

A Novel Bioerodible Deep Scleral Lamellar Cyclosporine Implant for Uveitis

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PURPOSE. To determine the feasibility, safety, and effectiveness of an episcleral or deep scleral lamellar sustained release cyclosporine (CsA) device in a naturally occurring animal model of uveitis.

METHODS. A two-compartment perfusion chamber was used to assess in vitro human and equine scleral permeability of fluorescein, dexamethasone-fluorescein, or CsA. A biodegradable, matrix-reservoir CsA implant was designed, and release rates of CsA were determined in vitro. Tissue CsA levels were measured in eyes with the implant. Horses with equine recurrent uveitis (ERU) received episcleral or deep scleral lamellar CsA implants and were monitored for up to 3 years.

RESULTS. Dexamethasone-fluorescein and CsA penetrated the in vitro equine sclera poorly; however, low but detectable levels of CsA were detected intraocularly in vivo. The implant placed episclerally failed to control inflammatory episodes in ERU. CsA implants placed in the deep sclera adjacent to the suprachoroidal space resulted in high levels of CsA in most ocular tissues. In clinical equine patients with ERU, frequency of uveitic flare-ups was significantly decreased after implantation of a deep scleral lamellar CsA implant.

CONCLUSIONS. Diffusion of CsA across the sclera from the episcleral space was not a feasible method of drug delivery to the equine eye. However, placing a deep scleral lamellar CsA implant adjacent to the suprachoroidal space was effective in achieving therapeutic ocular drug concentrations and controlling uveitis in horses with ERU. (*Invest Ophthalmol Vis Sci* 2006;47:2596–2605) DOI:10.1167/iops.05-1540

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Clinical uveitis in humans is a common disease and, in most cases, can be treated successfully by steroidal and nonsteroidal anti-inflammatory or immunosuppressive medications.^{1–7} However, these medications may have severe side effects and in many patients, the disease is not responsive or becomes refractory to steroidal or nonsteroidal therapy.¹ Immunosuppressive drugs, such as cyclosporine (CsA), are also successful in treating uveitis and may alleviate signs of uveitis with lower incidence of side effects.^{1,2,6,8} In humans, however, side effects of systemic CsA treatment can occur, notably renal toxicity, and so low-dose and local therapy is preferred.^{2,6} Local ocular injections or drug-delivery devices have been evaluated and may allow greater ocular drug concentration than is achieved with systemic therapy and may reduce or eliminate systemic adverse effects (Robinson MR, et al. *IOVS* 1999;40:ARVO Abstract 449).^{8–13}

Recent development of intravitreal implants, such as a fluocinolone implant for treatment of uveitis, diabetes, and age-related macular degeneration^{14,15}; a CsA implant for treatment of uveitis^{9–11,12}; and a ganciclovir implant for cytomegalovirus retinitis,^{13,16,17} have shown promise for implant-mediated ocular drug delivery.⁸ However, intraocular complications, such as retinal detachment and endophthalmitis, have been attributed to the devices or surgery.^{8,12–14,18} Other implant types designed for sustained ocular release of medications have been described, including scleral plugs,¹⁹ intrascleral implants,^{20–22} episcleral devices (Robinson MR, et al. *IOVS* 1999;40:ARVO Abstract 449),^{23,24} dissolvable intraocular implants,²⁵ and intravitreal encapsulated cell-based delivery.²⁶ The disadvantage of many of these approaches is that the internal aspect of the eye has to be entered, increasing the chance of complications.

Equine recurrent uveitis (ERU; also known as moon blindness and periodic ophthalmia) is the most common cause of blindness in horses.^{27–30} This immune-mediated panuveitis is characterized by recurrent episodes of intraocular inflammation. Similar to human uveitis, the pathogenesis of ERU is multifactorial and frequently autoimmune in origin.^{31–36} Horses with ERU have recurrent episodes of ocular inflammation that result in blindness in approximately 56% of horses over a 2-year period.^{37,38} The standard of care for treatment of ERU is symptomatic anti-inflammatory therapy.^{28,30} CsA, through its ability to block IL-2 transcription, leading to impaired proliferation of activated T-helper and T-cytotoxic cells among other immunologic effects,^{39–41} may be an ideal drug to prevent reactivation of ocular inflammation.^{1,2,6,11,42} CsA delivered via sustained-release drug devices that prevent recurrences of ocular inflammation in ERU without reliance on the patient's (or horse owner's) compliance with treatment would greatly facilitate the ability to control ERU. Previous studies have demonstrated that intravitreal sustained-release CsA devices are well tolerated in the equine eye, with and without ERU; however, late-onset traction retinal detachments occur.^{12,43,44} Placing implants episclerally or in the deep sclera prevents the need to enter the eye, which would eliminate the

chance of injuring the lens, minimize the chance of developing endophthalmitis, and decrease rates of retinal detachment, all complications of intravitreal delivery devices as observed in animal and human studies.^{8,10,12-14,18,43-46}

The purpose of this study was to determine the feasibility, safety, and effectiveness of an episcleral or deep scleral lamellar sustained-release CsA device in a naturally occurring animal model of uveitis.

METHODS

The use of animals in this study adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and was reviewed and monitored by the North Carolina State University Institutional Animal Care and Use Committee. For experimental studies, normal adult horses with complete vaccination and deworming schedules, which were donated to the North Carolina State University, were used.

In Vitro Transscleral Diffusion of CsA

The purpose of this portion of the study was to determine the transscleral penetration of CsA. Sclera was harvested from the equatorial region of normal horse eyes (removed immediately after euthanasia) where the mean thickness was comparable with human sclera.⁴⁷ Using a two-compartment perfusion chamber, as previously described,⁴⁸ in vitro human and equine scleral permeability to fluorescein (100 μ L of 10^{-4} M), dexamethasone-fluorescein (100 μ L of 10^{-4} M), or CsA (100 or 500 μ L of 50 mg/mL) were compared. The solution of interest was placed on the episcleral side of the sclera, while the uveal side was perfused with balanced saline solution continuously. Samples were collected hourly from the uveal side of the sclera for 24 to 40 hours.

