A Compartmental Model for the Ocular Pharmacokinetics of Cyclosporine in Rabbits

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Studies were conducted in rabbits to determine the ocular distribution and elimination of cyclosporine, with the objective of developing a comprehensive pharmacokinetic model. Following a bolus dose into the anterior chamber, drug levels were measured in the aqueous humor, cornea, iris/ciliary body, lens, sclera, and conjunctiva. Cyclosporine was rapidly eliminated from the aqueous, but drug levels in ocular tissues persisted for in excess of 48 hours, particularly in the cornea and iris/ciliary body. The terminal elimination half life from these tissues was 45 hr and 30 hr, respectively, providing evidence that these tissues could act as a reservoir for the drug. It was found that a compartmental model accurately described the experimental data. A single compartment was used for each of the tissues and fluids sampled, except for the cornea, which was subdivided into two compartments, representing its tissue and aqueous regions.

KEY WORDS: cyclosporine; ocular pharmacokinetics; compartment model; eye.

INTRODUCTION

Cyclosporine (CsA) is an immunosuppressive agent with several ocular indications, including autoimmune diseases, Sjogren's syndrome, some forms of uveitis, Bechet's disease, vernal keratoconjunctivitis, keratoconjunctivitis sicca, immune mediated keratitis, necrotizing scleritis, herpetic stromal keratitis, and corneal transplant therapy (1). Despite this broad range of ocular applications, only limited studies have been undertaken to examine the intraocular distribution and elimination of the drug.

The systemic pharmacokinetics of CsA in humans and rabbits have been well characterized as a result of earlier studies using CsA (2–5). However, only limited and incomplete ocular CsA pharmacokinetic data have been obtained following topical or subconjunctival administration, either in rabbits (6–11) or in humans (12,13). In many of these studies, only the cornea and aqueous humor were sampled, and if other tissues were sampled, the concentrations were very low or undetectable, particularly following topical administration. It is often difficult to accurately quantify the drug delivery rate following topical administration, primarily due to nasolacrimal drainage, a variable tear volume, and spillage of the eyedrop. Therefore, the existing data do not provide a good basis for developing a comprehensive ocular model which describes the distribution and elimination of cyclosporine.

Previous research has shown that the low intraocular levels of CsA following topical administration are due to the low transcorneal permeability of the drug and the rapid drainage of the drug into the nasolacrimal duct (14). The low corneal permeability also suggests that topical administration of CsA to treat ophthalmic disorders would be inefficient, and if effective, would likely require high doses which could lead to systemic side effects due to nasolacrimal absorption. Degradable implants, however, have the potential to provide therapeutic levels of CsA, depending upon the implant site and the site of action of the drug. To develop such an implant, a thorough understanding of the intraocular distribution and elimination of the drug is essential.

The objective of this work, therefore, was to develop a comprehensive pharmacokinetic model which describes the distribution and elimination of CsA in the eye. As part of this work, experiments were undertaken to obtain measurable and reproducible drug levels in a wide variety of ocular tissues and fluids, including the cornea, conjunctiva, sclera, lens, iris/ciliary body, and aqueous humor. It is expected that this model will provide important information which would aid in the development of drug delivery devices and protocols for treatment of a variety of ophthalmic disorders.

EXPERIMENTAL MATERIALS AND METHODS

Chemicals

CsA and cyclosporin D (CsD) were kindly donated by Sandoz Canada (Montreal, PQ). Monoclonal RIA kits (CYCLO-Trac SP RIA) were purchased from INCStar (Stillwater, MN), balanced salt saline (BSS) was purchased from Alcon (Mississauga, ON), ketamine-hydrochloride and xylazine hydrochloride were obtained from MTC Pharmaceuticals (Cambridge, ON), HPLC grade acetonitrile was purchased from Fisher (Toronto, ON), and T-61 was obtained from Ormond Veterinary Supplies (Ancaster, ON).

