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Blood Concentrations of Cyclosporin A During Long-Term Treatment With Cyclosporin A Ophthalmic Emulsions in Patients With Moderate to Severe Dry Eye Disease

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ABSTRACT

To quantify blood cyclosporin A (CsA) concentrations during treatment with CsA topical ophthalmic emulsions, blood was collected from 128 patients enrolled in a Phase 3, multicenter, double-masked, randomized, parallel-group study of CsA eyedrops for treatment of moderate to severe dry eye disease. Patients received 0.05% CsA, 0.1% CsA, or vehicle b.i.d. for 6 months; vehicle-treated patients then crossed over to 0.1% CsA b.i.d. for 6 months. CsA concentrations were measured using a validated LC/MS-MS assay (quantitation limit = 0.1 ng/mL). No patient receiving 0.05% CsA had any quantifiable CsA in the blood (n = 96 samples). All but 7 of 128 (5.5%) trough blood samples from the 0.1% CsA group were below the quantitation limit for CsA; none exceeded 0.3 ng/mL. CsA was also below the limit of quantitation in 205 of 208 (98.6%) of serial postdose blood samples collected from 26 patients during 1 dosing interval between months 9 and 12. The highest C_{max} measured, 0.105 ng/mL at 3 hours postdose, occurred in a 0.1% CsA-treated patient. These results indicate that long-term use of topical CsA ophthalmic emulsions at doses that are clinically efficacious for treating dry eye will not cause any system-wide effects.

INTRODUCTION

Keratoconjunctivitis sicca (KCS), or chronic dry eye disease, is a frustrating condition characterized by sensations of ocular grittiness, burning, photophobia, and blurred vision. Despite affecting

millions worldwide and substantially altering productivity and quality of life (1, 2), therapies for KCS have been palliative at best. Recent advances in understanding the pathophysiology of disease allow the development of effective therapies for the first time.

The ocular surface, lacrimal glands, and interconnecting nerves form a homeostatic functional unit that maintains normal tear production. Chronic dry eye disease results from a T cell-mediated inflammatory process leading to a disruption of this nerve traffic. Multiple factors, including an age-related drop in systemic androgen levels and chronic ocular surface irritation, create an environment in which activated T cells are recruited to the ocular surface and lacrimal glands in a vicious cycle of inflammation that ultimately results in destruction of the lacrimal glands (3). Cyclosporin A (CsA) topical ophthalmic emulsion targets the inflammatory basis of disease by acting as a local immunomodulator and anti-inflammatory agent. By inhibiting activation of infiltrating T cells on the ocular surface and lacrimal glands, production of inflammatory cytokines is prevented (4). CsA treatment has been shown to decrease several molecular markers of immune-based inflammation in conjunctival biopsies of dry eye patients (5–7).

Phase 2 and Phase 3 clinical trials have established both the safety and efficacy of topical CsA at concentrations ranging from 0.05% to 0.4% (8, 9). CsA is currently approved for systemic treatment of immune-related disorders at much higher doses than those given for KCS. Oral administration for treatment of psoriasis or rheumatoid arthritis produces blood CsA concentrations in the range of 75 ng/mL (C_{min}) to 655 ng/mL (C_{max}) (10). Because it is a potent immunosuppressant when used at these high doses, it is important to assess systemic exposure to CsA upon administration of the very low doses needed for treatment of KCS.

Topical ophthalmic treatment with CsA requires 2600-fold lower dosage than does systemic usage (10). A preliminary study of topical ophthalmic CsA conducted during Phase 2 development found that topical instillation produced negligible blood CsA concentrations, although sampling was sparse (8). The present study examines blood CsA concentrations of KCS patients participating in a much larger Phase 3 study of CsA topical ophthalmic emulsions. We show conclusively that CsA is practically undetectable in the blood of patients treated with these ophthalmic preparations.

PATIENTS AND METHODS

Clinical Trial Overview

Therapeutic blood monitoring was done at selected sites on a subset of patients enrolled in a Phase 3, multicenter, double-masked, randomized, parallel group study of cyclosporin A topical ophthalmic emulsions for the treatment of dry eye disease (9). Eligible study participants had moderate to severe dry eye disease as defined by 1) Schirmer test of $\geq$ 5 mm/5 min (without anesthesia) in at least one eye; 2) sum of corneal and interpalpebral conjunctival staining of $\geq +5$ in the same eye in which corneal staining was $\geq +2$; 3) indication of moderate to severe dry eye disease by the Ocular Surface Disease Index (11) and Subjective Facial Expression Scale. Patients were excluded if they had end-stage lacrimal disease, punctal plugs, were using medications that could interfere with the study results, wore contact lenses during the study, or were pregnant or lactating. The study was conducted from July 1997 to January 1999 and was in compliance with Good Clinical Practices, investigational site Institutional Review Board Regulations, Sponsor and Investigator Obligations, Informed Consent Regulations, and the Declaration of Helsinki.