The perfusate was collected and measured for fluorescence or CsA using previously described methods⁴⁸ and quantitated by our high-performance liquid chromatography (HPLC) method with a lower limit of CsA detection of 10 ng/mL.²⁴

Development and In Vitro Analysis of a Matrix-Reservoir CsA Implant

A biodegradable implant designed to be placed in the episcleral space was developed by investigators at the National Eye Institute (Robinson MR, et al. *IOVS* 1999;40:ARVO Abstract 449). The implant was made with a 10% CsA (wt/wt) drug load in a polyvinyl alcohol polymer, and the central drug reservoir was made by pelleting the CsA powder (12 mg, 4.5 mm diameter) with a customized pellet press (Parr Instrument Company, Moline, IL). The final total implant diameter was 6 mm (Fig. 1A). In vitro release rates from the implants were determined using previous methods by assaying CsA concentrations in the vial over time with HPLC.²⁴ The cumulative release of drug from the implant was determined by taking the area under the release rate curve, using the trapezoidal rule, and recorded as milligrams \pm 1 SD.

Pharmacokinetics of the CsA Implant in the Episcleral Space

Two normal adult horses were used to determine whether drug would diffuse through the intact sclera from the CsA implant in the episcleral space. With the horses tranquilized and with proparacaine topical anesthetic (Alcaine; Alcon, Fort Worth, TX) applied, a 6-mm incision was made in the superior temporal bulbar conjunctiva and episclera. Matrix-reservoir CsA devices were placed in contact with the sclera, approximately 1 cm posterior to the superior temporal limbus in one eye of each horse and the conjunctiva was reapproximated with absorbable sutures. Eyes and peripheral blood were collected from the horses at 7 and 30 days after implantation, to determine tissue and fluid concentration of CsA. After euthanasia, the eyes were enucleated and immediately frozen at -80°C for later dissection and drug extraction. The choroid-retina and vitreous humor were removed in toto and the CsA drug extraction procedure was performed as previously de-

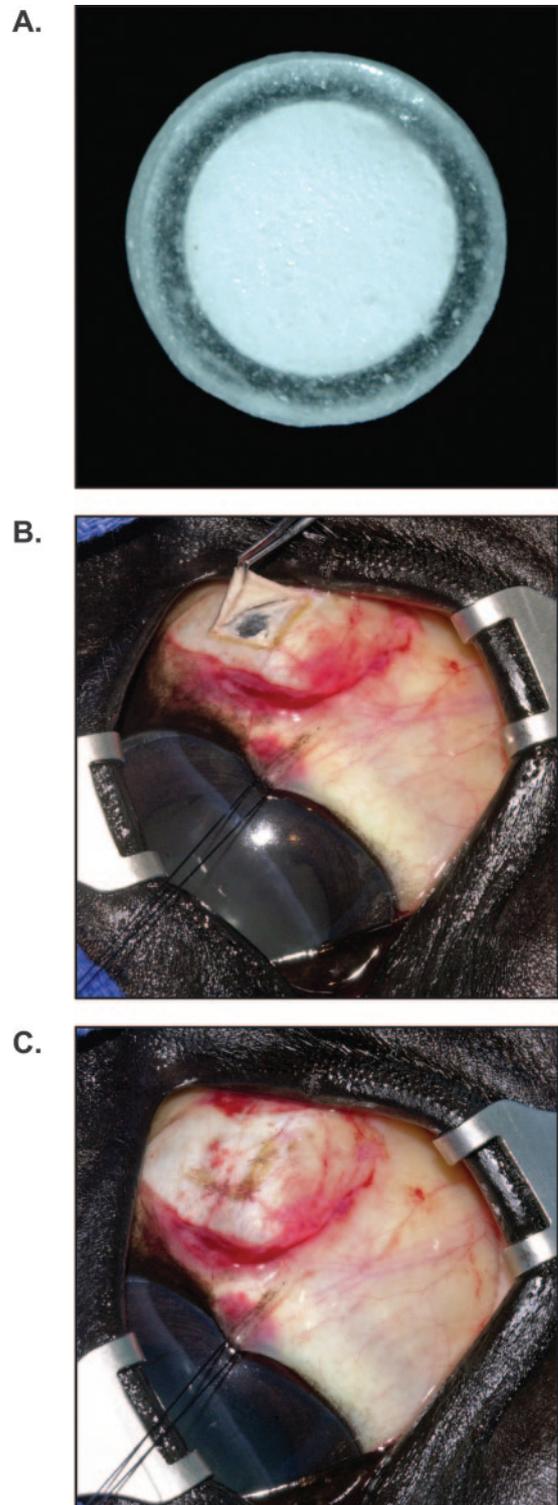


FIGURE 1. (A) Matrix-reservoir CsA bioerodible implant. (B) Surgical procedure and location of the deep scleral lamellar CsA implant. A 7-mm² partial-thickness scleral flap with 90% to 95% depth centrally was made 1 cm posterior to the limbus superotemporally. (C) The CsA implant was inserted under the flap and closed with absorbable suture material.

scribed.²⁴ The CsA concentrations were measured by HPLC and expressed in micrograms per gram of tissue. The efficiency of drug extraction was assessed in four freshly enucleated New Zealand White (NZW) rabbit eyes. The ocular tissues were isolated and spiked with

100 μ L of a stock solution of CsA (1 mg/mL) in acetonitrile. The tissues were subjected to the same drug extraction procedure, and the percentage of drug recovery (mean \pm 1 SD) was recorded.

Episcleral CsA Device in Naturally Occurring ERU

The potential benefits of an episcleral CsA implant prompted a small pilot study for the treatment of horses with ERU. Four horses with chronic ERU were entered into the study. Six of the eight eyes had ERU and received an episcleral implant as described earlier. The horses were examined at 7, 30, 90, and 180 days, then at 1 year after implantation. The number of postimplant flares, complications, and ophthalmic findings were recorded.

