Preclinical Evaluation of a Novel Episcleral Cyclosporine Implant for Ocular Graft-Versus-Host Disease

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P U R P O S E. To develop a local drug delivery system that provides therapeutic cyclosporine levels to treat lacrimal gland graft-versus-host disease after allogeneic hematopoietic stem cell transplantation.

M E T H O D S. Episcleral cyclosporine implants were manufactured with a silicone-based matrix design, and in vitro release rates were determined. Preclinical evaluation included toxicology (clinical examination, serial electroretinography, and histopathology) in normal rabbits and dogs, pharmacokinetics in normal rabbits, and pharmacodynamics in a canine model of aqueous tear deficiency and keratoconjunctivitis sicca.

R E S U L T S. The cyclosporine implants showed sustained release of drug over time with in vitro assays. Histopathology showed normal ocular tissues in both dogs and rabbits 6 months after implantation. The cyclosporine implant produced lacrimal gland drug levels 1 to 2 log units higher than those reported with a variety of topical cyclosporine formulations and oral administration. The cyclosporine implant was effective in a canine model of keratoconjunctivitis sicca, with all animals able to discontinue topical cyclosporine and maintain normal Schirmer scores over a 6-month follow-up.

C O N C L U S I O N S. This preclinical evaluation showed that the episcleral cyclosporine implant was safe, delivered potentially therapeutic cyclosporine levels to the lacrimal gland, and showed efficacy in a clinically relevant model of keratoconjunctivitis sicca. The episcleral cyclosporine implant shows promise in reducing the morbidity associated with lacrimal gland graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. In addition, continuous release of cyclosporine in the subconjunctival space with the episcleral implant was an effective means of delivering drug to the ocular surface and may have potential in treating other ocular inflammatory diseases. (Invest Ophthalmol Vis Sci. 2005;46:655–662) DOI: 10.1167/iovs.04-01076

G raft-versus-host disease (GVHD) is a major complication of allogeneic hematopoietic stem cell transplantation (allo-SCT) that commonly affects the skin, liver, gastrointestinal tract, and eye.¹² The most common clinical manifestations of ocular GVHD generally involve the lacrimal gland and the conjunctiva and occur in approximately 50% of patients after allo-SCT.³–⁵ Lacrimal gland biopsy specimens in patients with ocular GVHD show infiltration of T cells and CD34⁺ stromal fibroblasts into periductal areas, resulting in distal flow obstruction and proximal acinar damage.⁶,⁷ The destruction of the lacrimal gland results in aqueous tear deficiency and moderate to severe keratoconjunctivitis sicca (KCS),⁸ which is permanent in >90% of patients.⁴ At the National Institutes of Health (NIH), topical cyclosporine and topical corticosteroids have not been effective in treating lacrimal gland GVHD,⁹ probably because of the overlying conjunctival epithelium that prevents effective delivery of cyclosporine to deep ocular tissues.¹⁰ Topical cyclosporine preparations produce lacrimal gland concentrations 2 log units lower¹¹ than the in vivo inhibitory drug levels that prevent proliferation of fibroblasts,¹²,¹³ a predominant cell type in the pathogenesis of lacrimal gland destruction in the setting of GVHD.¹⁶,¹⁷ We sought to develop a novel sustained-release cyclosporine implant to deliver therapeutic drug levels for GVHD-related lacrimal gland involvement. Herein, we present a description of the implant’s development and in vitro release rate profile, pharmacokinetics in normal rabbits, safety in normal rabbits and dogs, and pharmacodynamics in a canine model of KCS.