After a 2-week run-in period with Refresh® artificial tears (Allergan, Inc., Irvine, CA), patients were randomly assigned 0.05% CsA topical ophthalmic emulsion eyedrop b.i.d., 0.1% CsA b.i.d., or vehicle for 6 months. The vehicle consisted of a sterile, non-preserved castor oil-in-water emulsion that was identical to the 0.1% CsA preparation except for the presence of medication. Patients instilled one drop in each eye every morning and evening, no later than 8:00 p.m. the night before clinical visits. They could use Refresh® as needed through month 4, but no more than 8 times daily of

ter that. At month 6, patients receiving vehicle were reassigned to 0.1% CsA b.i.d. for a further 6 months. Those initially placed in the 0.05% and 0.1% CsA treatment groups continued their assigned treatments. Table 1 lists sample numbers collected at each clinical visit.

Total daily doses of CsA were 0.057 mg/day (0.05% CsA treatment group) and 0.114 mg/day (0.1% CsA group).

Blood Sampling

Trough blood samples were collected immediately before the morning dose at day 0 and months 1 and 6. A final sample was collected at a single visit that occurred between months 9 and 12 (called the "month 9–12 visit"). In order to quantify peak CsA concentrations, 26 patients also provided serial blood samples at 1, 2, 3, 4, 6, 8, 10, and 12 hours after the morning dose during the month 9 - 12 visit. All blood samples were frozen until shipment to Allergan, Inc. (Irvine, CA) for analysis.

Blood Bioanalysis

A validated HPLC/tandem mass spectroscopy assay was used to quantify blood concentrations of cyclosporin A. Briefly, 1 mL of blood was acidified with 0.2 mL of 0.1 N HCl, then extracted with 5 mL methyl-*t*-butyl ether. The organic phase was neutralized by addition of 2 mL 0.1 N NaOH, evaporated, reconstituted in a water/acetonitrile-based mobile phase, and injected onto a 2.1 × 50 mm, 3 μm pore size C-8 reverse phase HPLC column (Keystone Scientific, Bellefonte, PA). Compounds were gradient-eluted at 0.2 mL/min and detected using an API III triple quadrupole mass spectrometer with a turbo-ionspray source (PE-Sciex, Concord, Ontario, Canada). Molecular reaction monitoring enhanced the sensitivity and selectivity of this assay. Protonated molecules for the analyte and internal standard were collisionally dissociated and product ions at m/z 425 were monitored for the analyte and internal standard. Under these conditions, cyclosporin A and the internal standard cyclosporin G eluted with retention times of ~3.8 minutes. The lower limit of quantitation was 0.1 ng/mL, at which concentration the coefficient of variation and deviation from nominal concentration was <15%.

For serial post-dose blood samples, the area under the blood concentration-time curve ($AUC_{0\text{-}12}$) was calculated using the linear trapezoidal rule (12). Since most concentrations were below the limit

Table 1. Numbers of Trough Blood Samples Collected for CsA Quantitation

Treatment Group	*Samples Per Treatment Group Per Visit*				
	Day 0 (Prestudy)	*Month 1*	*Month 6*	*Month 9–12*	*All Visits*
Vehicle	45	37	30	0[b]	112
0.05% CsA	43	40	30	26	139
0.1% CsA	43	36	34	58[c]	171
All treatment groups:	131[a]	113	94	84	422

[a]Samples were collected from 128 patients. Two patients from the vehicle group and 2 from the 0.1% CsA group each gave 2 samples. Baseline was not collected for 1 patient.

[b]Patients assigned to vehicle were reassigned to 0.1% CsA at month 6.

[c]Includes patients who had crossed over from the vehicle group at month 6.

of quantitation, an upper limit to AUC_{0-12} was calculated by assuming a concentration equal to the limit of quantitation at each sampling time, then expressing the mean AUC_{0-12} as below this theoretical upper limit of 1.2 ng•hr/mL. No statistical calculations were performed on serial postdose or trough blood concentrations because most data were below the limit of quantitation.

RESULTS

Study Participants

Blood was collected from a subpopulation of 128 patients out of 405 enrolled in a Phase 3 trial of cyclosporin A for the treatment of dry eye disease, yielding a total of 422 trough blood samples over the study period (Table 1). Patients ranged from 24 to 88 years of age (n = 128), and 78% (100/128) were women (9). Baseline characteristics of the subpopulation were similar to those of the larger study population. The rate of completion of the subpopulation (68%, 87/128) was slightly lower than the study population (77%, 671/877), but the reasons for discontinuation were similar in both populations and were primarily non-treatment-related.

