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Use of episcleral cyclosporine implants in dogs with keratoconjunctivitis sicca: pilot study

Laura Barachetti,* Antonella Rampazzo,† Carlo M. Mortellaro,* Stefania Scevola* and Brian C. Gilger‡

**Department of Veterinary Science and Public Health, Faculty of Veterinary Medicine, University of Milan, Milan, Italy; †Istituto Veterinario di Novara, Granozzo con Monticello, Italy; and ‡North Carolina State University, Raleigh, NC, USA*

Address communications to:

L. Barachetti

Tel.: +39 347 9530911
Fax: +39 02 503 17817
e-mail: laurabarachetti@yahoo.it

Abstract

Purpose To describe the use, tolerability, and efficacy of episcleral silicone matrix cyclosporine (ESMC) implants in dogs with keratoconjunctivitis sicca (KCS).

Methods Retrospective study. ESMC implants (1.9 cm length, 30% wt/wt CsA in silicone; with approximately 12 mg of CsA loaded into them) were used in dogs with KCS responsive to topical CsA (good candidate, GC) or not responsive (poor candidate, PC). Ocular surface inflammation scores, Schirmer tear test (STT) values, and ocular discharge quantity were evaluated and compared.

Results Twenty-seven eyes (15 dogs) received an ESMC implant for KCS; 15 eyes were considered GC, and 12 were considered PC. Both GC eyes and PC eyes showed a significant increase in STT values (increase of 7.7 and 8.5 mm/min; $P = 0.023$ and $P = 0.003$, respectively) after placement of ESMC implants (mean follow-up 18 ± 2 and 10.4 ± 15 months, respectively). Clinical signs improved significantly in both groups during the same follow-up, with reduction in conjunctival hyperemia ($P < 0.001$), corneal neovascularization ($P = 0.004$), corneal opacity ($P = 0.003$), and ocular discharge ($P = 0.002$). ESMC implants were well tolerated by all dogs, but two eyes lost the device at 12-months and 1-week follow-up, respectively.

Conclusions Results from this study suggest that the EMSC implants were well tolerated and efficacious in dogs with KCS responsive to topical CsA as well as dogs with poor response to topical therapy. Further study is needed to determine the duration of efficacy and optimal dose of CsA.

Key Words: cyclosporine, dog, dry eye, implant, keratoconjunctivitis sicca, sustained release delivery

INTRODUCTION

Keratoconjunctivitis sicca (KCS) is a common ocular disease in the dog.[1,2] It is characterized by aqueous tear deficiency resulting in desiccation and inflammation of the conjunctiva and cornea, ocular pain, and reduced vision.[1,2,3] While the list of possible causes of KCS is extensive, in many cases, the definitive cause is not determined.[1,2] Histopathologic studies of the lacrimal tissue from dogs affected with idiopathic KCS have revealed varying degrees of lymphocytic–plasmacytic cellular infiltrate and acinar atrophy, thereby suggesting an immunologic basis for the disease.[1,2] KCS may be associated with systemic autoimmune conditions, but canine KCS appears to occur more often as a tissue-specific immune-mediated disorder.[1,2,4,5] In animal models of immune-mediated lacrimal disease, the balance between T-suppressor and T-helper cells plays an important role in lacrimal gland regulation. In lacrimal tissue, T-suppressor cells normally predominate, but in immune-mediated KCS, T-helper cells become the prevalent T lymphocytes.[1,2] Therefore, by inhibiting T-helper cells in KCS, CsA may allow T-suppressor cells to sustain normal lacrimal function and stimulate tearing.[4,6–9] Therefore, CsA has both immunomodulating and tear-stimulating properties.[10–15] Topical ocular therapy with CsA in dogs with KCS is generally recommended every 8–12 h, several weeks of continuous treatment are usually needed before substantial improvement in Schirmer tear test (STT) values and/or clinical signs is observed.[9,15–19]

Sustained release ocular implants have been developed over the past decade that allows delivery of constant

therapeutic levels of drug to the eye.[20–25] Ocular implants are particularly useful in the treatment of chronic ocular problems, such as horses with equine recurrent uveitis (ERU) or immune-mediated keratitis (IMMK),[26–31] or dogs with KSC or chronic superficial keratitis (CSK).[32–35] This sustained release ocular drug delivery technology has also the advantage to eliminate or minimize the effect of patient and/or owner noncompliance in drug administration.[20,21,26]

