Efficacy and Safety of Cyclosporin A Ophthalmic Emulsion in the Treatment of Moderate-to-severe Dry Eye Disease

A Dose-Ranging, Randomized Trial

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Objective: To investigate the efficacy, safety, formulation tolerability, and optimal dosing of a novel cyclosporin A oil-in-water emulsion formulation for the treatment of moderate-to-severe dry eye disease.

Design: Randomized, multicenter, double-masked, parallel-group, dose-response controlled trial.

Participants: Total enrollment: 162 patients; cyclosporin A groups: 129 patients; vehicle group: 33 patients.

Intervention: Patients instilled study medication (cyclosporin A ophthalmic emulsion 0.05%, 0.1%, 0.2%, or 0.4%, or vehicle) twice daily into both eyes for 12 weeks, followed by a 4-week posttreatment observation period.

Main Outcome Measures: Efficacy: rose bengal staining, superficial punctate keratitis, Schirmer tear test, symptoms of ocular discomfort, and the Ocular Surface Disease Index (OSDI; a measure of symptom frequency and impact on vision-related functioning). Safety: biomicroscopy, cyclosporin A blood levels, conjunctival microbiology, intraocular pressure, visual acuity, and monitoring of adverse events.

Results: In a subset of 90 patients with moderate-to-severe keratoconjunctivitis sicca, the most significant improvements with cyclosporin A treatment were in rose bengal staining, superficial punctate keratitis, sandy or gritty feeling, dryness, and itching, with improvements persisting into the posttreatment period in some treatment groups. There was also a decrease in OSDI scores, indicating a decrease in the effect of ocular symptoms on patients' daily lives. There was no clear dose-response relationship, but cyclosporin A 0.1% produced the most consistent improvement in objective and subjective end points and cyclosporin A 0.05% gave the most consistent improvement in patient symptoms. The vehicle also performed well, perhaps because of its long residence time on the ocular surface. There were no significant adverse effects, no microbial overgrowth, and no increased risk of ocular infection in any treatment group. The highest cyclosporin A blood concentration detected was 0.16 ng/ml. All treatments were well tolerated by patients.

Conclusions: Cyclosporin A ophthalmic emulsions, 0.05%, 0.1%, 0.2%, and 0.4%, were safe and well tolerated, significantly improved the ocular signs and symptoms of moderate-to-severe dry eye disease, and decreased the effect of the disease on vision-related functioning. Cyclosporin A 0.05% and 0.1% were deemed the most appropriate formulations for future clinical studies because no additional benefits were observed with the higher concentrations. *Ophthalmology 2000;107:967–974 © 2000 by the American Academy of Ophthalmology.*

Recent population-based surveys indicate that dry eye disease, or keratoconjunctivitis sicca, affects millions of people worldwide.[1,2] Moreover, as many as 17%[3] to 25%[4] of patients visiting ophthalmic clinics report dry eye symptoms, making dry eye disease one of the most common complaints seen by ophthalmic specialists. Patients with dry eye disease typically complain of symptoms of ocular discomfort, including a dry, gritty feeling often accompanied by foreign body sensation. Depending on the duration and severity of disease, damage to the ocular surface may also be present. Patients with chronic, uncontrolled dry eye have an increased risk of ocular infections[5,6] and are more likely to have ocular infections that progress to endophthalmitis.[7]

A growing body of evidence suggests that chronic dry eye disease is the result of an underlying cytokine and receptor-mediated inflammatory process that affects the lacrimal gland acini and ducts, leading to abnormalities in the tear film and ultimately disrupting the homeostasis of the ocular surface.[8–11] Most conventional treatments for dry

Originally received: May 4, 1999.
Accepted: January 27, 2000. Manuscript no. 99216.

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Preliminary results of this study were presented at the annual meeting of the Association for Research in Vision and Ophthalmology, Fort Lauderdale, Florida, May 1997, and at the XIth Congress of the European Society of Ophthalmology, Budapest, Hungary, June 1997.
Supported by a grant from Allergan Inc.