M E T H O D S

Implant Manufacturing

We selected the implant release rate profile based on the typical use of immunosuppressants when treating GVHD, starting with an initial higher dose followed by a tapering maintenance dose.² To this end, we developed a matrix-style implant release system¹⁴ with the goal of delivering cyclosporine to the lacrimal gland for approximately 6 to 12 months. In our experience, this is the typical time course of lacrimal gland GVHD that leads to the destruction of the gland and irreversible tear loss. We selected a silicone-based matrix implant, by using methods of preparation previously described (Robinson MR, et al. IOVS 2002;43:ARVO E-Abstract 2297).¹⁵ In brief, the implants were made in a polytetrafluoroethylene mold with impressions on the surface measuring 0.75 inches long, 0.08 inch wide, and 0.04 inch in height or 0.5 inch long, 0.08 inch wide, and 0.04 inch in height (width was measured on the flat side; height was measured from the flat surface to the top of the rounded side). Cyclosporine powder (Xenos Bioresources, Inc., Santa Barbara, CA) was thoroughly 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 20% or 30%. This cyclosporine-silicone paste was placed in the impressions of the mold with a metal spatula and cured for a minimum of 24 hours at room temperature. Three

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Toxicology studies were performed with implant A, and two devices were inserted into one eye of both rabbits and dogs. Ocular toxicity was evaluated by clinical examination, serial electroretinography, and histopathology. All procedures adhered to the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Rabbit.** New Zealand White (NZW) rabbits of either sex weighing 2 to 3 kg (Covance Laboratories, Inc., Vienna, VA) were anesthetized with ketamine hydrochloride (35 mg/kg; Fort Dodge, Inc., Fort Dodge, IA) intramuscularly (IM) and xylazine (5 mg/kg; Phoenix Scientific, Inc., St. Joseph, MO) IM. Proparacaine 1% ophthalmic drops (Allergan America, Horimigueiro, PR) were used topically on the eye. The pupils were dilated with 1 drop each of phenylephrine hydrochloride 2.5% (Akorn, Inc., Decatur, IL) and tropicamide 1% (Alcon, Inc., Humacao, PR). A baseline eye examination, including fundoscopy with an indirect ophthalmoscope and intraocular pressure measurement, was performed. A toothed forceps was used to lift the conjunctiva and Tenon’s fascia in the superotemporal quadrant, and a 3-mm incision was made with Wescott tenotomy scissors. A pocket was formed in the sub-Tenon’s space, and two adjacent implant A devices were placed on the episclera, 5 mm posterior and parallel to the limbus in one eye. No sutures were used to secure the implants. To avoid bleeding, care was taken not to enter the venous sinus that surrounds the lacrimal gland. The conjunctiva and Tenon’s fascia were reapproximated with single 9-0 Vicryl suture. Bacitracin ophthalmic ointment was placed in the operative eye twice daily for 3 days. Clinical eye examinations were performed weekly for 1 month and monthly thereafter. Blood was obtained from the rabbits monthly, and serum chemistries, renal and liver function tests, complete blood count, and cyclosporine blood levels were determined. ERGs were recorded from each eye separately after 30 minutes of dark adaptation. A monopolar contact lens electrode (ERG-jet; Universo, La Chaux des Fondo, Switzerland) was placed on the cornea and served as the active electrode. A Barraquer eyelid speculum connected to an electrode wire served as the indifferent electrode, and a subdermal needle electrode inserted in the forehead area as the ground electrode. ERGs were elicited by brief flashes at 0.5 Hz delivered with a photostimulator (model PS22; Grass-Telefactor Instruments, W. Warwick, RI) at maximum intensity, coupled to an 18-inch long optic guide of 0.5-inch diameter. Responses were amplified, filtered, and averaged with a signal-averaging device (Spirit; Nicolet Instruments Corp., Madison, WI). Averages of 20 responses were measured to obtain peak amplitudes of a- and b-waves. Recordings were performed at baseline and every 4 to 8 weeks for 6 months. Differences in the mean amplitudes at each recording were compared with the baseline (preimplant) values for each eye and tested by analysis of variance (ANOVA) on computer (PSI-Plot, ver. 7.0; Poly Software International, Inc., Pearl River, NY). Differences were considered clinically significant if P ≤ 0.05. A subgroup of animals were anesthetized and then euthanized with an intracardiac pentobarbital overdose (Beuthanasia-D Special; Schering Plough Animal Health Corp., Kenilworth, NJ) at 6 months. The lacrimal glands were removed, and both eyes were enucleated, leaving the implants and overlying conjunctiva intact. All tissues were placed in 10% formalin for a minimum of 7 days. The globes were sectioned vertically across the long axis of the implants and through the optic discs. All tissue specimens were placed in increasing concentrations of ethanol, cleared with xylene using a tissue processor (Jung Histokinetette; Leica, Inc., Deerfield, IL), and embedded in paraffin (Shandon Embedding Center; Shandon, Inc., Pittsburgh, PA). Sections of 7-μm thickness were obtained with a microkeratome and stained with hematoxylin and eosin, and representative slide-mounted sections were examined by light microscopy.