Assays

Both HPLC and monoclonal RIA were used to analyze CsA concentrations. The HPLC assay, modified from the method of Sawchuk and Cartier (15), was as follows:

Samples and internal standard (20 mg/mL CsD) were combined in a 10:1 (v/v) ratio, and injected using an autoinjector (SIL-6B Shimadzu; Kyoto, Japan). A 250 × 4.6 mm (5 μm packing) C-18 column (Supelco; Oakville, ON) protected by a guard column (Supelco) was heated to 70°C in a column oven (CTO-6A Shimadzu). Samples were separated by iso-

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NOTATION: C_i, CsA concentration in compartment i, ng/μL or ng/mg tissue; k_{ij}, rate constant for mass transfer or elimination from compartment i to compartment j, hr^{-1}; K_{ow}, Octanol-water partition coefficient; V_i, volume of compartment i, μL or mg tissue; SUBSCRIPTS: 1 = aqueous humor; 2 = iris/ciliary body; 3c = cornea (cellular region); 3f = cornea (fluid region); 4 = lens; 5 = sclera; 6 = conjunctiva; E = systemic circulation.

cratic elution at 1.5 mL/min using 70% (v/v) acetonitrile in HPLC grade water. CsA and CsD concentrations were monitored at 204 nm using a photo-diode-array detector (SPD-M6A Shimadzu). The standard curve was linear ($r^2 = 0.995$) over the range from 250 ng/mL to 10,000 ng/mL; the deviation between replicate samples was less than 6 percent.

The RIA kit was used as directed by the manufacturer. Following sample preparation with the ^{125}I tracer and incubation with the monoclonal antibody, samples were loaded into the autosampling bed of a gamma scintillation counter (LKB Wallac 1282) for analysis. The standard curve was non-linear; as recommended by the manufacturer, concentrations were determined by spline interpolation. The reproducible lower and upper limits of the RIA assay were 15 ng/mL and 2,500 ng/mL, respectively. Concentrations beyond these limits were highly variable, and therefore considered unreliable. The deviation between replicate samples was less than 9 percent.

Experiments to Measure the Intraocular Distribution and Elimination of CsA

Dutch Belted rabbits (2 to 2.5 kg) were anesthetized by intramuscular injection of 1 mL of a 4:1 (v/v) mixture of ketamine hydrochloride (100 mg/mL) and xylazine hydrochloride (100 mg/mL). The saturated CsA solution (100 μL; 25 μg/mL) was injected into the anterior chamber using a 30 gauge needle. The needle was carefully inserted and removed to prevent leaks through the needle hole.

At 0.5, 1, 2, 3, 4, 8, 16, 24, and 48 hours following injection, rabbits were sacrificed. The aqueous humor was immediately aspirated, and the cornea, iris/ciliary body, conjunctiva, anterior sclera, and lens were subsequently dissected *in situ*. Preliminary studies produced undetectable levels of CsA in the vitreous, and hence, vitreous samples were not generally collected during these experiments. Tissue samples were dried on tissue paper and placed in a plastic petri dish (Fisher; Unionville, ON). Aqueous humor samples were placed in 2 mL glass vials, and blood samples were taken from the marginal ear vein just prior to sacrifice of the rabbit. The blood samples were placed in EDTA-lined tubes (Becton-Dickinson). The sample volume or wet weight was measured as appropriate. Six to eight animals were used for each time point.

To extract CsA from ocular tissues, a methanol extraction method was used, as follows: Samples were placed in a test tube containing 2 mL of HPLC grade methanol (Mallinckrodt, Paris, KT) for 48 hours. After 48 hours, the CsA content in the methanol was determined using RIA. For blood samples, the extraction procedure was as follows: 400 μL of methanol was combined with 100 μL of whole blood and vortexed for 15 seconds, then centrifuged at 3000 RPM for 5 minutes. The top layer was assayed for CsA using RIA. The efficiency of these extraction procedures was 78±7% and 87±10% for the lens and conjunctiva, and 91±5, 90±6, and 88±14% for the iris/ciliary body, cornea, and sclera, respectively (mean ± SD). Aqueous humor samples were assayed directly by RIA and by HPLC.