Trough Blood CsA Concentrations

Of 96 samples from the 0.05% CsA treatment group, none contained any quantifiable CsA. Mean trough blood CsA concentrations were below the limit of quantitation in both treatment groups at all time points, including month 9-12 (Table 2). The only study samples above the limit of quantitation were 7 samples from the 0.1% CsA treatment group, representing only 5.5% (7/128) of the samples collected from this group. Quantifiable blood CsA concentrations ranged from 0.104 ng/mL to 0.299 ng/mL and came from 7 different patients who had no obvious commonalities with respect to age, sex, or time point at which CsA was detected (Table 3). No individual patient had quantifiable CsA at more than one time point.

Serial Blood CsA Concentrations During One Dosing Interval

Serial post-dose blood samples (n = 208 samples) were collected from 26 patients during the 12 hours following the morning dose. As was the case with trough blood measurements, no patient in the 0.05% CsA treatment group had any quantifiable CsA. Overall, 99% of samples (205/208) did not contain quantifiable CsA. The 3 samples that were above the limit of quantitation came from 3

Table 2. Mean Trough Blood CsA Levels

Treatment Group	*Blood CsA Concentration (ng/ml)*			
	Day 0 (Prestudy)	*Month 1*	*Month 6*	*Month 9–12*
Vehicle	<0.1	<0.1	<0.1	NA
0.05% CsA	<0.1	<0.1	<0.1	<0.1
0.1% CsA	<0.1	<0.1	<0.1	<0.1[a]

[a]Includes samples from patients in the vehicle group who crossed over to the 0.1% CsA group.
NA = Not applicable

Table 3. Quantifiable Trough CsA Concentrations in Individual Patients

Age, Sex	*Treatment Group*	*Blood CsA Concentration (ng/mL)*			
		Day 0 (Prestudy)	*Month 1*	*Month 6*	*Month 9–12*
56, M	0.1% CsA	BLQ	0.134	BLQ	BLQ
56, F	0.1% CsA	BLQ	0.110	NS	NS
64, F	0.1% CsA	BLQ	0.143	BLQ	BLQ
86, F	0.1% CsA	BLQ	BLQ	0.299	BLQ
81, F	0.1% CsA	BLQ	BLQ	0.144	BLQ
52, F	0.1% CsA	BLQ	BLQ	0.125	BLQ
76, M	vehicle → 0.1% CsA	BLQ	BLQ	BLQ	0.104

BLQ = Below limit of quantitation.
NS = No sample collected.

different patients in the 0.1% treatment group. One of these patients had received 0.1% CsA for 9-12 months, while the other 2 had received vehicle for 6 months before being reassigned to the 0.1% CsA treatment group 3 to 6 months prior to the sampling period. CsA was barely detectable in these 3 samples, with C_{max} ranging from 0.102 ng/mL to 0.105 ng/mL. These values occurred early in the 12-hour time course, with t_{max} ranging from 1 to 3 hours (Table 4). Mean C_{max} was not calculable because most samples were below the limit of quantitation. Based on these results, the $AUC_{0\text{-}12}$ was less than 1.2 ng•hr/mL in both treatment groups.

DISCUSSION

Systemic exposure to CsA during treatment with CsA ophthalmic emulsions is barely detectable, even with an extremely sensitive assay capable of measuring blood CsA concentrations as low as 0.1 ng/mL. A striking result of this study is that no patient receiving 0.05% CsA had quantifiable CsA in the blood at any time during the yearlong study. Phase 2 and Phase 3 clinical trials have demonstrated this formulation of CsA to be clinically effective for the treatment of dry eye disease.

For those very few patients who did have quantifiable blood CsA in the present study, concentrations were no more than 2-fold higher than the limit of quantitation. No patient had quantifiable blood CsA at more than one time point, for either trough or serial postdose sampling, even for patients treated up to 1 year, demonstrating that long-term use of topical ophthalmic CsA emulsions produces insignificant systemic exposure. This is consistent with animal pharmacokinetic studies that have demonstrated ready penetration of topical CsA into ocular surface tissues but minimal absorption into the blood (13).

Table 4. Quantifiable Serial Postdose Blood CsA Concentrations in Individual Patients

Age, Sex	*Treatment Group*	*Blood C_{max} (ng/mL)*	*t_{max} (hr)*
59, F	0.1% CsA	0.104	2
64, F	Vehicle → 0.1% CsA	0.102	1
50, M	Vehicle → 0.1% CsA	0.105	3

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