Pharmacokinetics and Safety Evaluation of a Deep Scleral Lamellar CsA Implant

Six adult horses with normal ocular examinations were used in this portion of the study. The horses were tranquilized, and a retrobulbar nerve block was performed according to previously described methods.⁴⁹ After sterile ocular preparation, a conjunctival incision was made followed by a 7-mm² partial-thickness scleral flap (90%-95% depth centrally) 1 cm posterior to the limbus superotemporally, avoiding the rectus muscles (Fig. 1B). The CsA implant was placed under the flap, and the flap was closed with a 6-0 polyglactin 910 suture (Vicryl; Ethicon, Inc., Somerville, NJ; Fig. 1C). Two horses were euthanized for pharmacokinetic analysis at 4 weeks and 2 at 9 weeks after implantation of the deep scleral lamellar CsA device. An additional two horses were euthanized at 12 weeks after implantation for histopathologic evaluation. Eyes and peripheral blood were collected from the horses at 4 and 9 weeks for tissue and fluid concentration of CsA. After euthanasia, sections of bulbar conjunctiva were removed adjacent to the limbus superiorly and inferiorly, and the eyes were enucleated and immediately frozen at -80°C for later dissection and drug extraction. Eyes were dissected while frozen, and 5-mm² sections of superior and inferior sclera, 2 mm posterior to the limbus, were removed. The frozen globe was then cut 360° at the limbus with a razor blade and the cornea removed from 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. Other ocular tissues isolated for drug analysis included the combined iris/ciliary body, vitreous humor, choroid/retina (superior and inferior halves), and optic nerve. CsA was extracted as described. Differences in the mean tissue concentrations at the different locations for the conjunctiva, cornea, sclera, and choroid-retina were tested by ANOVA (JMP Statistical Software, ver 5.11; SAS Inc., Cary, NC). Differences were considered clinically significant if $P < 0.05$. Eyes from the horses euthanized at 12 weeks were fixed in 10% formalin for histologic examination. In addition, a complete blood count and serum chemistry profile was performed, and sections from the major organs were evaluated histologically for signs of toxicity.

Pharmacodynamics of a Deep Scleral Lamellar CsA Device in Naturally Occurring ERU

Horses entered into this study had severe ERU (i.e., blindness was imminent) despite standard-of-care therapy, with a mean of 5.5 ± 2.1 (SD) flares occurring in the year before enrollment. Previous studies have shown that $>90\%$ of eyes with five or more flares of uveitis progress to blindness, and frequently loss of the eye, within 1 year.³⁸ Aggressive control of clinically active ocular inflammation with traditional anti-inflammatory medications, such as topical and oral steroidal and nonsteroidal anti-inflammatory medications, was performed before placement of the deep scleral lamellar CsA device, so that the uveitis was not flaring at the time of surgery.

Presence of vision (positive menace response) and the owner's consent was required. Animals with significant cataract formation (i.e., more than 20% of the anterior cortical surface) were excluded from the study. If both eyes were affected with ERU and both fit into the

inclusion criteria, the owner had the option to have implants placed in both eyes.

Horses in this portion of the study had general anesthesia for the placement of the deep scleral lamellar devices. Otherwise, the surgical procedure was as described earlier. Horses were evaluated at 1, 3, and 6 months and then every 3 to 6 months after implantation. The number of postimplant flare-ups of uveitis, complications, and ophthalmic findings was recorded. We used the PROC MIXED procedure in the software system (JMP; SAS) to model repeated measures normal linear models and account for within-horse correlations, in looking at risk factors for change in flare-ups per month after surgery.

Effect of CsA on Growth of Leptospires In Vitro

Leptospirosis infection has been implicated in some cases of ERU.⁵⁰ Therefore, we examined the in vitro susceptibility of a stock culture of *Leptospira interrogans* to CsA in a broth culture system. Lyophilized CsA (product C1832, Sigma-Aldrich Corp., St. Louis, MO) was rehydrated in 95% ethanol to make a 10 mg/mL stock solution. This was then diluted in 2 mL of Ellinghausen, McCullough, Johnson and Harris (EMJH) medium (Difco-BD Biosciences, Sparks, MD) to make final concentrations of 25 and 50 $\mu\text{g/mL}$ CsA. Two types of growth control tubes were included. The first contained EMJH broth with no CsA. The second control was EMJH with 10 μL 95% ethanol (the amount of ethanol present in the 50 $\mu\text{g/mL}$ concentration.) *L. interrogans* serotype pomona (ATCC 23478; American Type Culture Collection, Manassas, VA) was maintained in semisolid Fletcher's agar medium (Difco-BD Biosciences). For susceptibility testing, leptospores were subcultured to EMJH broth and incubated at 30°C for 1 week, at which time a turbid suspension of approximately 1×10^8 leptospores/mL was prepared. Leptospores were counted in a Petroff-Hausser counting chamber and added to 2 mL EMJH broth to achieve a final concentration of 1×10^6 leptospores/mL.⁵¹ Culture tubes were incubated at 30°C with shaking, and leptospores were counted in the Petroff-Hausser chamber on days 1, 3, 5, and 7 after inoculation to determine the number of viable bacteria per milliliter. All experiments were performed in triplicate.

RESULTS

In Vitro Transscleral Diffusion of CsA

Diffusion of fluorescein across human and equine sclera was comparable (Fig. 2A); however, the lipophilic dexamethasone-fluorescein diffused poorly across the equine sclera (Fig. 2B). In addition, CsA, similar in molecular weight and solubility to dexamethasone-fluorescein, performed poorly in the perfusion chamber compared with human sclera (Fig. 2C). These results suggest that equine sclera was a significant barrier to CsA diffusion.

In Vitro Release Rates of the CsA Implant

The implant, a combination of a matrix and reservoir design, had two phases of drug release. The initial phase followed first-order release kinetics with an initial higher release of CsA from the matrix component (23.39 ± 2.5 $\mu\text{g/d}$), which declined to 6.35 ± 0.78 $\mu\text{g/d}$ by day 30 (Fig. 3). Phase two consisted of a steady state release of CsA of 5.96 ± 1.1 $\mu\text{g/d}$ after day 30. Assays longer than 2 months were not possible, because the PVA softened in the PBS over time, and the implant fragmented with the manipulation necessary to perform the fluid exchanges. The projected duration of release rates from the implant, assuming steady state release, was 3.18 years.