Published by Elsevier Science Inc.
ISSN 0161-6420/00/$–see front matter
PII S0161-6420(00)00035-X

eye disease focus on tear replacement or tear preservation and are incapable of affecting these processes. However, topical treatment with the immunomodulatory agent cyclosporin A has been shown to reduce cell-mediated inflammatory responses associated with inflammatory ocular surface diseases.[12,13] Preliminary studies have demonstrated that treatment with topical cyclosporin A can result in improvement of the signs and symptoms of dry eye disease (Foulks et al, Invest Ophthalmol Vis Sci 1996;37(Suppl): S646; Helms et al, Invest Ophthalmol Vis Sci 1996;37 (Suppl):S646).[12,14,15] In addition, several studies have established the efficacy of topical cyclosporin A in the treatment of keratoconjunctivitis sicca in dogs.[16–18] These findings suggest that topical cyclosporin A may provide a unique opportunity to move beyond treatments that only alleviate the symptoms of dry eye disease to therapies that effectively target the inflammatory processes contributing to disease pathogenesis.

The purpose of this study was to investigate the efficacy, safety, patient tolerability, and optimal dosing of a novel cyclosporin A oil-in-water emulsion formulation for the treatment of moderate-to-severe dry eye disease with or without Sjögren's syndrome.

Methods

Study Protocol

This report describes a randomized, multicenter, double-masked, parallel-group, dose-response study. The protocol was composed of three phases: a 2-week washout phase, a 12-week treatment phase, and a 4-week posttreatment phase. This study was conducted in compliance with the institutional review board regulations, informed consent regulations, sponsor and investigator obligations, and the Declaration of Helsinki. Written informed consent was obtained from all patients before the initiation of any study medication or study-related procedure.

Study Population. Patients were recruited between May 1995 and February 1996 from nine clinical sites throughout the United States. Eligible patients were at least 21 years of age and had a diagnosis of keratoconjunctivitis sicca with or without Sjögren's syndrome refractory to conventional management. Inclusion criteria included Schirmer test (without anesthesia) of 7 mm/5 minutes in at least one eye; mild superficial punctate keratitis defined as a corneal punctate fluorescein staining score of ≥ 1 in either eye (scale 0 [none] to 3 [severe]); and one or more moderate ($\geq +2$) dry eye–related symptoms, including itching, burning, blurred vision, foreign body sensation, dryness, photophobia, and soreness or pain. Both eyes were treated, but both eyes were not included in all analyses (see Statistical Methods).

Patients were excluded from study participation if they had any ocular disorder including ocular injury, infection, non-dry eye ocular inflammation, trauma, or surgery within the prior 6 months; were receiving concurrent treatment that could interfere with interpretation of the study results; had any uncontrolled systemic disease or significant illness; or were pregnant, lactating, or considering a pregnancy.

Study Medications. The medications used in this study were unit dose vials of unpreserved cyclosporin A 0.05%, 0.1%, 0.2%, and 0.4% ophthalmic emulsion; unit dose vials of unpreserved vehicle for cyclosporin A 0.2% ophthalmic emulsion; and REFRESH lubricant eye drops (Allergan, Irvine, CA). The vehicle for each concentration of cyclosporin A ophthalmic emulsion is formulated slightly differently because greater oil content is required to dissolve the higher concentrations of the active ingredient. The vehicle for cyclosporin A 0.2% ophthalmic emulsion (hereafter referred to as "vehicle") was chosen for the control because it was near the middle of the range of cyclosporin A concentrations used.

Study Treatments. During the washout phase, patients were instructed to discontinue use of all topical ophthalmic medications except for REFRESH. During this time, they were instructed to use REFRESH a minimum of four but no more than eight times daily in each eye. Patients who successfully completed the washout phase were then given their assigned medication (cyclosporin A 0.05%, 0.1%, 0.2%, or 0.4% ophthalmic emulsion or emulsion vehicle) and instructed to instill their medication twice daily (morning and evening) in both eyes for 12 weeks. The use of REFRESH (up to eight times daily in each eye) was allowed during the treatment phase.