Several types of ocular drug delivery methods have been described, such as solid implants (e.g., silicone), biodegradable implants (PLGA, chitosan, etc.), thermosensitive injectable gels, and microparticle/nanoparticle suspensions.[20–23,27,29,31,35–39] The method of ocular drug delivery must correlate with the intended disease in terms of site of drug target and duration of effect.[20,21,23,25] In general, subconjunctiva/episcleral implantation is used for anterior segment diseases,[26,32–34,40] whereas intravitreal or suprachoroidal methods are typically used to treat posterior segment diseases.[27–31] Intrascleral implants can be used for either ocular segment.[36]

Silicone matrix episcleral implants have been demonstrated to deliver drugs such as CsA to the cornea in a sustained release manner and are in development in humans for the treatment of high-risk corneal transplants and graft-versus-host disease.[40] These implants have been described for the long-term treatment of bilateral keratoconjunctivis sicca in a red wolf.[32,34,40] Episcleral silicon matrix cyclosporine implants allow sustained release of CsA below toxic levels and allow higher concentrations of the drug than topical therapy without systemic side effects.[21,32] These implants measure 1.9 cm by 2 mm by 1 mm, and contain approximately 12 mg of CsA.[26,32–34] CsA release has been determined *in vitro* at 27 μg/day of CsA for the first month, followed by a steady state release of 15 μg/day for the following 2–3 months, and an average of about 17 μg/day for the first 6 months.[32,40] The estimated duration of release *in vitro* is 18–24 months.[32,40]

The purpose of this retrospective study was to describe the use of episcleral silicone matrix cyclosporine (ESMC) implants in dogs with KCS, with evaluation of tolerability and retention of implants and their efficacy for management of KCS.

MATERIALS AND METHODS

Criteria for selection of cases

This retrospective study was performed by reviewing records of dogs diagnosed with immune-mediated KCS and implanted with one ESMC implant from 2010 to 2013 at North Carolina State University, University of Milan and Istituto Veterinario di Novara. The North Carolina State University Institutional Animal Care and Use Committee and the Veterinary Health Center Hospital Board approved this study for dogs enrolled at North Carolina State University. The cases were selected to be implanted based on the discretion of the attending ophthalmologist. All the dogs were considered affected by immune-mediated KCS, because all the other possible KCS etiologies were excluded. Dogs with residual STT values of >5 but <10 mm/min were considered good candidates (GC) for treatment, while dogs with STT of 5 mm or less were considered poor candidates (PC) for treatment. The GC dogs were usually included, because the owners could not afford treatment with more conventional topical therapy or because of the patient and/or owner noncompliance in drug administration. The PC received previously topical therapy with cyclosporine or tacrolimus without any improvement. Both groups of dogs were implanted and followed in response to therapy.

All dog owners signed a written consent before initiation of this experimental procedure and were fully informed that long-term outcome, complications, and efficacy of the implants in dogs with KCS were not known.

Implant manufacturing

The implants were made as described previously, using a polytetrafluoroethylene mold with impressions on the surface.[32] Implants measured 1.9 cm long, 2 mm wide, and 1 mm high, and they have a rounded side and flat side. Cyclosporine powder (Xenos Bioresources, Inc., Santa Barbara, CA) was mixed with medical grade silicone with a platinum cure system (Nusil Technology, Carpinteria, CA) so that the weight of the drug as a percentage of the total weight of the implant (wt/wt) was 30%, resulting in approximately 12 mg of cyclosporine loaded into each implant. The impressions were filled with the cyclosporine-silicone paste and cured for a minimum of 24 h at room temperature. The implants were sterilized with gamma irradiation (25–30 kGy).

Procedures

The surgical implantation was performed while the dogs were under general anesthesia. After surgical aseptic preparation of the eye, a 3-mm incision was made through the conjunctiva and episcleral tissue, 3–5 mm posterior to the dorso-temporal limbus. A pocket was formed in the episcleral space parallel to the limbus, and one implant was placed into this pocket. The flat side of the implant was applied to the episclera and the rounded side toward the overlying conjunctiva. The conjunctiva and Tenon's capsule were closed with a single interrupted or cruciate absorbable suture. Following surgery, a topical broad-spectrum antibiotic was applied for 5–7 days, and topical cyclosporine and/or artificial tears prescribed previously to manage the dog's KCS were continued for 30 days, then discontinued.

