Penetration of Cyclosporin A into the Rabbit Cornea and Aqueous Humor after Topical Drop and Collagen Shield Administration

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We used commercially available 12-hour collagen shields to deliver cyclosporin A (CsA) to the cornea and aqueous humor in rabbit eyes. Six New Zealand white rabbits were divided into three groups. The first group (four eyes) received 6 mg of CsA in castor oil and the second group (four eyes) received 6 mg of CsA in olive oil applied as topical drops to rabbit eyes within 12 hours. In the third group (four eyes) 12-hour collagen shields soaked in 6 mg CsA in olive oil were applied to rabbit eyes. The amount of CsA in corneal and aqueous samples from eyes treated with CsA castor oil and CsA olive oil were compared with each other and with collagen shield treated eyes. CsA concentrations were measured by radioimmunoassay. After the total dose of 6 mg CsA, percentage penetration was measured as follows: CsA castor oil—0.51% in aqueous and 20.75% in cornea; CsA olive oil—0.17% in aqueous and 11.13% in cornea; and with collagen shields—0.44% in aqueous and 11.84% in cornea. These results show that the CsA levels of castor oil drops were higher than those obtained with olive oil drops. In eyes with collagen shields, CsA levels were higher than olive oil drops but nearly equal to the castor oil drops. Collagen shields may be useful as an ocular delivery system for CsA.

Introduction

The successful treatment of many diseases of the eye depends upon adequate drug delivery. An important development in drug delivery has been the utilization of collagen shields. Collagen shields have been used as bandage lenses after radial keratotomy and keratorefractive surgery, to treat corneal abrasions, and as an alternative therapy for dry eyes.[1,2] The shields are made of porcine scleral collagen and are available in 12- 24- and 72-hour dissolution rates. Their oxygen permeability is high. After placement on the eye, they become hydrated with tears, take the shape of the cornea, and are degraded by proteolytic enzymes in the tear film. In addition to their capabilities of lubricating, protecting, and accelerating reepithelialization, they are used as vehicles for enhancing the ocular penetration of various drugs.[3] Recent investigations with collagen shields demonstrate that delivery of gentamicin,[4] vancomycin,[4] tobramycin,[5] dexamethasone,[6] amphotericin B,[7] heparin,[8] and cyclosporin A[9] to the eye are increased.

Cyclosporin A (CsA) is a cyclic polypeptide of fungal origin. First reported to have immunosuppressive properties by Borel in 1976,[10] it has dramatically improved the prognosis for solid organ transplantation because of its low myelotoxicity and suppression of specific T lymphocyte function. Systemic CsA has been used to suppress rejection after kidney transplantation, as well as bone marrow, heart, and liver transplantations.[10] It has also been used to treat various autoimmune diseases such as uveitis, psoriasis, rheumatoid arthritis, myasthenia gravis, and diabetes mellitus type 1.[10] It has been used with some success to treat patients with various ocular manifestations of systemic immune disease, including Grave's ophthalmopathy,[10] corneal peripheral melting syndrome,[11] Behcet's disease,[10], and Sjögren's syndrome. Nussenblatt and colleagues[10] suggested that topical

therapy was effective only if the serum CsA levels entered the therapeutic range of 50 to 300 ng/mL.

Delivery of therapeutic concentrations of CsA to the cornea and the anterior chamber has been difficult to accomplish because of its poor solubility in water.[9] Topical ophthalmic uses have included treatment of corneal graft rejections,[12,13] Mooren's ulcer, noninfiltrative marginal keratolysis,[14] and herpetic stromal keratitis.[15] Recently, investigations of CsA levels in the eye and various tissues after topical and systemic administrations have been made. Mosteller and coworkers[16] investigated the systemic and ocular absorption of topically applied 10% cyclosporine ointment. Ben Ezra and colleagues[17] determined the tissue levels of CsA after oral, intraperitoneal, and intravenous administrations. Reidy and associates[9] prepared collagen shields by mixing gelatinous collagen with crystalline CsA and compared them with topical eye drops.

The aim of this study was to investigate the effectiveness of commercially available collagen shields and CsA preparations and was performed to compare the penetration of CsA olive oil and castor oil forms administered as topical eye drops with each other and with commercially available collagen shields soaked in CsA olive oil.