**Dog.** Normal research beagles (Marshall Farms, Inc., North Rose, NY) were anesthetized with acepromazine (0.02 mg/kg; Abbott Laboratories, Abbott Park, IL) and hydromorphone HCl (0.11 mg/kg; Abbott Laboratories) IM. Using methods described earlier, we placed two implant-A devices in the superotemporal quadrant adjacent to the lacrimal gland in the sub-Tenon’s space 5 mm posterior and parallel to the limbus in one eye. Two sham implants (i.e., polymer without drug) were placed in the same location in the contralateral eye. No sutures were inserted into one eye of both rabbits and dogs. Ocular toxicity was evaluated by clinical examination, serial electroretinography, and histopathology. All procedures adhered to the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**FIGURE 1.** A representative photograph of a cyclosporine episcleral implant shows the flat surface, which has contact with the episclera, and the rounded anterior surface, which has contact with the conjunctiva.

different implants were manufactured: (1) 0.75 inch long, 0.08 inch wide, and 0.04 inch in height, 30% (wt/wt) cyclosporine (implant A); (2) 0.5 inch long, 0.08 inch wide, and 0.04 inch in height, 50% (wt/wt) cyclosporine (implant B); (3) 0.5 inch long, 0.08 inch wide, and 0.04 inch in height, 20% (wt/wt) cyclosporine (implant C). Each implant was sterilized with gamma irradiation (25–30 kGy). The implant was flat on one side (the posterior surface, which is applied to the episclera), and the anterior surface and ends were rounded (Fig. 1).
were used to secure the implant to the sclera, and a 6-0 Vicryl suture was used to close the conjunctival incision. After the implant surgery, clinical eye examinations including a Schirmer’s tear test, laboratory tests (serum chemistries, renal and liver function tests, complete blood count), and ERG recordings were performed over a 6-month period. ERG recordings were performed as described earlier, except the animals were awake and dark adapted for 5 minutes, and the averages of 10 responses were measured to obtain peak amplitudes of the a- and b-waves. Some of the animals were killed every 2 months over a 6-month period, and the eyes were enucleated and prepared for histopathology.