Outcome Measures. The efficacy measures were rose bengal staining (graded on a scale from 0 = none to 3 = severe); superficial punctate keratitis measured at nasal, temporal, pupil, and inferior and the scores summed (each graded on a scale from 0 = none to 3 = severe); Schirmer tear test (without anesthesia, with nasal stimulation only if needed to determine that the patient had some capacity to secrete tears); symptoms of ocular discomfort (graded by investigator queries on a scale from 0 = none to 4 = very severe, and in patient diaries on a scale from 0 = no discomfort to 4 = discomfort that interferes with normal daily activity); tear film debris (graded on a scale of 0 = none to 4 = very severe); tear breakup time; and the frequency and amount of REFRESH used.

In addition, patient response to treatment was evaluated using the Ocular Surface Disease Index (OSDI), a global assessment parameter consisting of 12 questions designed to assess the symptoms of ocular irritation consistent with dry eye disease and their impact on vision-related functioning. The questions covered three areas: ocular symptoms, environmental triggers, and vision-related function. Each question was phrased in terms of frequency (how often they were aware of a symptom, how often they experienced difficulty with a specific task because of their symptoms, etc) and graded on a scale from 0 to 4 (where 0 = "never" and 4 = "all the time"). Patient responses to all answers were then combined for a composite OSDI score ranging from 0 to 100.

Treatment safety was assessed by biomicroscopy, measurement of cyclosporin A blood levels, conjunctival microbiology, hematology and blood chemistry panels, intraocular pressure by applanation tonometry, and visual acuity by a 96% contrast Regan Letter Acuity Chart. Throughout the study, patients were monitored for signs and symptoms of adverse events and formulation tolerability. Any reported adverse event was graded by the investigator for severity (mild, moderate, or severe) and assessed for relationship to the study treatment (none, unlikely, possible, probable, definite, or unknown).

Patients were evaluated at weeks 4, 8, and 12 during the treatment phase. During these visits patients were evaluated for changes from baseline in Schirmer tear test, rose bengal staining, superficial punctate keratitis scores, symptoms of ocular discomfort, biomicroscopy, and visual acuity. After the completion of the treatment phase, patients were also evaluated at posttreatment weeks 2 and 4. During both visits patients were assessed for Schirmer tear test, rose bengal staining, superficial punctate keratitis, ocular symptoms of discomfort, biomicroscopy, and visual acuity.

Whole blood was obtained from all patients for evaluation of cyclosporin A trough levels at baseline; treatment weeks 1, 4, and 12; and posttreatment week 4. At week 12, additional blood samples were drawn at one study site only for evaluation of peak cyclosporin A concentrations. For evaluation of trough levels, blood was drawn immediately before the morning dose of study medication. For evaluation of peak levels, blood was drawn 1, 2, and 4 hours after instillation of the final dose of study medication at week 12.

Blood samples were sent to the Allergan Pharmacokinetics Laboratory, where they were assayed by liquid chromatography-mass spectroscopy/mass spectroscopy (LC-MS/MS) with a detection limit of 0.1 ng/ml. One milliliter of human blood was acidified with 2 ml of 0.1 N HCl solution and analytes extracted with 5 ml of methyl t-butyl ether. After separation from the acidified aqueous layer, the organic layer was made basic with 2 ml of 0.1 N NaOH, centrifuged, and the organic extract was then evaporated. The dried extract was reconstituted in 200 μl of mobile phase A and B (1:1 v/v) and 100 μl was injected into the LC-MS/MS system. The LC-MS/MS analysis was conducted on a PE-Sciex API III$^+$ triple quadrupole mass spectrometer (Perkin Elmer, Norwalk, CT) coupled to a Shimadzu HPLC system (Columbia, MD). Chromatography was performed on a Keystone BDS Hypersil C8 column (50 × 2 mm, 3 μm) with a binary mixture of 2 ammonium acetate/0.4% formic acid in water (mobile phase A) and 2 mmol/l ammonium acetate/0.4% formic acid in acetonitrile (mobile phase B) under gradient elution. The HPLC effluent flow rate of 300 μl/min was split, with 75 μl/min directed to the atmospheric ionization source. The mobile phase was 60% B at 0 to 0.5 minute, increased linearly to 95% B at 1 minute, held at 95% B from 1 to 2.5 minutes, and then decreased to 60% B at 3 minutes (held 1 minute). Cyclosporin G was used as the internal standard.