Materials and methods

Six New Zealand white rabbits, male and female, 2,038 to 3,220 g were divided into three groups.

Eye drops: In group 1, the eye drops consisted of a commercially available intravenous form of CsA (castor oil) in a concentration of 50 mg CsA/mL (0.5 mg CsA/10μL; Sandimmune, Sandoz Pharmaceuticals). In group 2, the eye drops consisted of the oral form of CsA (olive oil) in a concentration of 100 mg CsA/mL (1 mg CsA/10 μL; Sandimmune, Sandoz Pharmaceuticals). Over 12 hours, a total of 6 mg CsA was administered as topical drops with a micropipette.

*Collagen shields:*Four commercially available collage shields were used (12 hours [Bio-cor; Bausch & Lomb Pharmaceutics, Inc., Clearwater, FL]). For this purpose, 60 μL (1 mg CsA/10 μL) Sandimmune® oral solution (olive oil) was dropped over collagen shields. We used CsA olive oil because it was absorbed better than CsA castor oil. Within 1.5 to 2 hours, the total dose of 6 mg CsA was absorbed by the collagen shields.

*Experimental design:*The six rabbits were divided into three groups. In group 1, four eyes of two rabbits received CsA castor oil applied with a micropipette (10 μL [Socorex; Switzerland]) at one drop (10 μL) per hour for 12 doses for a total of 6 mg CsA. (Every 10 μL drop contained 0.5 mg CsA [12 × 0.5 mg = 6 mg CsA].) In the second group, four eyes of two rabbits received CsA olive oil applied to the eyes with a micropipette, one drop every 2 hours for 12 hours (six times). They also received a total of 6 mg CsA (every 10 μL drop contained 1 mg CsA [6 × 1 mg = 6.0 mg CsA]). In both groups, the total dose of CsA (6 mg) was equal to the amount of CsA delivered by a single collagen shield.

Four eyes of the two rabbits in the third group were treated with CsA olive oil soaked collagen shields. Rabbits were anesthetized by intramuscular injection of 0.5 mL 2% xylazine hydrochloride (Rompun; Bayer) and 1 mL ketamine hydrochloride (Ketalar, Parke-Davis). Collagen shields containing CsA were applied directly to the corneas and rehydrated with sterile saline. The eyes were closed by lid sutures to ensure retention of the shields. The shields remained in the rabbits' eyes for 12 hours.

Twelve hours after application of either the drops or the collagen shields, the rabbits were killed with sodium pentobarbital injections given intravenously. The eight eyes to which the topical eye drops had been administered were rinsed with sterile saline. Aqueous humor was collected with a sterile 25 gauge needle attached to a 2 mL syringe. The anterior chamber was entered 1 mm away from the limbal margin of the cornea, and 0.2 to 0.5 mL of aqueous humor was aspirated. The corneas were removed by 8.5 mm trephine. After rinsing them with sterile saline, the corneas were minced with a razor blade and transferred to a 1 mL volume of methanol. They were left at 4°C overnight and centrifuged at 2,000 revolutions per minute for 10 minutes.

When the four eyes to which the collagen shields were applied were opened, no shields were found on the corneas. Small fragments of collagen, combined with mucus, were lodged in the fornices. Eyes were rinsed with sterile saline, and samples were obtained in the same manner as previously described.

CsA concentrations in all samples were evaluated by monoclonal antibody based radioimmunoassay (RIA). The RIA laboratory used CYCLO Trac SP 125/RIA (INCSTAR Corp., Stillwater, MN) and a gamma scintillation counter.

