Intraocular Penetration of Topically Applied Cyclosporine

R.L. Kaswan

CYCLOSPORINE (CsA) is a potent immunosuppressive drug with specific T cell inhibitory activity. CsA appears to interrupt T cell activation at an early point by interfering with activation of intracellular mechanisms following antigen binding, with a resultant blockade of the lymphokine cascade.[1] CsA has proven to be very beneficial in organ transplantation and certain autoimmune disorders in man.[2] Recently, systemically administered CsA has been found to be beneficial in Sjögren's syndrome[3] and some forms of uveitis in both people and experimental species.[4-12] Additionally, topically applied CsA has been found to be useful in corneal allografts,[13-15] vernal keratoconjunctivitis,[16] keratoconjunctivitis sicca,[17] immune mediated keratitis,[18] necrotizing scleritis,[19] and herpetic stromal keratitis.[18,20] These studies suggest that local ocular immunosuppression provides adequate therapy while avoiding the risks of generalized immunosuppression and renal toxicity.

The efficacy of topically applied CsA for external ocular disorders led us to conjecture that if CsA can reach therapeutic levels inside the eye following topical application, it might be possible to treat intraocular diseases such as immune mediated uveitis with topical application. Specific advantages to use of topical CsA as compared to corticosteroids would include lack of collagenase activation when immune suppression is indicated in eyes with ulcerated corneas, and potentially improved immunosuppressive activity.

From the Departments of Small Animal Medicine and Veterinary Pharmacology/Physiology, College of Veterinary Medicine, University of Georgia, Athens.

Supported in part by the National Eye Institute, National Institutes of Health, RO3 EY05720-01, The National Society for the Prevention of Blindness, The University of Georgia Veterinary Medical Experimental Station 29-26-GR207-002, and Sandoz Pharmaceuticals Inc.

Address reprint requests to Renee L. Kaswan, DVM, MS, Department of Small Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA 30602.

0041-1345/88/2002-2121$03.00/0

MATERIALS AND METHODS

Rabbits

We used 33 adult female New Zealand White (NZW) rabbits (3 to 4 kg), divided into 11 groups of three rabbits. Tetracycline 4 g/gallon, was added to the drinking water for prophylaxis of *Pasteurella* infection.

Treatment

A solution of 1% CsA radiolabeled with ^{3}H specific activity of 8.5 μCi/mg (kindly supplied by William Robinson, Sandoz Pharmaceuticals, Hanover, NJ), was prepared in olive oil (Sigma, St Louis). Three rabbits received eyedrops containing olive oil without CsA as negative controls. A Varimetric positive displacement pipettor (Labindustries, Berkeley, CA) was used to deliver 7 μL eyedrops. This volume was chosen to avoid spillover since the tear volume in the rabbit averages 8 μL.[21] Increased drop size does not lead to greater corneal penetration[22] but confounds topical absorption with recirculation following absorption of drugs through the nasolacrimal system. Drops were applied to the dorsal corneoscleral limbus. One drop was applied every 15 minutes for six applications.[23] The total body dose per rabbit was 0.84 mg CsA, or 0.24 mg/kg. The time interval from the last application until enucleation was varied from one to 72 hours (Fig 1), with three animals killed at each interval.

Sample Collection

Rabbits were anesthetized with xylazine (5 mg/kg) and ketamine (35 mg/kg) intramuscularly IM. Samples of 200 μL of aqueous humor were drawn with a 30-gauge needle and tuberculin syringe inserted at the corneal limbus. After heparinized cardiac blood samples were drawn, the rabbits were killed with pentobarbital (325 mg) intracardiac injection. Both eyes were enucleated and frozen. Clean instruments were used for each rabbit.