Pharmacokinetics of the CsA Implant in the Episcleral Space

Despite the limited transscleral penetration of CsA in vitro, pharmacokinetic studies were performed using the CsA im-

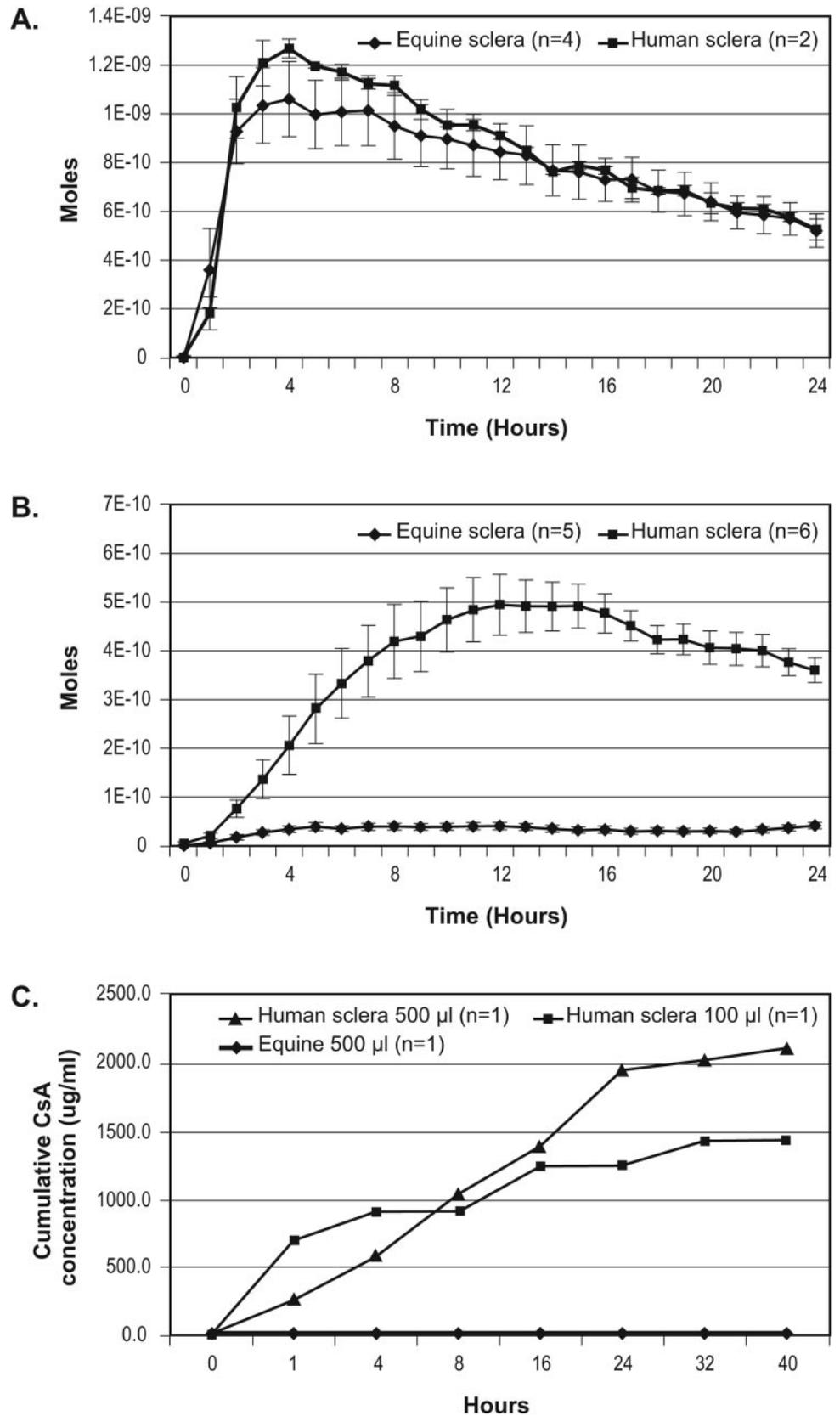


FIGURE 2. In vitro transscleral diffusion of CsA. A two-compartment perfusion chamber was used, as previously described,⁴⁸ and in vitro human and equine scleral permeability to fluorescein, dexamethasone-fluorescein, or CsA were compared. (A) The diffusion of fluorescein across the sclera of human and equine isolated sclera was comparable. (B) The lipophilic dexamethasone-fluorescein diffused poorly across the equine sclera compared with the human sclera. (C) CsA (100 or 500 µL of 50 mg/mL) diffused across the human sclera in a dose-dependent fashion and there was no perfusion of drug detected through the equine sclera.

plant in the episcleral space of normal horses. Clinical examinations performed during the course of the study revealed that the implants remained in place and there were no clinical abnormalities observed. At 7 days after implantation in vivo,

retina-choroid concentrations of CsA were 0.02 µg/g of tissue and vitreous CsA levels were 0.021 ± 0.05 µg/mL. At 30 days after implantation, retina-choroid concentrations of CsA were 0.07 µg/g of tissue and vitreous CsA levels were 0.043 ± 0.01