**Ocular Pharmacokinetics**

We studied the distribution of cyclosporine in the ocular tissues and the lacrimal gland over time after implant surgery in NZW rabbits. In rabbits, there are two lacrimal glands—one located in the superior orbit, the other in the inferotemporal aspect of the orbit—and they have identical histology.16 We have found that the inferotemporal lacrimal gland is larger and more consistent in NZW rabbits. It is called the lacrimal gland proper by some17,18 and the accessory lacrimal gland by others.19 In this study, we focused on delivery of cyclosporine to the inferotemporal lacrimal gland. In the inferotemporal quadrant, a single implant-A device was placed on the episclera, 5 mm posterior and parallel to the limbus in one eye using methods described earlier. The animals were examined weekly and killed with an intracardiac pentobarbital overdose (Beuthanasia-D Special; Schering Plough Animal Health Corp.) every 1 to 4 weeks for up to 6 months after implantation. After euthanasia, the implant-recipient eye was enucleated, and 5 × 5-mm sections of bulbar conjunctiva adjacent to the limbus were removed superiorly, nasally, inferiorly, and temporally, to examine the relative difference in cyclosporine concentrations around the eye. A fibrous capsule was present around the implant, firmly attaching it to the episclera, and an incision was made through the capsule for removal. The globes were immediately frozen at −70°C for later dissection and drug extraction. The time from enucleation to freezing was rapid (<10 seconds), which limited postmortem drug redistribution. The lacrimal gland was removed from the orbit, and blood was obtained to determine drug levels. The eyes were dissected while frozen, and 5 × 5-mm sections of sclera, 2 mm posterior to the limbus, were removed superiorly, nasally, inferiorly, and temporally. The frozen globe was then cut 360° at the limbus with a razor blade and the cornea lifted cleanly off of the frozen aqueous humor. The frozen cornea was cut into superior and inferior halves. A razor blade was passed parallel to the front surface of the iris, and the frozen aqueous humor lifted off the iris/lens diaphragm in two to three frozen pieces. A 3 × 5-mm section of tarsal conjunctiva including the underlying tarsus was removed from the center of the upper and lower eyelids at selected time points. Other ocular tissues isolated for drug analysis included the ciliary body and iris combined, vitreous humor, and optic nerve. Cyclosporine was extracted by placing the ocular tissues in HPLC-grade acetonitrile (Fisher Scientific, Pittsburgh, PA) in sealed vials for 24 hours at room temperature, sonicated with an ultrasonic processor (GEX 600; Daigger, Lincolnshire, IL) for 60 seconds, and stored in sealed vials for another 24 hours at room temperature. The samples were spun down in a centrifuge (Centra C12; Thermo IEC, Needham Heights, MA) for 30 minutes at 3500 rpm, and the supernatants were collected for HPLC analysis. The cyclosporine concentrations were expressed as microliters per milligram of tissue. Differences in the mean tissue concentrations at the different locations for the conjunctiva, cornea, and sclera, were tested by the ANOVA (PSI-Plot, ver. 7.0; Poly Software International, Inc.). Differences were considered clinically significant if P ≤ 0.05.

The efficiency of drug extraction was assessed in four freshly enucleated NZW rabbit eyes. The ocular tissues were isolated and spiked with 100 μL of a stock solution of cyclosporine (1 mg/mL) in acetonitrile. The tissues were subjected to the drug extraction procedure, and the percentage of drug recovery (mean ± 1 SD) was recorded.

**Ocular Pharmacodynamics**

To evaluate the effects of the cyclosporine implant in a model of ocular inflammation, canine studies were performed at North Carolina State University Veterinary College. The cyclosporine implant was evaluated in dogs with naturally occurring KCS, a disease similar to human GVHD, in which a lymphocytic infiltration occurs in the conjunctiva, cornea, and tear-producing glands.20 In normal dogs, both the orbital lacrimal gland and the nictitating membrane gland are involved in tear production.21 In dogs with KCS, these glands show coalescing areas of lymphocyte infiltration and atrophy of the acinar elements with fibrosis, resulting in aqueous tear deficiency and progressive KCS.22 Dogs with KCS were included in the study if they had Schirmer tear test scores under 5 mm/min and were dependent on daily topical ointment (Optimmune; cyclosporine 0.2% ophthalmic ointment, Schering Plough) to maintain tear function at 10 to 15 mm/min, the normal range in dogs. An implant B device was selected for this study, because the 0.5-inch length was most compatible with the size of the eye in the smaller dog breeds that are most prone to KCS. The cyclosporine implant was inserted in the subconjunctival space on the episclera adjacent to the lacrimal gland superotemporally, by a surgical method described earlier. Clinical examinations were performed monthly and the Schirmer tear test scores recorded. Topical cyclosporine formulations were discontinued after surgery, and the dogs were observed for 6 months. For proof of principal that the cyclosporine implant was able to deliver drug to the canine lacrimal gland, research beagles had an implant A device inserted on the episclera adjacent to the lacrimal gland superotemporally in one eye, and the animals were killed 1, 3, and 6 months after implantation. Both the orbital lacrimal gland and the nictitating membrane gland were removed and processed for drug extraction, as described earlier.

**RESULTS**

**In Vitro Release Rate**

In vitro release rates were determined on five randomly selected implants from each lot of implants A, B, and C. Total mean weights were 40.6 ± 1.82, 25.8 ± 1.64, and 32.4 ± 1.67 mg, respectively. The in vitro release pattern of all implants was typical of a matrix implant with release kinetics that are governed by diffusion from dispersed drug in a polymer (Fig. 2).23 During the first month, a cumulative mean drug release of 760, 604, and 415 μg occurred from implants A, B, and C, respectively. The cumulative mean drug release tapered down.
to 446, 325, and 282 μg between months 2 and 3 from implants A, B, and C, respectively. The total mean drug release during the 5-month assay period—2.537, 1.55, and 1.275 mg from implants A, B, and C, respectively—was approximately 20% of the initial drug loading. Compared with implants A and C, implant B showed a more rapid drug release taper after 100 days.