Schirmer tear test values were recorded before starting the topical therapy (t −1), at the time of surgery (t 0) and during the follow-up time. Degree of ocular discharge, conjunctival hyperemia, corneal neovascularization, corneal opacity was all scored from 0, if absent, to 4, if severe, at the same times as STT was evaluated. In partic-

ular, the ocular discharge was graded as 1 if only poor and mucous, 2 if moderate and mucous, 3 if moderate and mucous-purulent, and 4 if abundant and purulent. Conjunctival hyperemia was considered 1 if poor, 2 if moderate, 3 if severe, and 4 if severe with easily bleeding conjunctiva. Corneal neovascularization and corneal opacity were graded 1 if the involved cornea was <25%, 2 if more than 25% but <50%, 3 if more than 50% but <80%, 4 if more than 80%. Recommended follow-up examinations after the implant were 1, 2, and 4 weeks, then monthly.

Data analysis and statistical methods

Parametric normally distributed data (i.e., age, disease duration, follow-up time, STT) were compared by time point for each group using one-way ANOVA models with Tukey–Kramer *post hoc* analysis. In dogs that had both eyes treated, there were no significant differences in overall mean of values or grades between the right and left eyes; therefore, data from these eyes were averaged to give one value per dog to eliminate between-eye correlation. For nonparametric data (i.e., gender), Wilcoxon tests were conducted per animal by time point. Differences were considered significant at $P < 0.05$. Results and probabilities were calculated using computerized statistical software (JMP 10; SAS Inc., Cary, NC).

RESULTS

Overall, 27 eyes of 15 dogs were treated with ESMC implants for KCS, 15 eyes (seven dogs) were considered GC for CsA therapy, and 12 eyes (eight dogs) were considered PC. Several breeds were represented, including two shih tzu, two English bulldog, two mixed, one Border Collie, one Yorkshire T, one Jack Russel terrier, one West highland white terrier, one Maltese, one Dogue de Bordeaux, one Tibetan Spaniel, one Lhasa Apso, and one German Shepherd. There were five males, three neutered males, five females, two spayed females. Mean age of dogs treated was 5.63 ± 3.41 (standard deviation) years. The mean follow-up was of 18 ± 2 months for eyes considered GC and 10.4 ± 15 months for eyes considered PC.

Over the follow-up period, complications or signs of toxicity associated with implants or CsA were not observed. The devices were well tolerated and retained by the dogs, except for an implant lost 1 week after implantation and another lost at 12 months after surgery.

Statistically significant increases in STT values after implantation compared with the STT prior to surgery (baseline) were observed in both GC and PC groups (Fig. 1). The maximum STT increase was achieved at 90 days after surgery in both groups, including a 7.7 mm/min STT increase in the GC and an 8.5 mm/min STT increase in PC eyes compared with baseline. However, by 330 days in the GC eyes and 300 days in the PC eyes, there was no significant improvement in STT compared with baseline (Fig. 1).

All the clinical scores showed a significant improvement from 60 to 90 days, which remained significant up for 480–540 days (Figs 2–6). Dogs that showed severe preoperative conjunctival hyperemia, the presence of a mucous-purulent discharge, and severe corneal opacity and neovascularization had an improvement in the clinical signs after implantation, with significant reduction in the inflammatory scores at 60 with further improvement by 90 days FU (Fig. 2).

DISCUSSION

In this study, we describe the results of the use of solid, silicone matrix episcleral implants designed to release cyclosporine in therapeutic concentrations to the ocular surface for an extended period of time for the treatment of KCS in dog.