Development of the Model

A compartmental model (Fig. 1) was developed to describe CsA concentrations in the various ocular tissues and fluids. Separate compartments were provided for each of the aqueous humor, conjunctiva, sclera, lens, iris/ciliary body. The cornea was divided into two subcompartments, one to represent the lipophilic cellular layers, and one to represent the hydrophilic stroma. It was assumed that the concentration within each compartment was uniform. The transport of CsA between compartments was modelled using a material balance expression and a first order transport coefficient (k, hr^{-1}). The transfer of CsA to and from the various compartments is given by Eq. (1) to (7). Subscripts 1, 2, 3f, 3c, 4, 5, and 6 refer to the aqueous humor, iris-ciliary body, cornea fluid, corneal tissue, lens, anterior sclera, and conjunctiva, respectively, and a subscript E refers to elimination to the systemic circulation. Potential pathways for the elimination of CsA from the aqueous to the vitreous, and from the cornea, conjunctiva and sclera to the systemic circulation were not included in the model because there were insufficient experimental data to warrant their inclusion; preliminary experiments indicated undetectable CsA levels in the vitreous, and in these studies, CsA levels in the systemic circulation were also below the detection limit at all times.

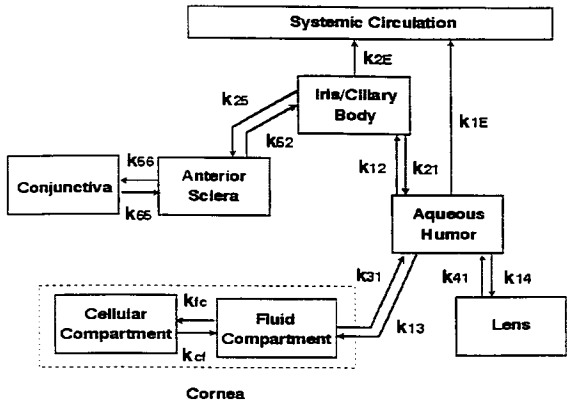

Fig. 1. Compartmental model for cyclosporine pharmacokinetics in the eye.

$$V_1(dC_1/dt) = -(k_{12}+k_{13}+k_{14}+k_{1E})V_1C_1 + k_{21}V_2C_2 + k_{31}V_{3f}C_{3f} + k_{41}V_4C_4 \quad (1)$$

$$V_2(dC_2/dt) = -(k_{21}+k_{25}+k_{2E})V_2C_2 + k_{12}V_1C_1 + k_{52}V_5C_5 \quad (2)$$

$$V_{3f}(dC_{3f}/dt) = -(k_{31}+k_{fc})V_{3f}C_{3f} + k_{13}V_1C_1 + k_{cf}V_{3c}C_{3c} \quad (3)$$

$$V_{3c}(dC_{3c}/dt) = -k_{cf}V_{3c}C_{3c} + k_{fc}V_{3f}C_{3f} \quad (4)$$

$$V_4(dC_4/dt) = -k_{41}V_4C_4 + k_{14}V_1C_1 \quad (5)$$

$$V_5(dC_5/dt) = -(k_{56}+k_{52})V_5C_5 + k_{25}V_2C_2 + k_{65}V_6C_6 \quad (6)$$

$$V_6(dC_6/dt) = -k_{65}V_6C_6 + k_{56}V_5C_5 \quad (7)$$

C and V represent the concentration (ng/μL or ng/mg tissue) and volume (μL) of the specified compartment. The subscripts on k indicate the direction of CsA movement, where the first subscript indicates the origin and the second, the terminus. As an example, in Eqn (2), the term on the left side

of the equation represents the instantaneous rate of change in concentration in the iris-ciliary body. The negative terms on the right side of the equation represent movement of CsA from the iris-ciliary body to the aqueous humor, sclera and systemic circulation, respectively, while the positive terms on the right side represent transport of CsA to the iris-ciliary body from the aqueous humor and sclera.

The value of V for each compartment, except for the aqueous humor, was obtained experimentally by measuring the mass of the separated tissues and assuming a tissue density of 1.0 g/mL. For the aqueous humor, the physiological volume of 0.3 mL was used (16). The volume of the whole cornea was divided between the two corneal compartments based upon the volumes of the cellular and fluid regions in the cornea. Physiologically, the cornea is a tri-laminate composed of cellular layers sandwiched around an aqueous core, and therefore, it is reasonable to have more than one compartment to represent the cornea, especially given the lipophilic nature of CsA. In principle, three compartments could be justified; one each for the epithelium, the stroma, and the endothelium. However, the experimental data were average corneal concentrations, rather than concentrations in each layer, and therefore, there was insufficient information to justify a three compartment model. The two compartment corneal model may be thought of as a fluid compartment, representing the stroma, and a cellular compartment, representing both the epithelium and endothelium.