The PE-Sciex MacQuan software (PE-Sciex Instruments, Concord, Ontario, Canada) was used to determine peak areas of analyte and internal standard, peak area ratios of analyte/internal standard, calibration curves, and calculated concentrations of unknowns. The accuracy and precision of the LC-MS/MS method was assessed within each run using quality control blood samples at 0.1, 0.2, 1, and 5 ng/ml. The intraday accuracy (percent ratio of observed to nominal concentration) ranged from 100% to 109%, with precision (coefficient of variation) ranging from 3% to 10%. The interday accuracy ranged from 102% to 113%, with precision ranging between 1% and 13%.

At four selected study centers, ocular samples for microbiologic evaluation were collected from the conjunctival cul de sac at baseline, treatment week 12, and posttreatment week 4 and sent to a centralized laboratory for culture and organism identification.

Statistical Methods. Efficacy variables from subjective measurements with data collected on both eyes were analyzed by averaging the data from both eyes. Efficacy variables from objective measurements with data collected on both eyes were analyzed using data from the worse eye. The worse eye was defined as the eye with the worse Schirmer value and the worse superficial punctate keratitis value (pupil and nasal areas only) at baseline.

Because of the heterogeneity of patient disease profiles, subgroup analyses of patients who had various degrees of disease severity were analyzed separately. Only patients with moderate-to-severe dry eye disease at baseline were included in the efficacy analysis described in this report. All patients who received study medication were included in the analysis of safety variables.

Demographic parameters were summarized with descriptive statistics and frequency tables. Efficacy parameter comparisons among treatment groups were analyzed with the Kruskal-Wallis test. Pairwise comparisons between treatment groups were analyzed with the Wilcoxon rank sum test. Within-group changes from baseline were evaluated with the Wilcoxon signed-rank test. REFRESH use, intraocular pressure, and laboratory variables were analyzed by analysis of variance. Within-group changes from baseline were evaluated with the paired t test. Adverse event data were summarized by frequency tables. A two-sided test with $P = 0.05$ was considered statistically significant for all main effects.

The null hypothesis was that there were no differences among the treatment groups with regard to changes from baseline values. The alternative hypothesis was that there was a change.

Power was calculated to detect an among-group difference in change from baseline in categorized Schirmer tear values at week 12. For a sample size of 12 to 15 patients in the moderate-to-severe subgroup analysis, a standard error of 0.394 and standard deviation of 0.881, the power to detect a one grade difference was 0.69.

Patient Treatment Assignment

Qualified patients within each investigator's population were assigned equally to one of the five masked treatment groups sequentially, corresponding to a randomization schedule generated by the sponsor and using a block of five design.

Study Masking

All study medications were liquids of similar appearance, dispensed in identical unit dose vials, sealed in identical two-compartment plastic pouches, and packed in identical boxes of 16 pouches each. Each pouch and packing box was coded with a shipment number and the patient number.

When each box was dispensed, the tear-off portion of the label was attached to the patient's case report form. If necessary (because of a serious or severe adverse event), the investigator could irreversibly unmask the tear-off portion of the patient's medication label to determine which study medication the patient had received to institute appropriate patient care.

Results

Because this was the first clinical trial conducted with this new cyclosporin A formulation, it was designed to function as a pilot study for future investigations. Therefore, patients who varied widely in the severity of their dry eye were enrolled. The data from all patients who received study medication (intent-to-treat population) were analyzed. However, a subgroup analysis revealed a sizable population of patients who had moderate-to-severe dry eye disease at baseline. Moderate-to-severe dry eye disease was defined as a Schirmer tear test 5 mm/5 minutes at baseline in at least one eye and superficial punctate keratitis (pupil and nasal average) of 1.5 averaged over both eyes. Because these patients represent the greatest therapeutic challenge for any dry eye treatment, the efficacy analysis presented here is confined to the evaluation of this moderate-to-severe subgroup. This subgroup also represents the most appropriate target population for future clinical studies of dry eye therapeutics because these patients have sufficient manifestations of the disease to allow the response to therapeutic intervention to be more objectively evaluated. Data from all patients were included in the safety analysis.