Globes were dissected by the method of Abel and Boyle.[24] Briefly, the globes were transected at the equa-

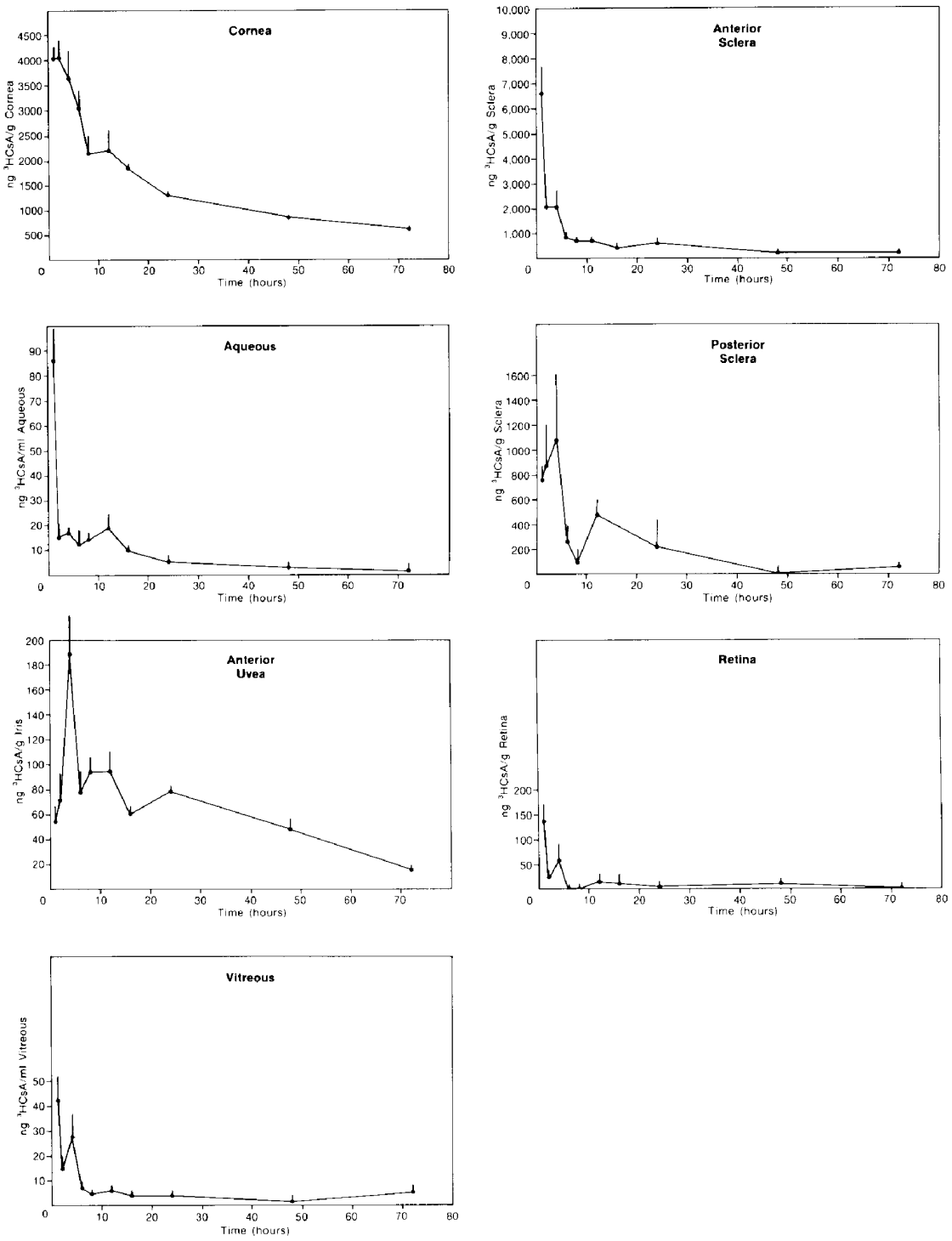

Fig 1. CsA content in various ocular tissues of the rabbit eye following six applications of 7 μL of 1% ^{3}HCsA in olive oil. Concentrations have been calculated from the specific activity of ^{3}HCsA and expressed as ng/g tissue or ng/mL fluid. Six eyes have been used for each individual point.