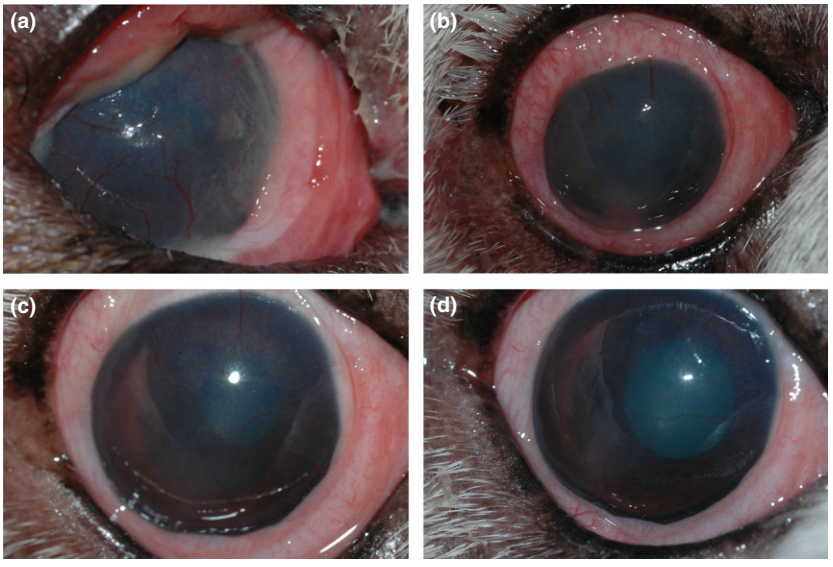

Figure 1. One of the PC cases, the eye of an English bulldog. (a) In the preoperative picture of the eye, there were severe conjunctival hyperemia, the presence of a mucous-purulent discharge and severe corneal opacity and neovascularization. (b) Then it is possible to appreciate the improvement of the clinical signs over the FU periods with significant reduction in the inflammatory scores at 60 (c) and better at 90 (d) days FU. PC, poor candidate.

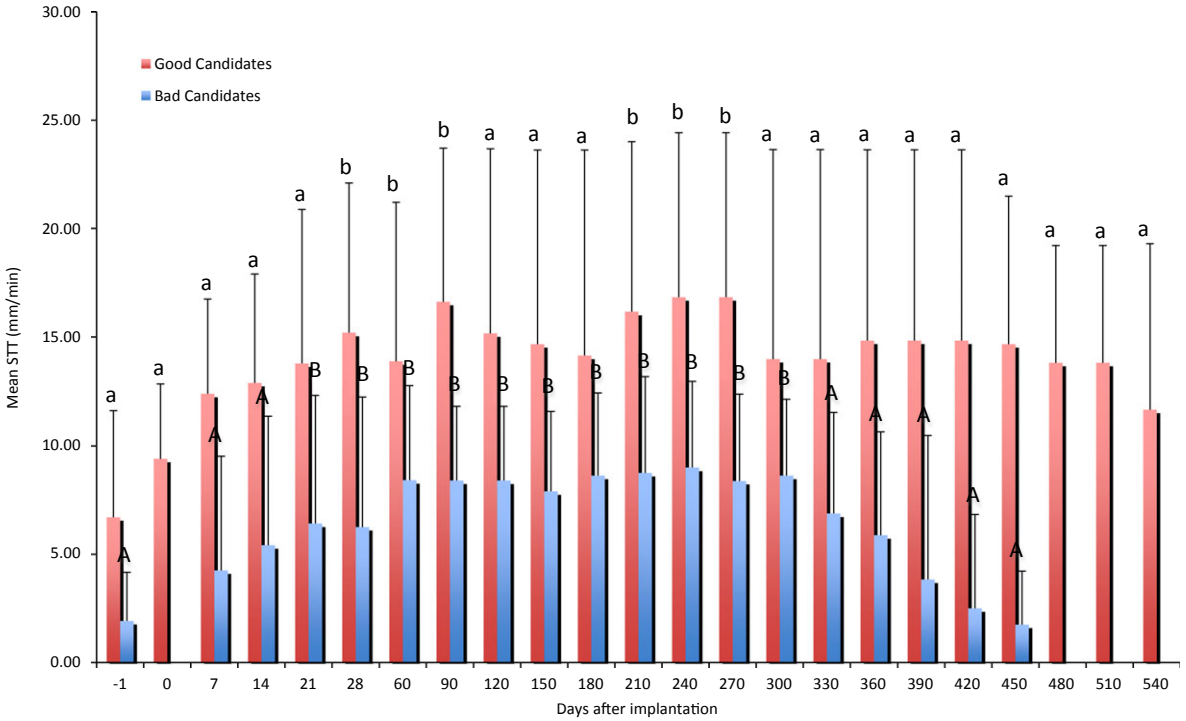

Figure 2. A statistically significant increase in Schirmer tear test (STT) values was achieved in both good candidate (GC) and poor candidate (PC) groups. The maximum increase was detected at 90 days in both groups, and was of 7.7 mm/min in the GC ($P = 0.023$) and 8.5 mm/min in PC ($P = 0.003$). A significant reduction in the STT started from 330 days in the GC and 300 days in the PC. Means with different letters are significantly different ($P < 0.05$).