To obtain an estimate of the overall corneal concentration which could be compared to experimental data, the predicted mass of CsA from each corneal compartment was summed and divided by the total volume. The rate constant for elimination of CsA from the aqueous humor to the systemic circulation (k_{1E}) was set equal to the aqueous humor drainage rate of 0.037 μL/s (16) divided by the volume of the aqueous humor.

In the pharmacokinetic model, the systemic circulation was assumed to be a perfect sink. This assumption is valid for these experiments, since only 2.5 μg of CsA was injected into the anterior chamber, which would result in very low systemic levels.

The 13 unknown rate constants (k_{12}, k_{21}, k_{13}, k_{31}, k_{14}, k_{41}, k_{25}, k_{52}, k_{fc}, k_{cf}, k_{56}, k_{65}, and k_{2E}) were estimated by minimizing a difference function using a quasi-Newton algorithm with a line search method (16). The difference function calculated the sum of the squared residuals based upon the relative error between the predicted concentrations and the experimental results. Numerical integration of equations (1) to (7) was performed using a subroutine which can dynamically select either the Gear or Adams-Moulton multi-value methods (16). Parameter estimations were carried out in three steps. First, the transport pathways between the compartments for the aqueous humor, iris-ciliary body, lens, corneal fluid and systemic circulation were considered, and the relevant rate constants estimated. Second, the corneal tissue compartment was added, which was linked to the corneal fluid compartment, and all the rate constants were re-estimated. Third, the sclera and conjunctiva compartments were added to complete the ocular pharmacokinetic model. All the rate constants were then re-evaluated. To ensure that a global minimum was reached, a sensitivity analysis around each parameter was performed.

RESULTS

The ocular distribution and elimination of CsA were measured following an injection of CsA in BSS into the anterior chamber. CsA was broadly distributed from the anterior chamber, with detectable levels in the lens, iris-ciliary body, sclera, conjunctiva, and cornea (Fig. 2). In the aqueous humor, CsA elimination followed a biexponential decay profile; the concentration declined exponentially from 8 ng/μL at the time of injection to 0.28 ng/μL after three hours, and another slower elimination phase was observed from 6 hours until 48 hours, when only 0.04 ng/μL remained. Similar biexponential profiles were observed in the cornea and iris/ciliary body. CsA levels in the cornea increased to 15 ng/mg of corneal tissue within 0.5 hours, then declined exponentially to 7 ng/mg tissue after 4 hours. Slower elimination was observed thereafter, with levels declining to 1.5 ng/mg tissue after 48 hours. In the iris/ciliary body, levels increased to 10 ng per mg of iris/ciliary body tissue within 0.5 hours, then exponentially decayed to 2 ng/mg tissue after 4 hours. From 8 to 48 hours, slower elimination was observed, with approximately 0.2 ng/mg tissue remaining in the iris/ciliary body after 48 hours. Kinetic profiles for the lens, sclera, and conjunctiva showed that CsA concentration quickly reached a maximum within 2 to 4 hours following injection, and decayed very slowly in approximately a first order fashion over the next 48 hours. CsA levels in these tissues did not exceed 1.5 ng/mg tissue at any time. CsA was not detected in the systemic circulation at any time, and metabolic products of CsA were not observed. The terminal elimination half life of CsA estimated from the data was 45 hours for both the cornea and sclera, and 30 hours for the iris-ciliary body.

The effectiveness of the ocular pharmacokinetic model in describing the experimental data is shown in Fig. 2. The model parameters are summarized in Table I.