tor. The frozen vitreous was extruded and collected. The anterior uvea (iris and ciliary body), choroid with retina, lens, cornea, anterior sclera, and posterior sclera were dissected, individually processed, and assayed. In rabbits killed at two hours post-CsA treatment the lacrimal gland of the third eyelid was also removed and assayed. Each ocular tissue was digested in three times its weight of collagenase (Sigma type I) in Michaelis' barbital buffer.[25] Next, 100 μL of collagenase buffer solution was added to each vitreous sample. Tissue digests, 250 μL whole blood, and 100 μL aqueous samples were transferred to scintillation vials and mixed with 10 mL Scintiverse I[a]. Blood samples were decolorized with BTS-450 (Beckman, Atlanta), 30% H_2O_2, and glacial acetic acid.[26] The disintegrations were assayed as counts per minute (cpm) using a Beckman LS 7000 liquid scintillation counter. Counting efficiency for each assay was determined by H-number quench correction using commercial external standards for data conversion to disintegrations per minute (DPM).[26] Conversion to mg CsA was made by the following equation:

$$\frac{\text{DPM of sample} \times 10^6 \text{ ng/mg}}{2.2 \times 10^6 \text{ DPM}/\mu\text{Ci} \times 8.5\ \mu\text{Ci } ^3\text{H/mg CsA} \times \text{tissue wt (g)}} = \text{ng CsA/g tissue}$$

Negative control tissues were used for background subtraction of DPM for each tissue type.

Blood samples from rabbits from each time group were assayed in a blind manner by Sandoz Research Institute using a tissue-burning technique to assay the vaporized tridium from each blood sample, because the tissue-burning assay has higher sensitivity for whole blood samples than can be attained with liquid scintillation.[b]

RESULTS

Figure 1 illustrates the CsA content of the aqueous, vitreous, and ocular tissues. Peak concentrations were reached within the first four hours in all tissues. Tissue concentrations in excess of minimal therapeutic levels (50 to 300 ng CsA/g tissue)[6] were achieved within one hour and maintained for at least 24 hours in the cornea, anterior, and posterior sclera. Levels of CsA were below therapeutic values within two hours in the retina, vitreous, and aqueous samples. The average level of CsA in the lacrimal gland of the third eyelid was 2,850 ng/g at two hours post-CsA treatment. Iris and ciliary body CsA concentrations were at equivocal levels for therapy of uveitis,[6] between 50 and 200 ng/g tissue, (Fig 1) for all samples taken between one and 24 hours.

The elimination rates of CsA in the cornea, anterior uvea, and anterior and posterior sclera were determined as the slopes of the terminal phase of the concentration v time curves using a visually fit two compartmental model.[27] The elimination half-life was calculated as 0.693 divided by the elimination rate and was found to be 33.5 hours, 26.7 hours, 19.5 hours, and 20.0 hours, respectively.

Amongst 20 blood samples assayed for radioactivity by both liquid scintillation and tissue burning techniques, only one sample had significant radioactivity (greater than three times baseline) and it was identified by both methods. The CsA content of this sample was 57 ng. No radioactivity was detected in the remaining 10 samples, which were assayed by liquid scintillation but not by tissue-burning techniques.

The pigmented tissues and especially the whole blood samples were most affected by chemiluminescence. The presence of biologic pigments, especially red pigments, causes high control level cpm values, confounding the background activity. The use of antioxidants and bleaching agents reduced this error but did not eliminate it, making small amounts (less than 50 ng/mL) of CsA in the blood impossible to detect by liquid scintillation. Use of the tissue-burning technique on portions of these samples confirmed that no detectable CsA occurred in blood samples except one sample taken at 8 hours posttreatment.