Toxicity Evaluation

Rabbits. Eight rabbits each received two implant A devices in one eye, and after 6 months there were no implant extrusions or signs by clinical examination of ocular toxicity, such as infection, conjunctival erosions over the implant, or cataract (Fig. 3A). ERG recordings were obtained in five rabbits, and there was a significant decrease in the mean a-wave amplitude ($P = 0.04$) from baseline and a smaller decrease in mean b-wave amplitude ($P = 0.08$) at the 1-month recording (Fig. 4). By the 5-month recording, the mean a- and b-wave amplitudes had recovered to baseline. ERG recordings in the contralateral eye showed no significant changes in the mean a- and b-wave amplitudes from baseline during the study period. Three rabbits that did not undergo ERG were euthanized 6 months after implantation, and the histopathology of the enucleated eyes showed a fine, fibrous encapsulation of the implants that secured them to the surrounding tissues (Figs. 3B, 3C). The histopathologic appearance by light microscopy in all eyes showed a normal lacrimal gland, cornea, anterior chamber, iris, ciliary body, lens, vitreous, and retina (Fig. 3D). Monthly laboratory data were normal, with no measurable levels of cyclosporine in the blood over the first 4 months in all animals; therefore, subsequent laboratory testing was discontinued.

Dogs. Six dogs each received two implant A devices in one eye, and two sham implants in the contralateral eye. Over the 6-month period, clinical examinations showed no signs of ocular toxicity (Figs. 5A, 5B). One cyclosporine implant (of 24 total) extruded at 5.5 months after implantation. There were no significant changes in the ERG recordings compared with baseline during the 6-month study in both eyes. The Schirmer tear scores, which reflected the functional integrity of the lacrimal gland, remained normal throughout the study period. Two animals were killed every 2 months, and histopathology was performed on both eyes and lacrimal glands. The histopathologic appearance by light microscopy in all eyes showed a normal lacrimal gland and ocular tissues. There was a fine, fibrous encapsulation surrounding the implant that secured it to the episclera (Fig. 5C). There were no signs of retinal toxicity in all quadrants of the eye (Fig. 5D). There were no abnormalities in the laboratory data throughout the study period.

Ocular Pharmacokinetics

Twenty-one rabbits received a single implant A device adjacent to the lacrimal gland in one eye, and three rabbits were killed at each of seven time points over a 6-month period. The efficiency of drug recovery was moderate, ranging from 65.1% to 75.1% in the solid ocular tissues (Table 1). Lacrimal gland cyclosporine levels ranged from a mean of 0.029 to 0.423 μg/mg of tissue (Table 1). There were no significant differences in the cyclosporine concentrations in the different areas sampled for the conjunctiva, sclera, and cornea, in each rabbit.
and 6 months) were 0.242 mg/mg. As

The mean of the spatial means for each tissue of the three rabbits sampled at each time point is given in Table 1. The lowest drug concentrations at each time point were in the vitreous humor. Mean drug levels in the upper and lower eyelid tarsus and tarsal conjunctiva (pooling data from 3, 4, 5, and 6 months) were 0.242 ± 0.128 to 0.213 ± 0.149 µg/mg. As anticipated from the in vitro drug release kinetics of the implant, tissue cyclosporine concentrations trended downward during the study period (Table 1). However, the drug concentrations remained in the therapeutic range for the inhibition of fibroblast proliferation and T-cell activation at all time points in the lacrimal gland, conjunctiva, and cornea, the principal tissues involved with GVHD (Fig. 6).