Results

In group 1, CsA castor oil treated eyes, the CsA concentration in the aqueous humor ranged between 22.8 ng/mL and 38.2 ng/mL, with an average of 30.6 ng/mL (Table I); the concentrations in the cornea ranged between 865 ng/mL and 1,622 ng/mL, with an average of 1,245 ng/mL (Table II). In group 2, the CsA concentrations in aqueous humor of eyes that received topical CsA olive oil drops were below levels that could be measured with RIA (< 10 ng/mL; Table I). The values obtained in the cornea ranged between 472 ng/mL and 855 ng/

TABLE I Cyclosporine concentrations in aqueous humor (ng/mL)

	Mean	SD	Range
CsA, castor oil	30.625	8.531	+22.8 – +38.2
CsA, olive oil*	10.00	0	0
Collagen shield	26.475	13.192	+14.7 – +42.8

**CsA, olive oil < 10 ng.*
SD = standard deviation

TABLE II Cyclosporine concentrations in cornea (ng/mL)

	Mean	SD	Range
CsA, castor oil	1245	426.792	+865 – +1622
CsA, olive oil	667.8	157.559	+472 – +855
Collagen shield	710.5	129.935	+589 – +857

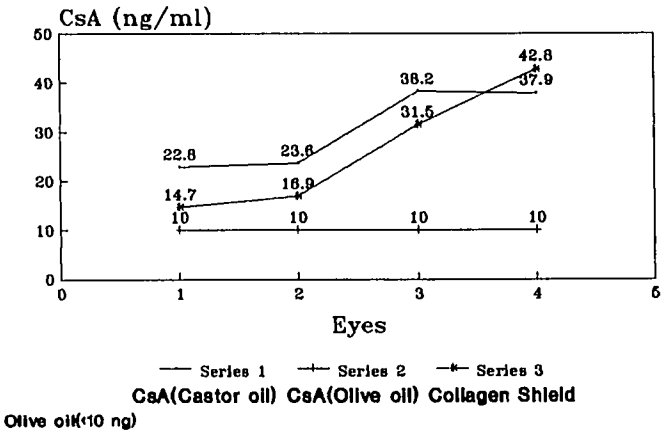

Figure 1 *Aqueous humor levels.*

mL, with an average of 667.7 ng/mL of CsA (Table II). Thus, after 12 hours, the concentrations in the aqueous humor (Figure 1) and the cornea (Figure 2) were statistically higher in the group that received topically applied castor oil CsA (aqueous humor, $P < 0.017$; cornea, $P < 0.035$, paired t test).

In group 3, CsA applied by collagen shields, aqueous humor values ranged between 14.7 ng/mL and 42.8 ng/mL, with an average of 26.5 ng/mL (Table I), and the values of the cornea ranged between 589 ng/mL and 857 ng/mL, with an average of 710.5 ng/mL (Table II). Whereas the mean aqueous humor concentrations of CsA in eyes treated with collagen shields were significantly greater than the mean concentrations of CsA in eyes treated with topically applied CsA olive oil drops, no significant difference in corneal concentrations could be demonstrated between these two groups ($P > 0.316$).

When the results obtained by using collagen shields were compared with the results of topically applied CsA castor oil drops, the concentrations were nearly equal in aqueous humor (Figure 1). According to paired t-test, no significant difference could be demonstrated ($P > 0.25$), but the corneal concentrations were higher with topically applied CsA castor oil drops (Figure 2), and this was significant ($P < 0.038$).