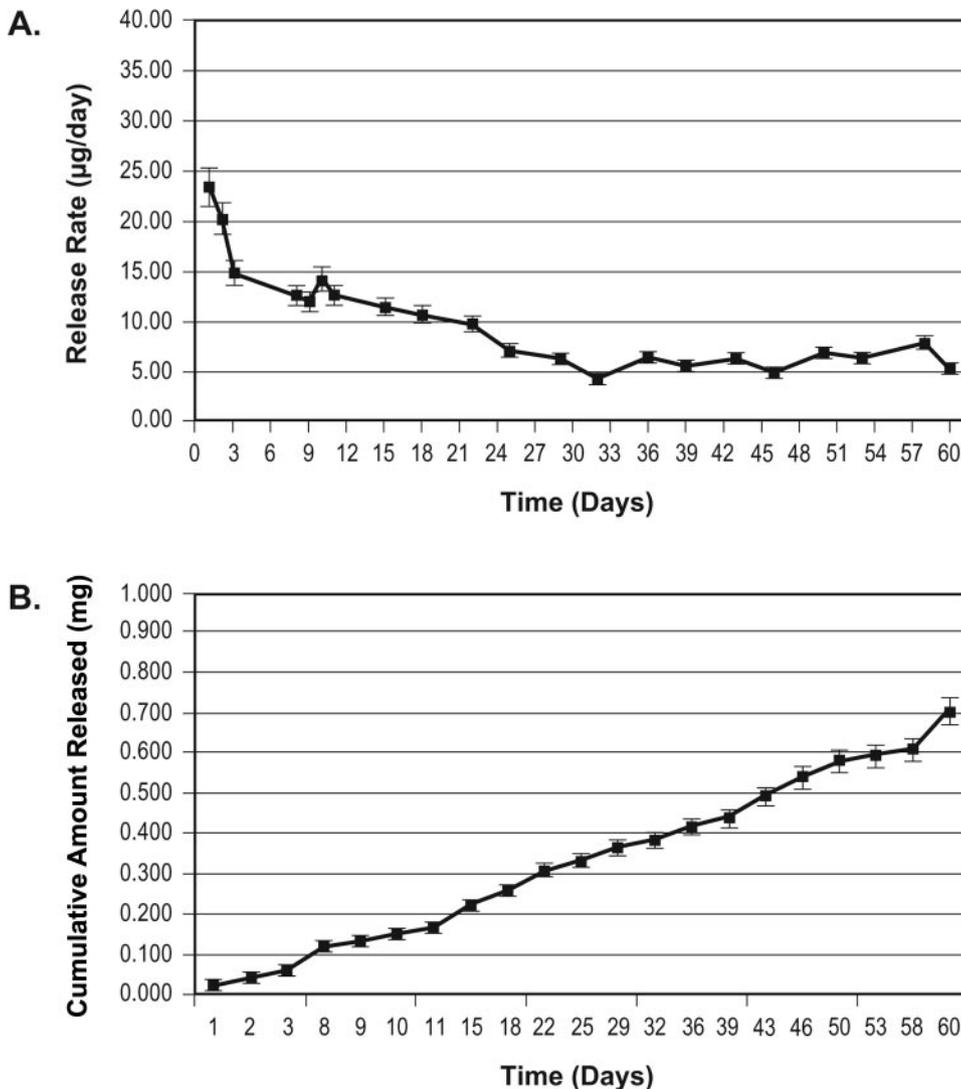


FIGURE 3. (A) In vitro mean release rates ($\mu\text{g}/\text{d} \pm 1 \text{ SD}$) for the CsA implant. There is a higher release rate for the first month followed by a steady state maintenance release. (B) Cumulative mean release of CsA ($\text{mg} \pm 1 \text{ SD}$) from the implant over time.

$\mu\text{g}/\text{mL}$, lower than the minimal drug concentrations necessary to treat ocular inflammation.¹⁰ Peripheral whole blood had no detectable levels of CsA at 7 or 30 days after implantation.

The percentage of drug recovery, as assessed by HPLC in four freshly enucleated NZW rabbit eyes that were spiked with 100 μL of CsA (1 mg/mL), was $65.7\% \pm 4.19\%$ for conjunctiva, $69.5\% \pm 1.75\%$ for sclera, $65.1\% \pm 1.96\%$ for cornea, and $72.3\% \pm 5.64\%$ for ciliary body. The percentage of drug recovery for aqueous and vitreous humor was $63.8\% \pm 4.41\%$ and $81.1\% \pm 2.98\%$, respectively.

Episcleral CsA Device in Naturally Occurring ERU

Although the in vitro transscleral diffusion studies indicated that CsA showed poor transit through equine sclera, the in vivo studies suggested low but detectable CsA delivery into the eye by the episcleral implant. Therefore, four horses with ERU (three with bilateral disease) with a mean age of 11.1 years were entered into the pilot study. Horses exhibited ocular signs of uveitis for a mean of 11.3 months before receiving the implant. Mean frequency of flares before surgery was 0.49 flares/mo (i.e., approximately 1 flare every 2 months). There was no reduction in the mean frequency of uveitis flares per month (mean of 0.5 flares/mo after implantation) with a mean follow-up of 12.4 months. One horse receiving bilateral episcleral CsA implants progressed to bilateral blindness over a

1-year postoperative period from unabated uveitis flares. Our in vitro observations that CsA poorly penetrated the equine sclera were demonstrated clinically, since there was no reduction in uveitis flares when the implant was placed in the episcleral space.

Pharmacokinetics and Safety Evaluation of a Deep Scleral Lamellar CsA Implant

Normal horses had the CsA device placed in the deep sclera adjacent to the suprachoroidal space under a scleral flap in one eye. No abnormalities were observed on ophthalmic examination of these horses for up to 12 weeks after surgery, except for mild conjunctival hyperemia over the surgical site for the first week after surgery. No abnormalities were noted on histopathologic examination of the eyes of horses 12 weeks after implantation with the CsA device. The implant was encapsulated with a fine layer of fibrous tissue, which helped prevent implant migration (Fig. 4). Signs of retinal or uveal toxicity were not observed. In addition, no abnormalities were observed in histologic sections of renal, cardiac, lung, or hepatic tissue from the implant-recipient horses. Furthermore, no abnormalities were observed in a complete blood count or serum chemistry profile.

Ocular tissue CsA concentration was measured at 4 and 9 weeks after implantation, and peripheral blood CsA concentra-