**Ocular Pharmacodynamics**

Eight eyes of six dogs with KCS were included in this study. After a follow-up of 6 months, the implant-recipient eyes of all dogs maintained Schirmer tear test scores of more than 10 mm/min, and we 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. Nine research beagles received a cyclosporine implant in one eye, and three animals were killed at each of three time points during a 6-month period. The lacrimal gland cyclosporine concentrations were 0.082 ± 0.0984, 0.025 ± 0.0047, and 0.034 ± 0.0057 µg/mg at 1, 3, and 6 months, respectively. The nictitating membrane gland cyclosporine concentrations were

![Image](image_url)

**Figure 5.**Normal dog toxicity evaluation 6 months after implantation. (A) An external photograph shows a cyclosporine episcleral implant (arrow) in the subconjunctival space. A second implant was present posteriorly and is not shown in the photograph. (B) A fundus photograph demonstrating a normal retina and optic disc. Light microscopy shows (C) the subconjunctival region from which a cyclosporine implant (✱) had been removed at the time of euthanasia. A fibrous capsule (arrows) is present around the implant; and (D) a normal retina and choroid in the same quadrant of the cyclosporine implant. (C, D) Hematoxylin and eosin. Original magnification, ×10.

**Data are expressed as the mean micrograms per milligram of tissue ± 1 SD, except as noted.**

<table>
<thead>
<tr>
<th>Lacrimal Gland</th>
<th>Conjunctiva</th>
<th>Cornea</th>
<th>Sclera</th>
<th>Ciliary Body/Iris</th>
<th>Aqueous Humor</th>
<th>Vitreous Humor</th>
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<tbody>
<tr>
<td>1 Week</td>
<td>0.059 ± 0.0040</td>
<td>1.872 ± 0.4886</td>
<td>1.033 ± 0.2826</td>
<td>0.479 ± 0.2118</td>
<td>0.227 ± 0.0547</td>
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<td>3 Weeks</td>
<td>0.264 ± 0.2523</td>
<td>1.804 ± 0.5263</td>
<td>2.792 ± 1.0971</td>
<td>0.306 ± 0.1566</td>
<td>0.152 ± 0.0497</td>
<td>0.116 ± 0.1459</td>
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<td>6 Weeks</td>
<td>0.077 ± 0.0401</td>
<td>1.052 ± 0.3927</td>
<td>0.910 ± 0.0649</td>
<td>0.570 ± 0.2962</td>
<td>0.144 ± 0.0256</td>
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<td>3 Months</td>
<td>0.169 ± 0.1252</td>
<td>0.557 ± 0.2820</td>
<td>0.418 ± 0.1571</td>
<td>0.202 ± 0.0589</td>
<td>0.106 ± 0.0766</td>
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<td>4 Months</td>
<td>0.423 ± 0.5219</td>
<td>0.591 ± 0.0668</td>
<td>0.346 ± 0.2070</td>
<td>0.432 ± 0.0884</td>
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<td>5 Months</td>
<td>0.123 ± 0.0057</td>
<td>0.144 ± 0.0495</td>
<td>0.056 ± 0.0336</td>
<td>0.163 ± 0.1065</td>
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<td>6 Months</td>
<td>0.029 ± 0.0049</td>
<td>0.098 ± 0.0402</td>
<td>0.102 ± 0.0364</td>
<td>0.108 ± 0.0326</td>
<td>0.049 ± 0.0046</td>
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<td>Drug recovery (%)</td>
<td>75.1 ± 5.78</td>
<td>65.7 ± 4.19</td>
<td>65.1 ± 1.96</td>
<td>69.5 ± 1.75</td>
<td>72.3 ± 5.64</td>
<td>63.8 ± 4.41</td>
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</tbody>
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TABLE 1. Drug Concentration in Ocular Tissues over 6 Months with a Cyclosporine Episcleral Implant

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0.188 ± 0.0583, 0.486 ± 0.05, and 0.056 ± 0.0309 μg/mg at 1, 3, and 6 months, respectively. These data demonstrate that the implant was successful in delivering drug to both tear-producing glands of the dog.

