcers (Aifa et al., 2012; Arvola et al., 2016; Guerra et al., 2017) and corneal defects post-photorefractive keratectomy (Aslanides et al., 2015). However, the present study did not find a therapeutic advantage of using Cacicol for accelerating epithelial healing in canine SCEEDs when

administered every 48 hours for 14 days. The use of Cacicol as an adjunct therapy to corneal debridement for SCCED resulted in a healing rate of 60% after 2 weeks of therapy, a finding that was not statistically different from placebo-treated eyes (50%). Of note, all the canine eyes that failed to heal with corneal debridement have subsequently re-epithelialized with a diamond burr and/or grid keratotomy, and not a single case developed bacterial keratitis or other complications. Thus, it is appropriate to consider corneal debridement as a first-line therapy for SCCED, since it provides the opportunity to initiate antimicrobial therapy and results in a reasonable success rate of 50-60%, consistent with findings of a meta-analysis (Bentley, 2005). This is especially true for small surface area SCCEDs, given the positive association found between ulcer size and healing time in the present study.

The present study has some limitations. It is possible that every other day administration of Cacicol was not the optimum dosage for treating canine SCCEDs. Although the manufacturer's labeled dosage is once a week, the frequency of Cacicol administration varies greatly among studies, from twice daily (Aslanides et al., 2015), once daily (Kymionis et al., 2014), once every 2 days (Aifa et al., 2012; Arvola et al., 2016) to once (Chebbi et al., 2008) or twice a week (Guerra et al., 2017). The frequency and timing of administration seem to be critical and have to reflect the stage of healing (Barritault et al., 2017); in fact, too much Cacicol may be counter-productive for corneal healing, since the excess may compete with heparan-binding growth factors in the extracellular matrix (Barritault et al., 2017). The sample size was small and the timing of rechecks may have attenuated potential differences between groups that might have been identified if the dogs had been evaluated more frequently. Finally, our study focused primarily on the rate of re-epithelialization and did not evaluate other key parameters of corneal healing for which Cacicol

may be beneficial, such as ocular pain (Aslanides et al., 2015), stromal edema and haze (Brignole-Baudouin et al., 2013; Xeroudaki et al, 2016).

Although well tolerated by dogs, there was no therapeutic advantage of using Cacicol for accelerating re-epithelialization of SCCED. Future studies could evaluate the use of Cacicol for neurotrophic keratopathy in veterinary species, a particularly frustrating disease with no ideal therapeutics to date.

Conflict of interest statement

None of the authors of this paper have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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Figures

Fig. 1. Box-and-whisker plots showing the time to re-epithelialization of spontaneous chronic corneal epithelial defects treated with either Cacicol (dark gray) or placebo (light gray). Median values are shown by a horizontal line. First and third quartiles (25th and 75th percentiles) are represented by the lower and upper limits of the box, respectively. The minimum and the maximum values are shown as the lower and upper whiskers, respectively.