DISCUSSION

Two major questions must be determined before topical CsA would prove to have practical importance for uveitis. Can CsA reach the target organ in therapeutic levels following reasonable therapeutic regimens? Is the effect of CsA mediated locally, in the eye, or does the inhibition of intraocular inflammation require systemic immunosuppression?

CsA is an undecapeptide with a molecular weight of 1,202. The therapeutic range for organ transplantation is 200 to 600 ng/mL

serum, but the intraocular level speculated to be needed for control of uveitis is 50 to 300 ng/mL.[6] Alternately, if the key event is inhibiting the triggering of lymphokine release from activated T cells, the required intraocular dose would be only 5 to 199 ng/mL CsA.[1] Compared to most ophthalmic medications, CsA is a large molecule, but it is highly lipophillic. If compounds are lipid soluble and pass through the corneal and conjunctival epithelium their size does not appreciably alter the rate of transport.[28] Intraocular absorption of large molecular weight drugs occurs across the conjunctival-scleral interface, bypassing the necessity of passage through the aqueous humor.[29] CsA's absence in the aqueous humor[30,31] was therefore not discouraging, because the aqueous is an inappropriate compartment for sampling of intraocular drug absorption for those drugs that depend upon noncorneal absorption routes.[29]

Since the total volume of CsA given to each experimental animal in the present study was 6 doses of 7 μL 1% CsA in each eye, the total body dose per rabbit was 0.84 mg, or 0.24 mg/kg.[2] The recommended systemic dose of CsA is 10 to 20 mg/kg[2] or 60 to 120 times higher than the doses used in this study.[2] Following therapeutic dosage in people with uveitis, intraocular levels were only 40% of serum levels.[32] Bell and coworkers report aqueous levels of 12 to 49 ng/mL[33] following topical or systemic administration to rabbits, but the lower limit of sensitivity of the RIA assay used was 45 ng/mL.[34] Based on the absence of detectable CsA in 29 of 30 blood samples and the minimal total body doses used, intraocular levels in this study probably were not due to blood redistribution.

Wiederholt and coworkers studied intraocular penetration of 1% CsA dissolved in castor oil, but reported their results in cpm, rather than in a universally comparable term.[26] The corneal half-life in both studies is remarkably long, 34 hours in this study and 52 hours in Wiederholt's.[35] Our findings do not support their conclusion that the intraocular levels occur due to blood redistribution to both eyes following CsA administration to either eye.[35]

The concentration of CsA reached in the present study was well above therapeutic levels in the cornea, and in anterior and posterior sclera, but the intraocular CsA levels achieved were low. The anterior uvea approached hypothesized therapeutic levels with levels ranging from 50 to 200 ng/gm. An assay of steady state pharmacokinetics with chronic administration for five elimination half-lifes and assays from inflamed eyes may be required to determine whether or not higher therapeutic levels can be achieved in the anterior uvea and retina under typical clinical conditions.

Where is the target site? If CsA works by entering the eye and blocking intraocular T cells responding to ocular antigens within the inflamed eye, then local administration can be advocated. If, however, ocular antigens are disseminated to lymphatic organs where T cell activation occurs, systemic CsA therapy would be mandated.

Topical CsA had demonstrated efficacy in immune-mediated diseases of the cornea and sclera, including experimental corneal graft transplantation,[13-15] vernal keratoconjunctivitis,[16] keratoconjunctivitis sicca,[17] chronic immune mediated keratitis,[18] necrotizing scleritis,[19] and experimental herpetic stromal keratitis.[18,20] In dogs with keratoconjunctivitis sicca, topical CsA ameliorated the chronic keratitis and increased the average Schirmer tear test by 9 mm/min.[17] These studies strongly suggest that local immune suppression is adequate to control immune-mediated disorders with CsA, providing the CsA reaches therapeutic levels at the target tissue. The present pharmacokinetic data confirm that CsA levels are very high in the lacrimal gland of the third eyelid, cornea, and sclera following topical dosage.