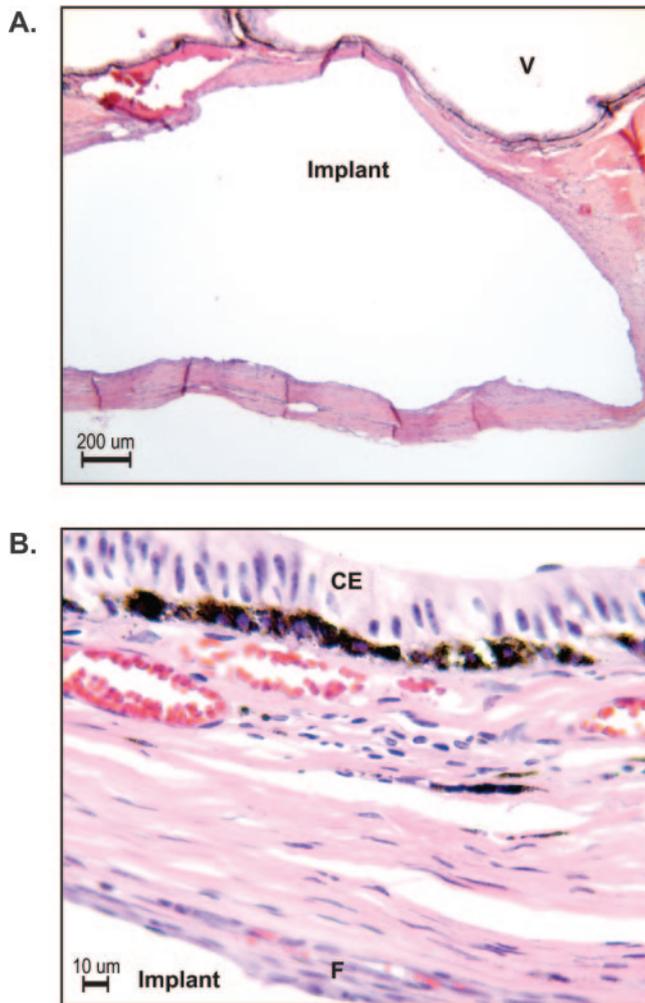


FIGURE 4. Histologic section of a normal equine eye 12 weeks after placement of a CsA implant under a scleral flap. (A) Site of implant placement in sclera (Implant). V, vitreous body. (B) Normal pars plana nonpigmented epithelium (CE) near the area where the implant (Implant) approached the suprachoroidal space. There was moderate fibrosis (F) surrounding the implant site. Hematoxylin and eosin stain.

tion was measured at 4, 9, and 12 weeks after surgery. High concentration of CsA was measured at both 4 and 9 weeks in the superior sclera, inferior sclera, superior retina-choroid, inferior retina-choroid, ciliary body, and optic nerve (Table 1).

TABLE 1. Tissue Concentrations of CsA at 4 and 9 Weeks after Placement of a Deep Scleral Lamellar CsA Sustained-release Device

	4 Weeks	9 Weeks
Superior conjunctiva	22.0 ± 6	0.1 ± 0.1
Inferior conjunctiva	21.0 ± 7	0.1 ± 0.03
Superior sclera	811.0 ± 740	45 ± 19.6
Inferior sclera	3.0 ± 3	3.0 ± 1
Superior cornea	0.0	0.0
Inferior cornea	0.0	0.0
Ciliary body	28.0 ± 2	12.0 ± 12
Lens	1.0 ± 0.1	2.0 ± 1
Superior retina-choroid	76.0 ± 6	65.0 ± 16
Inferior retina-choroid	58.0 ± 8	65.0 ± 16
Optic nerve	96.0 ± 65	66.0 ± 60

* Data are expressed as the mean micrograms per gram of tissue ± SD.

Mean vitreous concentrations were $0.20 \pm 0.14 \mu\text{g/mL}$ (SD) at 4 weeks and $0.14 \pm 0.04 \mu\text{g/mL}$ at 9 weeks. There were no significant differences in CsA tissue concentration in tissues at 4 weeks; however, at 9 weeks the superior sclera, superior choroid-retina, inferior choroid-retina, and optic nerve tissue CsA concentrations were significantly higher than levels measured in the other ocular tissues ($P < 0.001$; i.e., conjunctiva, cornea, ciliary body, and lens). CsA was not detected in the cornea, aqueous humor, or peripheral blood at either 4 or 9 weeks after implantation.

Pharmacodynamics of a Deep Scleral Lamellar CsA Device in Naturally Occurring ERU

Sixty-seven horses at seven locations (Table 2) met criteria for inclusion in the study. Each horse received at least one implant, and 13 horses received implants in both eyes. The mean age was 11.1 ± 5.1 years (SD; range, 3–23; median, 11). Nineteen (28%) of the horses were female and 48 (72%) were male. The sample was made up of a variety of breeds: with 15 (22%) quarter horses, 13 (19%) Appaloosas, and 8 (12%) thoroughbreds as the most common study sample breeds (Table 2). Horses had exhibited ocular signs, on average, 16.4 ± 16.0 months (SD; median 12; range 3–120) before receiving the implant. Mean follow-up time (time after surgery at last recheck) was 14.2 ± 9.9 months (range, 3–36; median, 11.5).

The number of uveitis flare-ups was significantly decreased after surgery ($P < 0.0001$), with a mean of 0.544 flare-ups per month before surgery and 0.096 per month after surgery. There was no significant correlation between duration of disease, signalment of the animal, location of surgery, and preoperative ocular clinical signs (e.g., presence of aqueous flare, synechia, vitreous opacity, and cataract) to postoperative frequency of uveitis flare-ups. However, there was a significant correlation between the presence of preoperative glaucoma, even if medically controlled, and an increase in postoperative uveitis flare ups ($P = 0.0016$).

Non-vision-threatening adverse effects or complications after surgery included development of a superficial corneal ulcer ($n = 3$), controlled glaucoma ($n = 2$), and mild progression of cataract ($n = 3$). Twelve of the 80 (15%) eyes became blind after surgery. Blindness was attributed to uncontrolled uveitis ($n = 4$), glaucoma ($n = 4$), complete cataract ($n = 2$), retinal

TABLE 2. Signalment Data of 67 Horses with Deep Scleral Lamellar CsA Implants

Breed (n)	Gender (n)	Location of Surgery (n)
Quarter Horse (15)	MC (47)	OSU (28)
Appaloosa (13)	F (19)	NCSU (23)
Thoroughbred (8)	M (1)	UPENN (5)
Arabian (5)		UFL (4)
Paint (5)		CONN (3)
Hanoverian (4)		TX (2)
Warmblood (4)		UM (2)
Others (9)		

n, number of horses that had a CsA implant in each category; MC, male neutered; F, female; M, male; OSU, The Ohio State University College of Veterinary Medicine, Columbus, OH; NCSU, North Carolina State University College of Veterinary Medicine, Raleigh, NC; UPENN, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA; UFL, University of Florida College of Veterinary Medicine, Gainesville, FL and Animal Eye Specialty Clinic, West Palm Beach, FL; CONN, Animal Eye Clinic, Norwalk, CT, and Animal Vision, West Hartford, CT; TX, Texas A&M College of Veterinary Medicine, College Station, TX; UM, University of Missouri College of Veterinary Medicine, Columbia, MO.

detachment ($n = 1$), and fungal keratitis ($n = 1$). Overall mean time after surgery of development of blindness was 17.3 ± 10.6 months (SD; median, 12; range, 6-32). The time after surgery was not significantly different from eyes that were visual (mean 14.0 ± 10.0 months; median, 11.0; range, 3-36). Although eyes with uveitis (mean, 10.8 ± 8.1 months) and retinal detachment (9 months) had the shortest time to blindness after surgery, these times were not significantly different from time after surgery for development of blindness associated with glaucoma (mean, 23.8 ± 9.5 months) or cataract (mean, 22.0 ± 14.0 months).