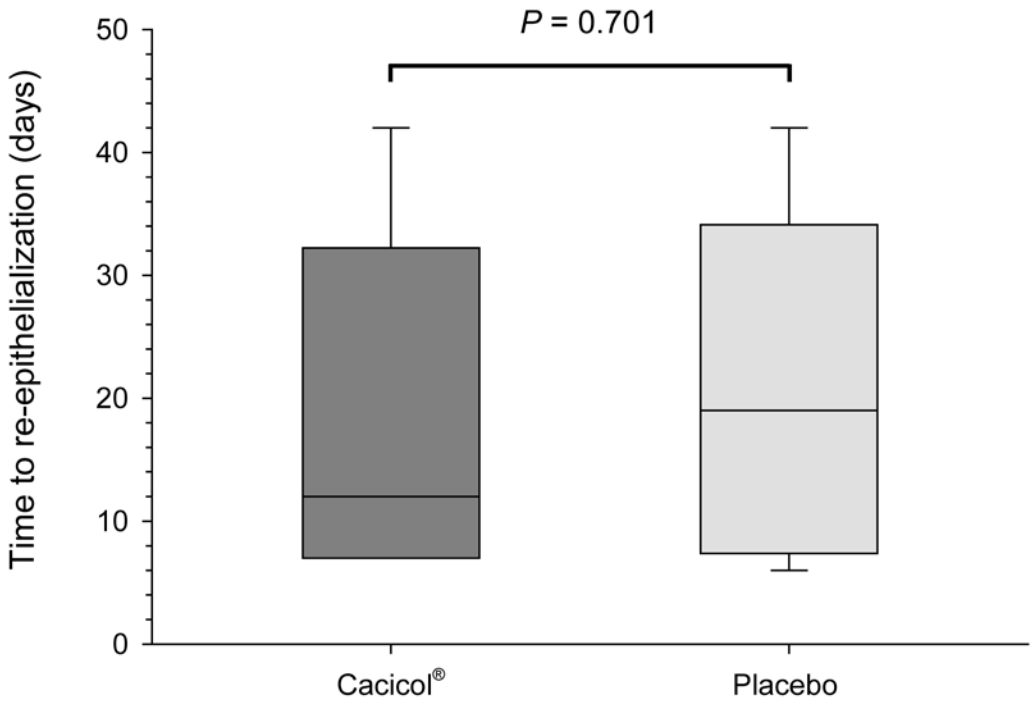


Fig. 2. Scatterplot showing a positive correlation between the initial size of ulceration (pre-debridement) and the time to ulcer re-epithelialization (Spearman's correlation test; $r = 0.555$, $P = 0.011$).

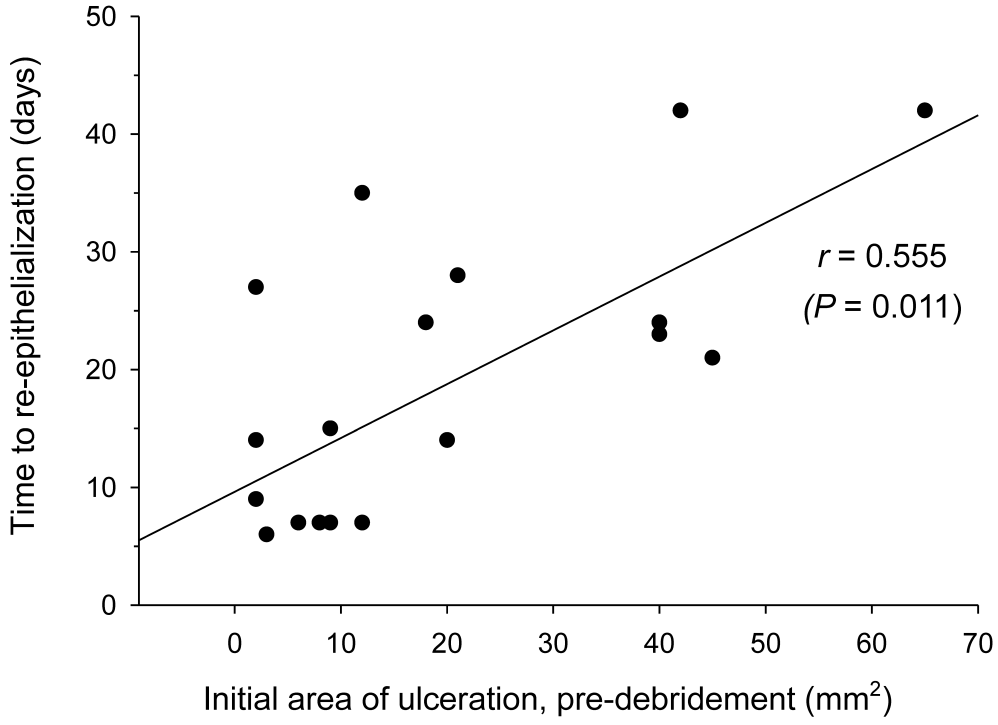
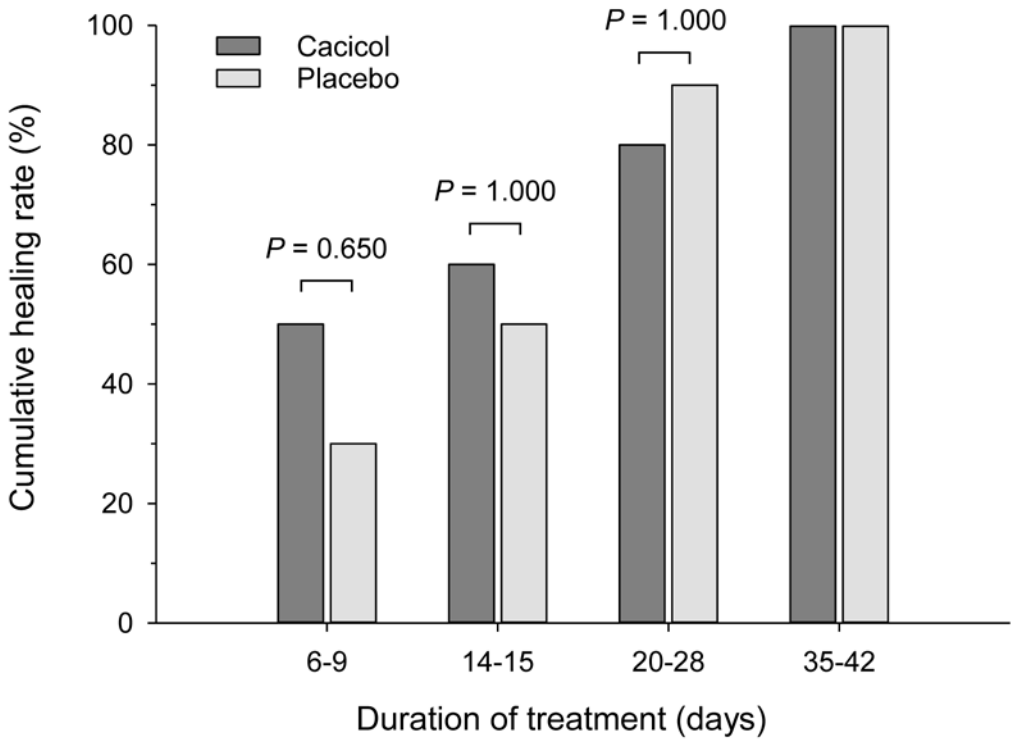
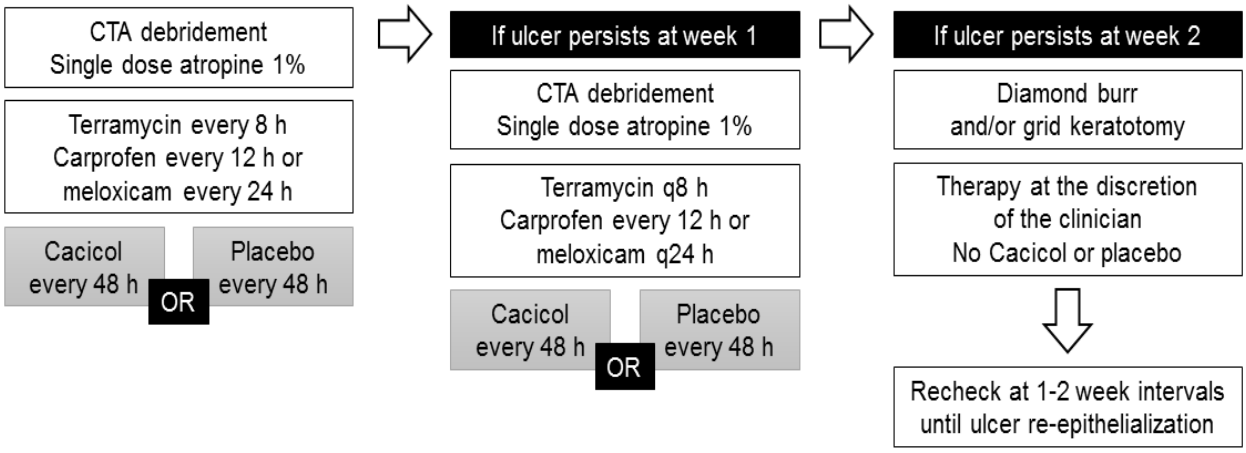