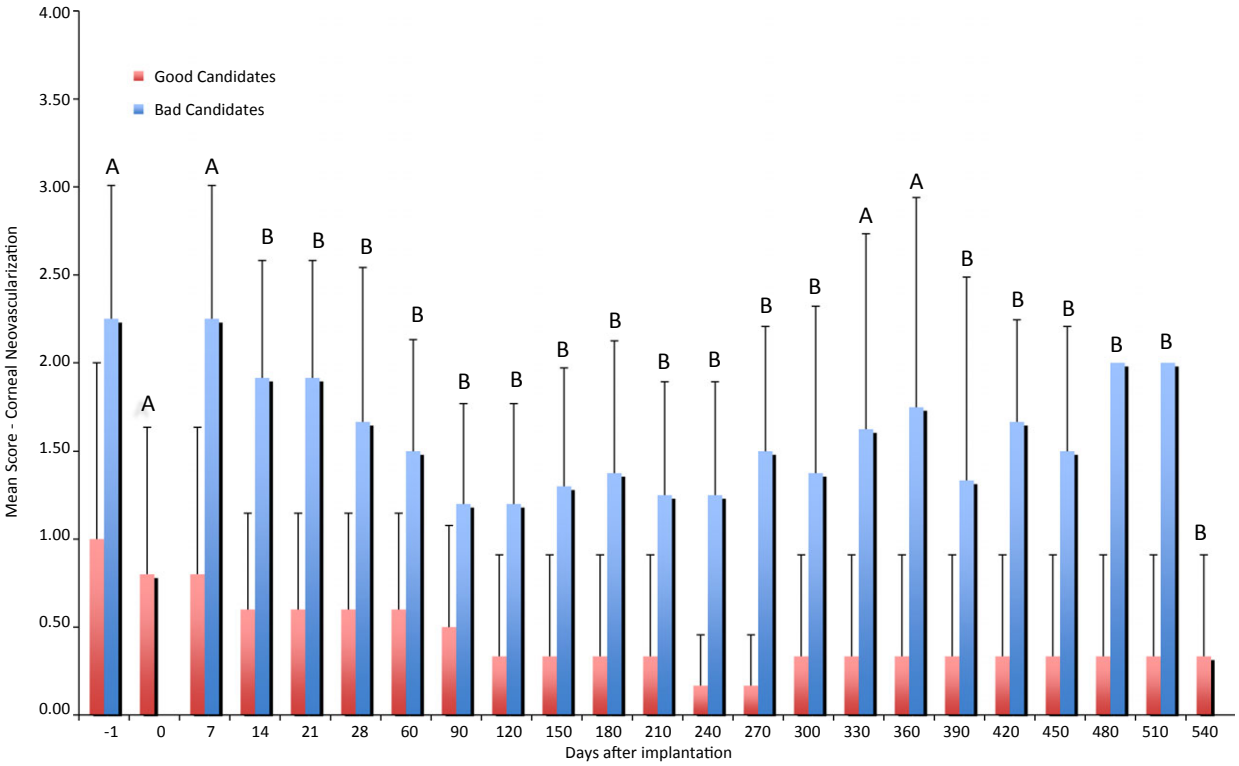

Figure 3. Overall the maximum statistically significant reduction in corneal neovascularization was achieved at 90 days ($P = 0.004$) and remained significant up for 540 days. Means with different letters are significantly different ($P < 0.05$).