There was no significant correlation between signalment of the animal, location of surgery, and preoperative ocular clinical signs (e.g., presence of aqueous flare, synechiae, vitreous opacity, cataract, or glaucoma) to postoperative development of blindness. Duration of clinical signs before surgery was significantly longer in eyes that became blind (mean, 31.0 ± 9.1 months) compared with eyes that retained vision (mean, 13.7 ± 10.1 months; $P=0.0003$). The frequency of uveitis flare-ups before surgery did not significantly correlate with blindness; however, the frequency of flare-ups after implantation was significantly higher in eyes that became blind (mean, 0.42 ± 0.10 flare-ups/mo) than those that retained vision (mean, 0.04 ± 0.04 flare-ups/mo; $P = 0.0005$).

On follow-up, the percentage of eyes with vision at 6 months after surgery was 98% (68/69), after 12 months was 93% (43/46), after 18 months was 90% (28/31), and after 24 months was 96% (22/23). Overall, 85% (68/80) of eyes had vision after surgery (Fig. 5).

Effect of CsA on *Leptospira* spp. Growth In Vitro

In the control tubes (EMJH), the leptospires multiplied to a concentration of 10^8 organisms/mL at the end of the 1-week incubation period. In contrast, the number of viable leptospires in the presence of $50 \mu\text{g/mL}$ CsA was diminished by 24 hours and was undetectable at subsequent time points (Fig. 6).

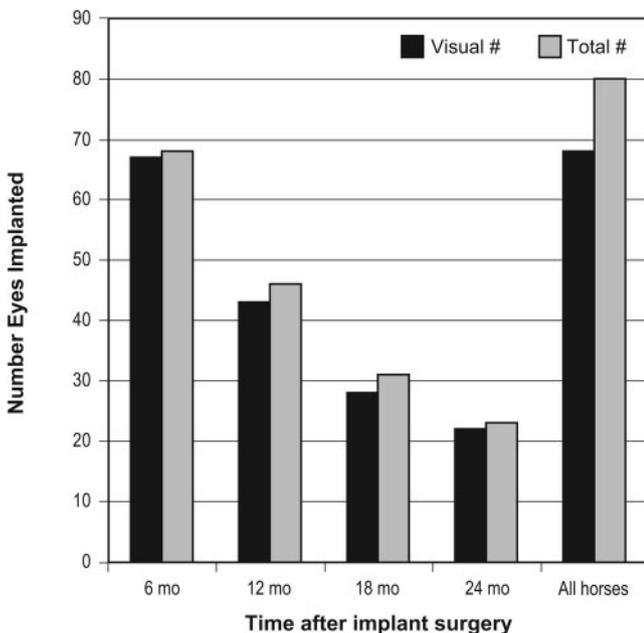


FIGURE 5. Number of eyes that were visual after surgical implantation of a deep scleral lamellar CsA implant. The percentage of eyes with vision at 6 months after surgery was 98% (68/69), after 12 months was 93% (43/46), after 18 months was 90% (28/31), and after 24 months was 96% (22/23). Overall, the percentage of eyes with vision after surgery was 85% (68/80).

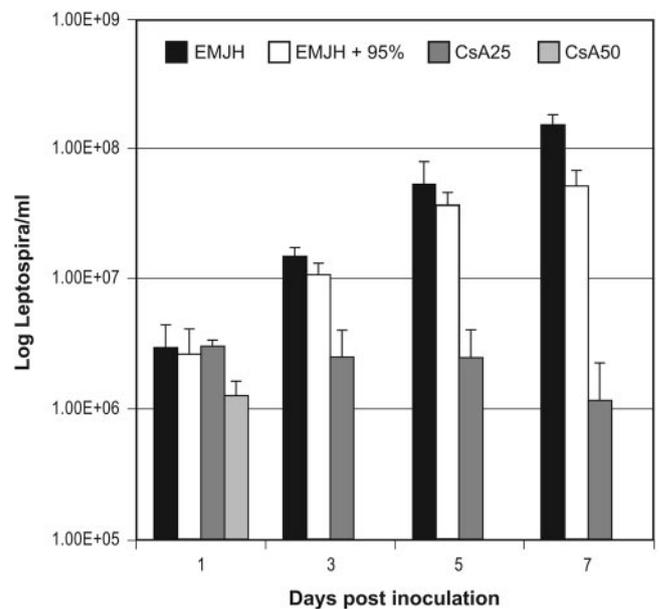


FIGURE 6. Viable leptospires, as determined by counting with the Petroff-Hausser chamber on days 1, 3, 5, and 7. EMJH is a growth control. EMJH+95% EtOH is a growth control containing the amount of 95% ethanol in the highest concentration of CsA tested. CsA 25 and CsA 50 are cultures with 25 and 50 $\mu\text{g/mL}$ CsA, respectively. Data represent the mean of three replicates. Error bars, SD.

The 25- $\mu\text{g/mL}$ concentration of CsA inhibited the growth of leptospires with a gradual decrease in viable organisms noted over the 7-day incubation period. Thus, CsA was rapidly bactericidal at a concentration of 50 $\mu\text{g/mL}$ and bacteriostatic for 7 days at 25 $\mu\text{g/mL}$ (Fig. 6). As these CsA concentrations are in the range of those achieved in the uveal tissues in the presence of the implant in vivo (Table 1), this supports the possibility that CsA released from the implant may have a direct inhibitory effect on potential leptospires that may contribute to uveitis.