Fig. 3. Bar chart showing the comparative cumulative healing rate of spontaneous chronic corneal epithelial defects over several visits in dogs treated with either Cacicol or placebo.



Appendix 1. Diagram of the study design used to treat 19 dogs (20 eyes) with spontaneous chronic corneal epithelial defects, randomized to receive either Cacicol (10 eyes) or placebo (10 eyes) once every-other-day following cotton-tipped applicator (CTA) corneal debridement. If the ulcer failed to heal within 2 weeks, the study drug was discontinued and the eye was treated with a stromal altering procedure (diamond burr and/or grid keratotomy).



Appendix 2. Baseline characteristics and descriptive statistics of the study participants. Nineteen dogs (20 eyes) with spontaneous chronic corneal epithelial defects were randomized to receive either Cacicol (10 eyes) or placebo (10 eyes) once every-other-day following corneal debridement.

	Cacicol	Placebo	*P* value
Breed (number of dogs) Boxer Non-Boxer	 3 7	 1 9	0.582
Sex (number of dogs) Male Female	 4 6	 3 7	1.000
Eye affected (number of eyes) Right Left	 6 4	 5 5	1.000
Age (years)	9.5 (7-11)	9.5 (7-11.5)	0.907
Body weight (kg)	12 (3.2-42.8)	29.1 (3-48)	0.438
Schirmer tear test-1 (mm/min)	23.5 (19-35)	24 (18-28)	1.000
Duration of ulcer prior to referral (days)	14 (7-35)	14 (7-42)	0.816
Size of ulcer pre-debridement (mm^2)	12 (2-65)	15 (2-42)	0.790
Size of ulcer post-debridement (mm^2)	115.5 (20-240)	70 (8-210)	0.198