Figure 3 shows the penetration levels of CsA in aqueous

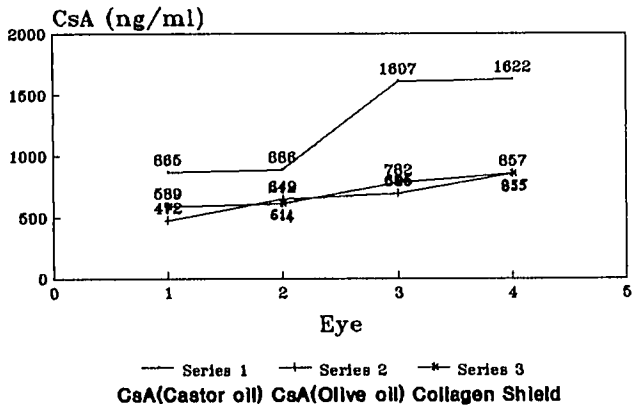

Figure 2 *Cornea levels.*

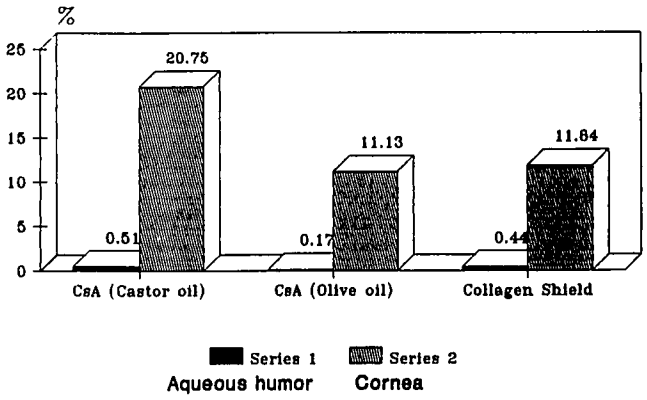

Figure 3 *Aqueous humor and cornea levels.*

humor and cornea with topical castor oil, olive oil drops, and olive oil soaked collagen shields as a percentage of the total dose of 6 mg.

Discussion

The results of this study indicate that in rabbits topically applied CsA castor oil drops resulted in higher concentrations of the drug in the aqueous humor and cornea than when CsA olive oil drops were used. An additional finding is that a collagen shield soaked in CsA olive oil form is an equally effective means of delivering CsA to the cornea and aqueous humor as topically applied castor oil drops.

The topical application of CsA to the eye has been studied in an attempt to reduce the risk of systemic toxicity while maximizing its local therapeutic potential. Because of the hydrophobic nature of the corneal epithelium, CsA would be expected to penetrate it easily. Conversely, the stroma should be quite impermeable to the drug because it is hydrophilic. By using a 2% solution of CsA (castor oil), Wiederholdt and colleagues[18] evaluated its penetration into the different layers of rabbit eyes. Following a single dose of 10 µL of 2% CsA, a concentration of 900 to 1,400 ng/mL was detected in the cornea at 6 hours, of which 67% was found in the epithelium, 25% in the stroma, and 8% in the endothelium. Concentrations in the remaining tissues (lens, vitreous, uvea, retina) were < 45 ng/mL.[18] Kaswan[19] measured the absorption of topically administered 1% CsA in olive oil. After the total dose of 0.84 mg was given, tissue levels (cornea, anterior and posterior sclera) greater than 50 ng/mL were achieved within 1 hour, but lower levels were obtained in the retina, vitreous, ciliary body, iris, and aqueous humor.

In our study, the penetration of topically applied CsA castor oil and olive oil drops were compared with each other. After a total dose of 6 mg of topically applied CsA castor oil, 0.51% was found in aqueous humor and 20.75% in the cornea, compared with 0.17% in aqueous and 11.13% in the cornea with topical CsA olive oil drops. Thus, topical drops prepared using CsA in castor oil are more effective than CsA in olive oil.

Reidy and coworkers[9] prepared collagen shields by mixing gelatinous collagen with crystalline CsA as a means of delivering CsA to the cornea and aqueous humor in rabbit eyes.

They found that both the corneal and aqueous humor concentrations of CsA achieved with the shield delivery system were higher than those obtained with topical CsA olive oil drops. In our study, 12-hour collagen shields were soaked in CsA olive oil and applied to the eyes. CsA levels in the aqueous humor were 0.44% of the total dose, and 11.84% of the total dose was found in the cornea.

Commercially available collagen shields can be utilized as a means of delivery system for CsA and in addition to commercially available oral and intravenous forms of CsA dispensed as topical drops. Our study is the first to show the efficacy of using commercially available collagen shields as a means of delivering CsA.

The application of CsA in a collagen shield delivery system is easy to apply to patients with diseases such as Mooren's ulcer, corneal peripheral keratolysis, herpetic stromal keratitis, and corneal allograft reactions, thus minimizing systemic toxicity and maximizing local penetration.

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ERRATUM The article "Deposition of Ciprofloxacin, Prednisolone Phosphate, and Prednisolone Acetate in SeeQuence Disposable Contact Lenses," which appeared in our July 1993 issue (*CLAO J* 1993;19:166-168), failed to indicate a legal name change for one of the article's co-authors. The correct spelling of the second author's name is Ameet K. Goyal, MD. The editors apologize for this error.