DISCUSSION

This study describes the use of a novel bioerodible, deep scleral lamellar CsA ocular implant in a naturally occurring model of uveitis. Placement of the device to facilitate delivery of CsA to the suprachoroidal space was chosen because of the lack of significant scleral penetration of the drug in vitro and in vivo. Sustained delivery of CsA to the deep sclera-suprachoroidal space achieved high levels of drug throughout various intraocular tissues, including the ciliary body, choroid-retina, and optic nerve. No toxicities or detrimental effect of the surgery, device, or medication in the equine eye was found. Use of the device in the deep sclera of a naturally occurring model of severe uveitis in horses resulted in a significant reduction of postoperative uveitis flare-ups and rates of blindness. In horses with severe ERU, 15% of eyes became blind a mean of 14.2 months after implantation, which compares favorably with the natural history of severe ERU with traditional therapy in which blindness at 1 year is $>90\%$.^{37,38}

The philosophy of using sustained-release CsA implants in the treatment of ERU is based on the predominant T-cell infiltration present histopathologically in eyes with ERU.^{33,35} In many cases, animals with ERU can have low-grade chronic ocular inflammation and present with blindness as the initial sign of uveitis.⁵⁰ Therefore, treating only clinically evident flares may not be the optimal approach for ERU, since the structural eye damage may progress in times of a clinically

inactive disease. This supports the use of a sustained-release implant to have ocular tissues continuously exposed to CsA to treat both clinically active and indolent ocular inflammation.

An intravitreal CsA implant was found to be effective in an experimental model of equine uveitis.⁴⁴ In this study, horses were immunized peripherally with *Mycobacterium tuberculosis* (MTB) antigen twice and then received MTB antigen intravitreally. After receiving an intravitreal CsA device, the horses were rechallenged with intravitreal MTB antigen. Clinical signs of uveitis in eyes with the CsA device were less severe and significantly shorter in duration than signs with the polymer-only implanted eyes. Duration and severity of inflammation, cellular infiltration, tissue destruction, and proinflammatory cytokines RNA transcript levels were significantly less in those eyes implanted with the CsA device.⁴⁴ Similar results were seen in a rabbit model of uveitis after receiving a CsA implant.¹¹

A year-long study was performed to determine the long-term toxicity of an intravitreal device releasing continuous CsA in normal eyes of horses.⁴³ Ten eyes were implanted with an intravitreal CsA device releasing 2 $\mu\text{g}/\text{d}$ of CsA. Two eyes experienced complications related to the surgical procedure (lens penetration, bacterial endophthalmitis). The other eyes had only minimal postoperative inflammation for 1 to 2 days after the procedure and no changes on scotopic electroretinography. Histologic findings revealed only a mild lymphoplasmacytic cellular infiltrate in the ciliary body and pars plana near the implantation site and no retinal or lens toxicity.⁴³ Similar toxicity studies have been performed in rabbits and primates with intravitreal CsA.¹⁰

Another study evaluated prospectively 23 horses with naturally occurring ERU that received an intravitreal CsA implant.^{12,46} All horses had a chronic history (>3 months) of recurrent episodes of uveitis that was typical of ERU. After implantation, of the 23 horses evaluated, only 8 exhibited a recurrent episode of uveitis with a total number of 9 recurrent events in all horses. At follow-up, vision remained in 18 (78.2%) of 23 horses. The mean follow-up in visual eyes was 11.1 months and in blind eyes 8.2 months. Complications observed included development of glaucoma ($n = 2$), mature cataract ($n = 2$), retinal detachment ($n = 3$), and phthisis bulbi ($n = 2$).⁴⁶ Although these results were good, the high rate (3/23 eyes, 13%) of retinal detachment prompted the search for less invasive methods of sustained drug delivery. In the series of horses that received a deep scleral lamellar implant in our study, only 1 horse (of 80 eyes; 1.2%) had a retinal detachment.

Although ERU is widely considered an immune-mediated disease,^{33,35} *Leptospira* spp. may be implicated in some cases.^{50,52,53} The CsA concentrations observed in vitro for *L. interrogans* inhibition were in the same range of those observed in the uveal tissues in vivo with a deep scleral lamellar implant. Based on these results, the CsA deep scleral lamellar implant may be therapeutic in known leptospiral uveitis by reducing the bacterial burden as well as treating the inflammatory component. CsA has been shown to inhibit growth of other microorganisms including viruses such as hepatitis C⁵⁴ and herpesvirus⁵⁵; protozoa such as *Leishmania*⁵⁶ and *Toxoplasma*⁵⁷; other parasites such as *Toxocara*⁵⁸ and *Angiostrongylus* spp.⁵⁹; and fungal organisms.^{60,61} Although the CsA implant may have a bifunctional role as an anti-infective and an anti-inflammatory agent in ERU, live leptospire are not likely to be a major contributor to ERU, because specific antibiotic therapies³⁸ or vaccination⁶² do not alter the course of the disease.^{12,29,38} Recent studies have demonstrated that the immunogenic potential of fragments of infectious organisms may have a role in development of immune-mediated

disease in the equine eye⁶³⁻⁶⁵ and support the use of immunosuppressant therapy for ERU.

The suprachoroidal space is situated beneath the sclera but external to the choroid.^{66,67} This space has been used without complication for shunting of aqueous humor^{68,69} and as a site for drug delivery to the posterior segment of the eye.⁷⁰ The advantage of this site is that medications can bypass the scleral barrier and be delivered directly to the choroid and potentially the retina without the need to enter the vitreous cavity surgically. The main disadvantage is that the high choroidal blood flow may prevent many medications from reaching internal ocular structures. Despite this concern, the deep scleral lamellar CsA device, which was near to or in contact with the suprachoroidal space, delivered high levels of drug to the uveal tissues in the large equine eye. Furthermore, the tissues on the opposite side of the globe, in general, had levels similar to those in the tissues proximal to the CsA implant. It is possible that the suprachoroidal space acts as a conduit to allow medication to quickly bathe the external choroid instead of relying on simple drug diffusion.

Based on the results of this study, sustained delivery of medications to the suprachoroidal space is feasible and warrants evaluation of the potential of the suprachoroidal space for sustained drug delivery to human eyes.

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