**DISCUSSION**

In this preclinical evaluation, the episcleral cyclosporine implant appeared to be safe in both rabbits and dogs. Typical ocular implant-related complications when placed on the episclera, such as conjunctival erosion, migration, and extrusion, were minimized with our implant by using a low-profile design with soft, medical-grade silicone that conformed to the globe. Manufacturing the implant with a flat surface placed on the episclera has been found to reduce implant migration, and the rounded ends help reduce erosion of the overlying conjunctiva (Robinson MR, et al. IOVS 1999;40: ARVO Abstract 449). Increasing the implant length increases both the drug load and surface area, which enhances the implant release rate from these matrix devices. Anchoring sutures for this implant were not necessary because the fibrous encapsulation adequately secured the implant to the episclera. The significance of the ERG amplitude depressions in the rabbits with the cyclosporine implants was not clear. Declines in the b-wave amplitude of the ERG have occurred in previous rabbit studies in which intravitreal cyclosporine was delivered by injection (Ivert L, et al. IOVS 2000;41:ARVO Abstract 1878) or by an implant; however, there were no signs of retinal toxicity by clinical evaluation or histopathology. At a cellular level, cyclosporine binds with cyclophilin, which inhibits calcium-dependent intracellular functions. Alterations in retinal calcium metabolism can reduce transmembrane potentials and have a negative effect on the generation of the ERG, without causing cellular damage. The ERG changes may be species dependent, since they were not observed in horses and monkeys that received intravitreal cyclosporine implants.

There were no systemic toxicities observed with the cyclosporine implant. Such reactions would not be expected when the total amount of drug release over a 5-month period was 2.5 mg with the highest release device (implant A). There was considerably less drug exposure from the implants compared with topical application (50 μL twice daily in one eye) over a 5-month time frame, which exposes the patient to 7.5 and 300 mg, with 0.05% and 2% eye drop concentration, respectively. Although the cyclosporine implant was designed to treat lacrimal gland GVHD for 6 to 12 months, it is not known how long drug therapy is necessary to maintain a durable treatment response.

The implant maintained cyclosporine levels in the rabbit lacrimal gland ranging over time from a mean of 0.029 to 0.423 μg/mg of tissue. These levels are well within the 0.01- to 0.05-μg/mg range necessary for fibroblast inhibition with in vitro assays. In addition, the cyclosporine levels in the lacrimal gland were 1 to 2 log units higher than the 0.0001 to 0.001 μg/mg range necessary for T-cell activation with in vitro assays. The implant also delivered potentially therapeutic levels of cyclosporine in the conjunctiva and cornea, both commonly involved with GVHD. In contrast, topical formulations produce cyclosporine levels in the lacrimal gland ranging from 0.000062 μg/ mg with a 0.05% oil emulsion formulation from Allergan (Irvine, CA) to 0.0001 μg/ mg with a 0.2% ointment from Novartis (Basel, Switzerland) after multiple applications in rabbits. Similar low drug concentrations (0.00017 μg/ mg) were observed in humans after oral cyclosporine (5 mg/kg) in the lacrimal gland tears, probably explaining why systemic cyclosporine is rarely successful in treating lacrimal gland dysfunction in the setting of GVHD. Although intensive topical cyclosporine 1% eye drop applications (6 – 8 times per day) have been reported to be useful in GVHD-related epithelial keratitis and corneal melting, reversal of the aqueous tear deficiency in these patients did not occur.

There was variability in the drug levels at each time point, with the standard deviation exceeding the mean in some tissues. The intersubject variability in cyclosporine pharmacokinetics after systemic administration has been well described. The etiologies include the marked differences in the population of liver cytochrome p 450 3A4 activity, the enzyme prin-
ciscally involved with cyclosporine metabolism. Cytochrome P450 enzymes, as well as other drug metabolizing enzymes, are heavily expressed in mammalian eye tissues, and differences in enzyme activity between rabbits may have led to some of the variability in drug tissue concentrations observed in our study. Further study is warranted to understand the pharmacokinetics of cyclosporine when locally released in the episcleral space.