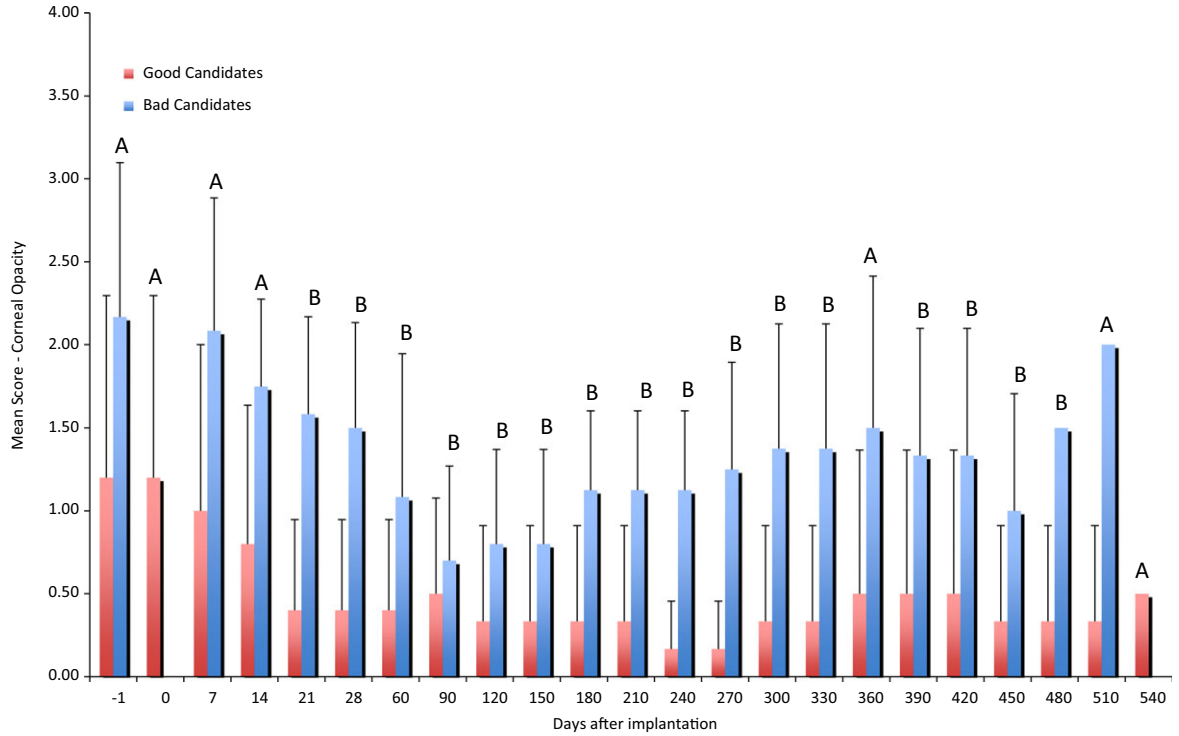

Figure 4. Overall the maximum statistically significant reduction in corneal opacity was achieved at 90 days ($P = 0.003$) and remained significant up for 480 days.