DISCUSSION

CsA delivered into the anterior chamber was quickly distributed to the surrounding tissues, primarily into the cornea and iris-ciliary body. Elimination from ocular tissues was very slow, especially from the cornea (Fig. 2). The experimental results also showed that during the elimination phase following the initial period of absorption, corneal tissue levels exceeded aqueous humor levels by approximately a factor of 40 (Fig. 2), suggesting an affinity of CsA for lipophilic ocular tissues. These results agree with those reported by Wiederholt *et al.* (6) and Kaswan (7), who reported, respectively, that the elimination half life from the cornea was 52 hrs and 34 hrs. Furthermore, the model was able to accurately predict the limited elimination-phase data published by Wiederholt et al. (6) and by Kaswan (7) (data not shown). However, the absorption phase of their data could not be satisfactorily described, primarily due to the implicit assumptions required to model uptake from eyedrop administration.

The long half-lives and observed partitioning of CsA into ocular tissues suggest that the cornea and iris/ciliary body may act as a reservoir capable of releasing CsA over a long period of time. Also, CsA should be highly soluble in the cellular layers of the cornea, suggesting that the cornea

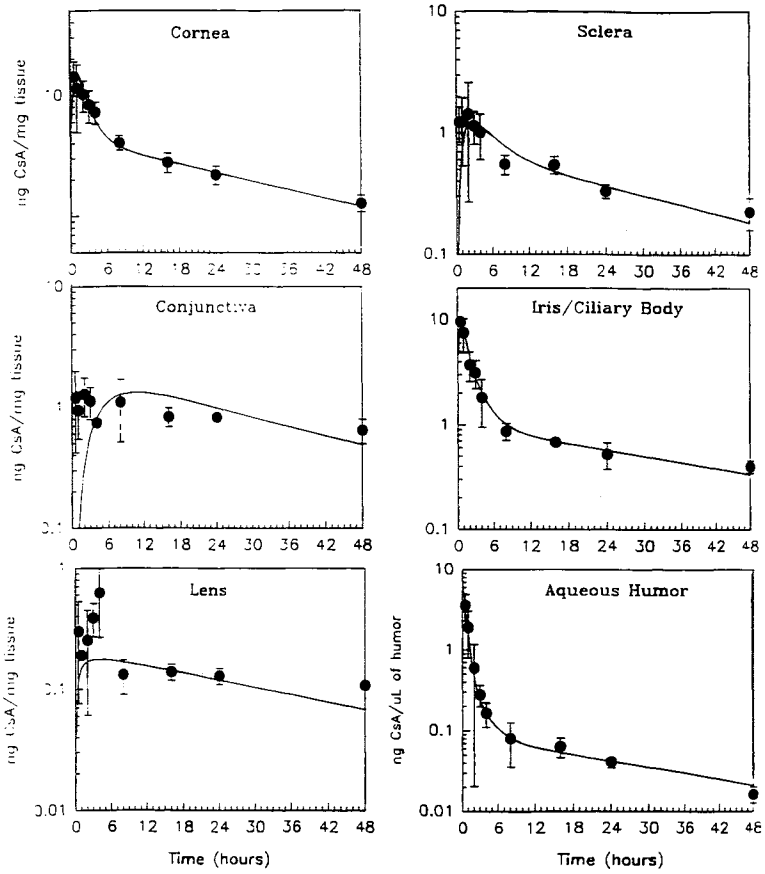

Fig. 2. CsA pharmacokinetics following delivery into the anterior chamber. Experimental data (•) are shown as mean ± one standard deviation. The solid line represents the model prediction.

and iris/ciliary body may be capable of storing a large amount of CsA. The reservoir effect of the ocular tissues, especially the cornea and iris/ciliary body, has important consequences since, following an initial loading dose, these tissues may act to supply a therapeutic quantity of drug to the aqueous humor and to other tissues over an extended period of time.

Pharmacokinetic Modelling

A multi-compartment model (Fig. 1) was developed to simulate the pharmacokinetic data obtained from injection of CsA into the anterior chamber (Fig. 2). It was found that a single compartment representing the cornea could not reproduce the bi-exponential CsA profile in the cornea that was observed experimentally. However, a two compartment model of the cornea reproduced the bi-exponential profile and agreed well with the data (Fig 2). The fact that the time scale for significant distribution of CsA into the cellular tissues (2hr, Fig. 1) was much less than the terminal elimination half life of CsA from the cornea (45hr) justifies the assumption that the epithelium and endothelium can be combined as a single tissue compartment. This assumption is also supported by the fact that the octanol-water partition coefficient (K_{ow}) for CsA is almost 1000, suggesting significant distribution into the tissue regions of the cornea.