The mechanisms of drug transport around the eye from an episcleral implant are not known. Studies examining the movement of a lipophilic compound in the rabbit brain from a matrix implant with release kinetics similar to those used in this study showed the highest drug concentrations in tissues closest to the implant. In our present study, there were no significant differences in cyclosporine concentrations in the conjunctiva, cornea, and sclera, relative to the position of the implant, starting at the earliest time point (1-week). It appears unlikely that diffusion alone could be responsible for the rapidity with which cyclosporine dispersed from the implant to these other locations. We can estimate the characteristic time to achieve a particular diffusion distance using transport theory. For diffusion in one dimension in the absence of clearance processes, the concentration (C) at a distance from a source (i.e., implant) of constant concentration (C0) is given by the equation:

\[
C = C_0 e^{-\text{erfc} \left( \frac{x}{2 \sqrt{Dt}} \right)},
\]

where D is the diffusion coefficient of solute diffusion through the rabbit sclera and corneal stroma, t is time, erfc is the complementary error function, and x is the distance between the source and the measurement position.

From in vitro measurements of diffusion of other hydrophilic and lipophilic model solutes, the diffusion coefficient of cyclosporine in the sclera and corneal stroma in vivo at 37°C was estimated to be \(1.0 \times 10^{-6} \text{ cm}^2/\text{sec}\). The distance between the implant and the superior sclera sampling site (x) was 2 cm. If the release from the implant maintained the cyclosporine concentration in the adjacent scleral tissue at C0, then diffusion would only increase C in the superior sclera to 50% of C0 at 7 weeks according to the equation. In contrast, in the present experiments, C in the superior sclera was 50% of C0 by 1 week. This relatively high cyclosporine concentration in the superior sclera suggests that there were mechanisms other than simple diffusion involved with the movement of drug released from the episcleral implant. Previous work with a lipophilic fluorescein molecule released by an episcleral implant showed a similar pattern of rapid drug distribution around the eye that could not be explained by simple diffusion (Kim H, et al. IOVS 2002;43:ARVO E-Abstract 2303). Mechanisms facilitating drug transport from an episcleral implant may include the extensive superficial and deep lymphatics that are found throughout the ocular surface. Other investigators have shown facilitation of water and particle movement in Tenon’s fascia immediately adjacent to the episclera. In this tissue plane, there is considerable fluid egress from the episclera through uveoscleral flow capable of moving molecules with molecular weights as large as albumin. This topic of drug transport in the subconjunctival space is important when deciding the optimal implant position to enhance drug concentrations in specific ocular tissues and deserves further study.

There are differences in the anatomy and physiology of the rabbit eye that have to be considered before extrapolating the results of the ocular drug distribution in this study to humans. The lacrimal gland is located in the superotemporal orbit in humans and inferiorly in rabbits. Differences in the location of the implant relative to the lacrimal gland were not specifically examined. Although the permeability of rabbit and human sclera to a number of compounds is similar, the mean scleral thickness is less in the rabbit and this may affect drug transit around the eye. We have shown in a prior study that choroidal blood flow is a significant barrier to the influx of a drug surrogate from the episcleral space into the vitreous in normal rabbits. The choroidal blood flow is 16% higher in rabbits than in primates, and therefore the elimination of drug may be increased in rabbits, and vitreous drug concentrations in human may be greater from an episcleral implant. Another difference between rabbits and humans that may affect drug clearance from the eye is the degree of retinal vascularization. The rabbit possesses a merangiotic retina (i.e., partially vascularized retina), whereas the human possesses a holangiotic retina (i.e., completely vascularized retina). Clearance of drugs from the vitreous via the retinal circulation may be higher in humans because the retinal vascularization is complete. Last, because the interactions of cyclosporine with ocular pigments were not examined in this study, our pharmacokinetic data in NZW (albino) rabbits may not have general applicability to pigmented mammals.

In summary, in this preclinical evaluation, the episcleral cyclosporine implant was safe, delivered potentially therapeutic cyclosporine levels to the lacrimal gland, and showed efficacy in a clinically relevant model of KCS. The episcleral cyclosporine implant shows promise in reducing the morbidity associated with lacrimal gland GVHD after allo-SCT. In addition, continuous release of cyclosporine in the subconjunctival space with the episcleral implant was an effective means of delivering drug to the ocular surface and may have potential in treating other ocular inflammatory diseases.

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