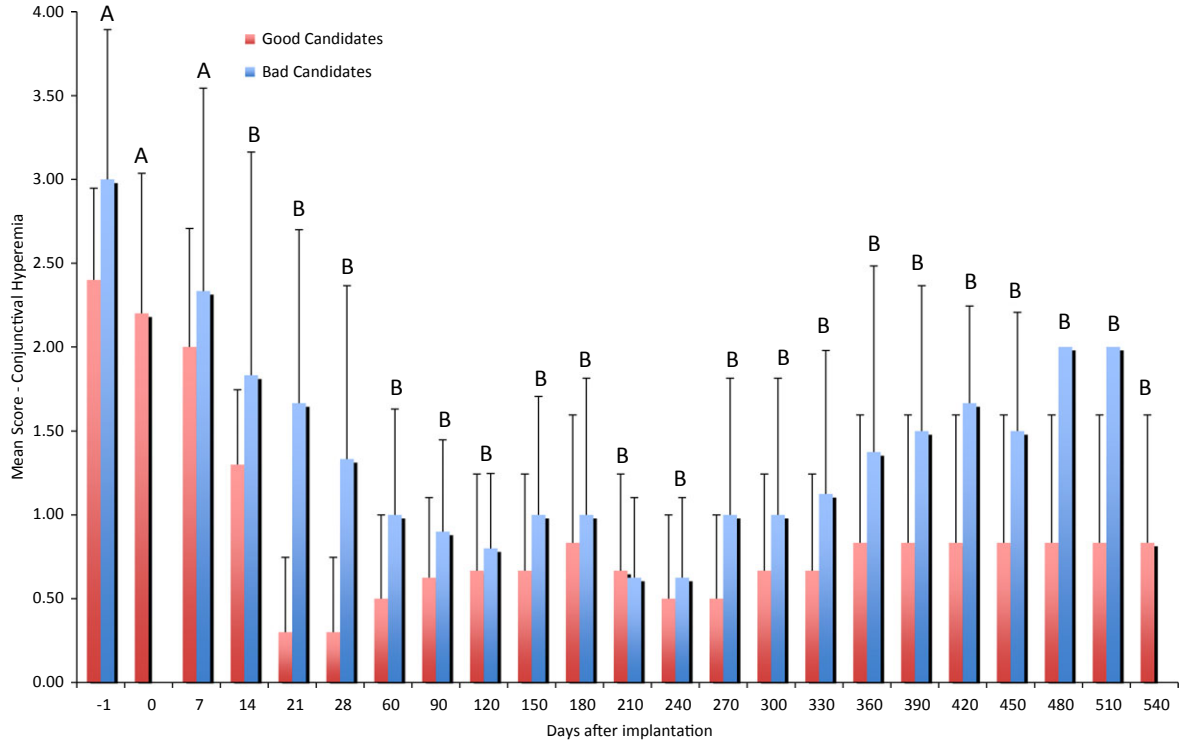

Figure 5. Overall the maximum statistically significant reduction in conjunctival hyperemia was achieved at 60 days ($P < 0.001$) and remained significant up for 540 days. Means with different letters are significantly different ($P < 0.05$).

In a preclinical evaluation of silicone matrix episcleral implants, Kim *et al.*[32] evaluated the toxicity associated with two devices placed into the superotemporal episcleral space of rabbits and dogs. Over a 6-month period, clinical examinations showed no signs of ocular toxicity and only one cyclosporine implant (of 24 total) was