Table I. Rate Constants for the Ocular Pharmacokinetics of CsA

i	j	k_{ij}/k_{ji} (hr^{-1})
Aq Humor (1)	Iris-Ciliary (2)	0.922/0.070
Aq Humor (1)	Lens (4)	0.065/0.043
Aq Humor (1)	Cornea (fluid) (3f)	1.726/0.462
Iris-Ciliary (2)	Central Compartment	0.752/—
Iris-Ciliary (2)	Sclera (5)	0.349/0.186
Sclera (5)	Conjunctiva (6)	0.157/0.272
Cornea (fluid) (3f)	Cornea (cellular) (3c)	0.122/0.069

The subscripts of k indicate the direction of movement from compartment i to j.

The one compartment representation of the sclera, iris/ciliary body, and aqueous humor fit the data well using the optimal rate constants (Fig. 2). This suggests that the model equations used to predict CsA transport to and from these compartments are appropriate. However, for the conjunctiva and lens, only an adequate fit to the data was obtained. This may be due to the low concentrations measured in these tissues, or possibly due to a limitation of the single compartment representation for these tissues. For example, Francoeur et al. (18) observed that the transport of timolol through the lens could not be adequately described using a single compartment, and concluded that concentrations in the lens could be better represented by considering drug diffusion through the entire tissue. Since the model for CsA

provided a reasonable prediction of the data, and more importantly, since the experimental concentrations were very low, the one compartment representation of the conjunctiva and lens was considered to be appropriate.

Redistribution of CsA from the systemic circulation to the ocular tissues was not observed in this study, which is not surprising in light of the very low dose of CsA (2.5 μg). Concentrations in all fluids and tissues declined monotonically once the initial absorption phase had passed (Fig. 2), unlike the ocular tissue levels that were recorded following topical delivery. Wiederholt *et al.* (6), following a topical dose of 200 μg, noted two sharp spikes in the ocular tissue levels of CsA which were unrelated to the initial absorption of the drug. The first spike occurred 8 hours after the absorption phase ended, and the second spike occurred approximately 36 hours later. A similar pattern was observed by Kaswan (7) following a topical dose of 840 μg. The absence of any spikes in tissue levels following the delivery of CsA to the anterior chamber is very likely due to the lower dose used in this study, which led to undetectable levels of CsA in the systemic circulation. In the studies by Weiderholt *et al.* (6) and Kaswan (7), significantly higher doses were needed because CsA was delivered topically; up to 95% of a topical dose is rapidly drained into the systemic circulation. The higher systemic loading of CsA which occurs from topical delivery could lead to significant recirculation of CsA from the systemic circulation to the eye, and account for the observed spikes. Furthermore, the higher systemic levels which follow topical administration would invalidate the perfect sink assumption which was appropriate for the studies reported in this manuscript, and would necessitate the use of additional differential equations to represent the systemic distribution and elimination of CsA.

CONCLUSIONS

1) Following a single bolus injection into the anterior chamber, cyclosporine was rapidly distributed into ocular tissues. However, elimination from ocular tissues was slow; half-lives of 30–45 hours were observed for the cornea, sclera, and iris/ciliary body.
2) Consistent with its high octanol-water partition coefficient (K_{ow}), cyclosporine demonstrated an affinity for lipophilic tissues; during the terminal elimination phase, concentrations in the cornea exceeded concentrations in the aqueous by approximately a factor of 40.
3) The time-dependent CsA concentrations in the eye were successfully described using a compartmental model, with single compartments for the aqueous, iris/ciliary body, lens, conjunctiva, and sclera, and two compartments for the cornea. The model provided an accurate description of concentrations in the aqueous humor, cornea, sclera, and iris/ciliary body. However, the fit was not as good for the lens and conjunctiva, possibly due to the low levels in these tissues or due to limitations of using a single compartment to represent concentrations in these tissues.

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