The question of whether or not uveitis can be controlled with topical CsA has been more elusive. The intraocular dosage of CsA may not be sufficient to control inflammation at all

areas of the globe. The current investigation and previous kinetic work has determined the elimination half-life, but has not addressed the steady state intraocular concentrations one would anticipate during multiple chronic dosage.

Previous uveitis studies that used intrasubject positive control eyes may have inadvertently triggered an afferent immune reaction in the positive control eye, causing a systemic immune response with an efferent response occurring in both the control and treated eye.[6] Studies that treated both eyes with CsA and used an extrasubject positive control were more successful.[6,10] Two studies have examined the potential for topical CsA in experimental uveitis. When Nussenblatt and coworkers[6] tested CsA in a limited number of rats with experimental autoimmune uveitis, they could not separate systemic effects from topical effects because the ophthalmic drops used were so large (50 μL), and the subjects so small (200 g rats), that therapeutic serum levels were reached via nasolacrimal absorption. Considering the mechanism of CsA activity, the problem of differentiating the effects of systemic absorption of topical drug was not well addressed by unilateral drug dosing. CsA prevents initiation of the immune response by blocking T cell recognition of antigens and thereafter production of interleukin 2, which activates the cascading immune response. Antigenic recognition and lymphocyte activation within the untreated eye would be expected to trigger a systemic immune response[36] affecting both eyes; therefore, unilateral treatment designs would likely fail. Re-evaluation of Nussenblatt's work demonstrates that bilateral topical therapy was effective in preventing experimental autoimmune uveitis (EAU) at concentrations of 2%, 0.5%, 0.2%, but unilateral treatment did not show a significant beneficial effect in the eye treated with 2%, 0.5%, and 0.2% CsA.[6] This result was attributed to the fact that rats receiving bilateral drops got more volume of CsA, but actually, the rats receiving unilateral 2% CsA had more total CsA than rats with bilateral 0.5% or 0.2% treatments. An alternate explanation is that it is the intrasubject positive control eye that instigates EAU in the CsA-treated eye,[37] and that bilateral CsA is effective in EAU. Based on the small sample size (two rats per treatment group) and the ambiguity of results, further investigation into use of topical CsA for uveitis may be warranted.

Advocacy of topical CsA for intraocular disease still awaits proof positive of intraocular penetration. What is most encouraging from our data is that CsA does reach very high levels in the cornea, sclera, and lacrimal gland of the rabbit. Considering the results from topical trials of CsA in corneal transplantation,[13-15] herpes stromal keratitis,[18,20] keratoconjunctivitis sicca,[17] and chronic immune mediated keratitis,[18] it is predictable that other immune-mediated disorders of the cornea, conjunctiva, and sclera may also respond positively to topical CsA.

ACKNOWLEDGMENT

The author thanks Susan Gardner for her expert advice on ocular pharmacokinetics, Antoinette Jernigan for assistance with the pharmacokinetic determinations, and William Robinson for furnishing ^{3}HCsA and for performing corroborative assays.

REFERENCES

1. Borel JF, Ryffel B: In Schindler R (ed): Ciclosporin in autoimmune disease. New York, Springer-Verlag, 1985, p 24

2. Kahan BD: Cyclosporine, Biological Activity and Clinical Applications. Philadelphia, Grune & Stratton, 1983

3. Leuenberger PM, Miescher PA: Klin Mbl Augenheik 190:290, 1987

4. Nussenblatt RB, Palestine AG, Chan CC: J Ocular Pharmacol 1:369, 1985

5. Nussenblatt RB, Salinos-Carmona M, Waksman BH, et al: Int Arch Allergy Appl Immunol 70:289, 1983

6. Nussenblatt RB, Dinning WJ, Fujikawa LS, et al: Arch Ophthalmol 103:1559, 1985

7. Nussenblatt RB, Rodriques MM, Salinas-Carmona MC, et al: Arch Ophthalmol 100:1146, 1982

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.