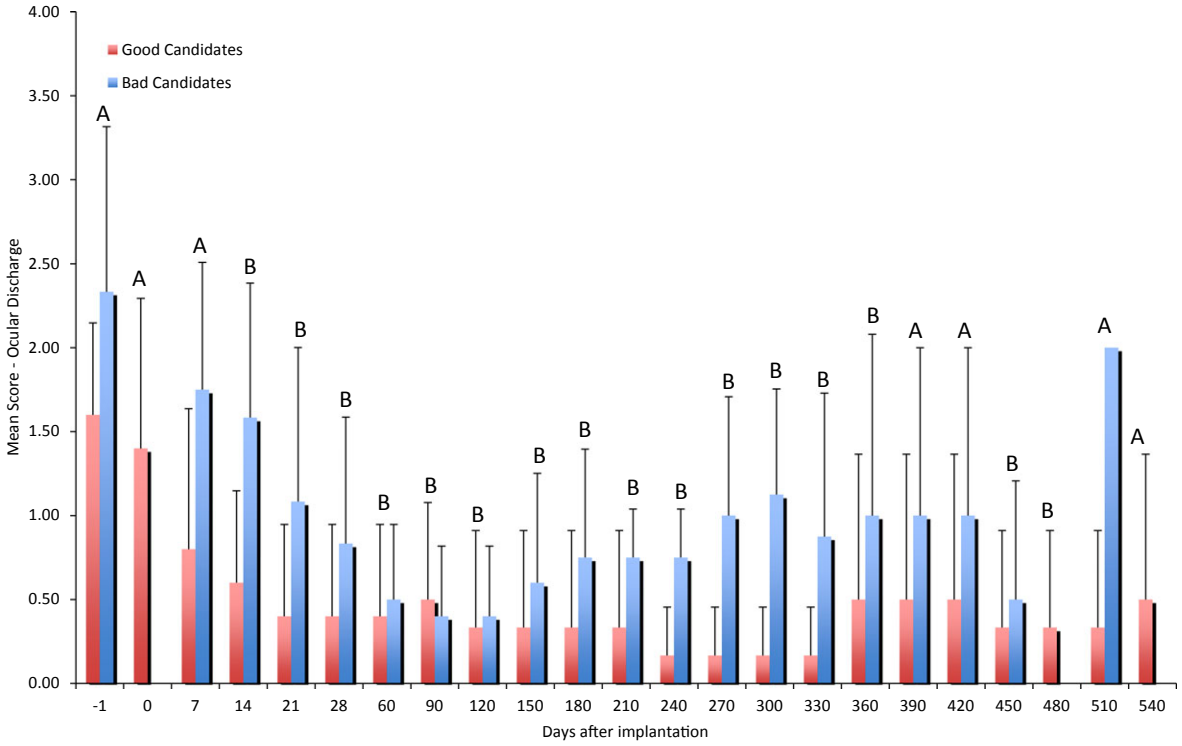

Figure 6. Overall the maximum statistically significant reduction in ocular discharge was achieved at 60 days ($P = 0.002$) and remained significant up for 480 days. Means with different letters are significantly different ($P < 0.05$).

extruded at 5.5 months after implantation. Histologically, these eyes demonstrated no toxicity of the ocular tissues or lacrimal glands; however, a fine, fibrous encapsulation surrounding the implant that secured it to the episclera was observed. Over a 6-month period, the mean CsA concentration in the conjunctiva, lacrimal gland, and cornea was significantly higher than drug concentrations necessary for inhibition of fibroblast proliferation and T-cell activation.[32] CsA tissue levels of implantation were also higher than tissue drug concentrations in the lacrimal gland achieved with topical and oral cyclosporine formulations.[32,41,42]

In our study, one implant was lost at 1 week after implantation and another implant at 12 months after surgery. The cause of the implant extrusions is unknown, but the early loss was likely from loss of surgical incision integrity. No other evidence of implant complications or toxicity was observed in these clinical patients.

Another purpose of this study was to determine whether ESMC implants have efficacy in the treatment in canine KCS. Use of an ESCM has been described for the treatment of KCS in a red wolf, which had the disease controlled for >12 months after bilateral implantation.[34] In a preclinical evaluation of ESCM for graft-versus-host disease in humans, the pharmacodynamics of the implants were studied in a canine model of aqueous tear deficiency and KCS.[32] The CsA implant was evaluated in dogs with naturally occurring KCS (STT <5 mm/min). A smaller implant (1.3 cm long, 2 mm wide, and 1 mm high) was inserted in the superotemporal episcleral space of eight eyes of six dogs. After a follow-up of 6 months, all the implant-recipient eyes maintained STT scores of more than 10 mm/min and were able to discontinue topical cyclosporine. None of the treated eyes exhibited recurrence of KCS symptoms such as conjunctival hyperemia or discharge. In addition, there was no implant extrusion or toxicity related to the cyclosporine implant in this group of dogs.[32]

In our study, the maximum effect on STT values and clinical scores was evident from 60 to 90 days after implantation, and was effective for 10 months for STT and 17 months for clinical scores. This supports a therapeutic effect near, or slightly below, the estimated therapeutic release time determined from *in vitro* studies.[32,40]

As it could be expected in a small retrospective study, the present study has its limitations. Clinical scores were subjective, a negative or sham implant control group was not included, and the number of eyes treated was small. Furthermore, measuring CsA concentrations in tears, conjunctiva, or lacrimal gland would have further supported our clinical findings. However, the improvement of clinical signs and STT values compared to baseline in our study and the similarity of our results to previous studies support our conclusion that the use of the implants resulted in sustained therapy of KCS in the dogs of this study.

The episcleral implant that is described and used in this report has many advantages, including the ability to deliver

constant therapeutic levels of drug directly to the site of ocular disease, while minimizing systemic side effects.[20,21] In addition, cases that are refractory to treatment due to patient and/or owner non-compliance in drug administration would benefit from this sustained release ocular drug delivery technology. This nonbiodegradable implant has also the advantage of steady, controlled release of drug during potentially long periods of time but the disadvantage of removal and/or replacement when the drug is depleted.[20,21,23,32] In addition, it requires a surgical procedure to be implanted, so its routine use would require multiple surgeries in a dog over its lifetime.[20,21] Devices with longer duration of release and injection technologies using microparticles, nanoparticles, or gel-forming solutions that do not require surgery for application would be desirable for treatment of chronic ocular disease in dogs.

The results of this study support the further development and evaluation of episcleral cyclosporine implants in dogs with KCSU. Additional studies, using prospective, controlled studies, are needed to determine the absolute therapeutic role, tolerance, duration of effect, and optimal number of ESMC implants for the treatment of canine KCS.

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