Participant Flow and Follow-up

A total of 162 patients was enrolled: 129 in the cyclosporin A groups and 33 in the vehicle group (Table 1). Eight patients were discontinued for administrative reasons. Of the four patients discontinued because of adverse events, two were in the vehicle group (one with a visual disturbance and ocular burning, and one with conjunctivitis and a contact irritation dermatitis), one was in the cyclosporin A 0.2% group (ocular burning) and one was in the cyclosporin A 0.4% group (myocardial infarction). Only the ocular adverse events were considered to be possibly or probably related to the study medication.

Table 1. Patient Disposition

Treatment Group	Moderate-to-Severe Dry Eye Disease (n = 90)				Intent-to-Treat Population (Total Enrollment) (n = 162)			
	Completed		Discontinued		Completed		Discontinued	
	n	%	n	%	n	%	n	%
Vehicle	16	100.0	0	0.0	30	90.9	3	9.1
CsA 0.05%	17	100.0	0	0.0	30	96.8	1	3.2
CsA 0.1%	18	94.7	1	5.3	30	93.8	2	6.3
CsA 0.2%	20	100.0	0	0.0	32	94.1	2	5.9
CsA 0.4%	17	94.4	1	5.6	28	87.5	4	12.5
Total	88	97.8	2	2.2	150	92.6	12	7.4

CsA = cyclosporin A.

Of the 90 patients with moderate-to-severe dry eye disease, 16 were in the vehicle group, 17 in the cyclosporin A 0.05% group, 19 in the cyclosporin A 0.1% group, 20 in the cyclosporin A 0.2% group, and 18 in the cyclosporin A 0.4% group. One patient in the cyclosporin A 0.1% group was discontinued for personal reasons, and one patient in the cyclosporin A 0.4% group was discontinued because of a myocardial infarction (same patient as mentioned earlier). No patients' medications were unmasked during this study.

Patient Demographics

The demographic characteristics of the patient population are listed in Table 2. Note that the mean patient age was approximately 58 years, that more than 80% of patients were women, and that approximately 90% were white. Approximately 32% of the patients in the moderate-to-severe dry eye group were also Sjögren's syndrome patients. Sjögren's syndrome was defined as the presence of one or more of the following in the blood: antinuclear antibodies (>0 titer); rheumatoid factor (30 international units/ml); Sjögren's syndrome A (>10 IU/ml) or B (>5 IU/ml) antibodies. No significant differences were noted among the treatment groups for either the intent-to-treat or moderate-to-severe dry eye populations.

Efficacy Analysis

At baseline, mean scores for conjunctival rose bengal staining ranged from 1.2 to 2.0 for both temporal and nasal regions in all treatment groups. Significant improvements from baseline in temporal conjunctival rose bengal staining scores were observed with cyclosporin A 0.1% at all treatment and posttreatment visits ($P \leq 0.016$), with cyclosporin A 0.2% at week 12 and both posttreatment visits ($P \leq 0.047$), with cyclosporin A 0.4% at week 8 ($P = 0.031$), and with the emulsion vehicle at week 12 ($P = 0.047$) (Fig 1). Cyclosporin A 0.1% produced significantly greater improvements in temporal conjunctival rose bengal staining scores than vehicle ($P = 0.006$), cyclosporin A 0.05% ($P = 0.022$), and cyclosporin A 0.4% ($P = 0.007$) at posttreatment week 2.

Significant improvements from baseline in nasal conjunctival rose bengal staining scores were observed with cyclosporin A 0.2% at all treatment and posttreatment visits ($P \leq 0.022$), with cyclosporin A 0.1% and 0.05% at treatment week 4 through posttreatment week 2 ($P \leq 0.031$), in the cyclosporin A 0.4% group at posttreatment week 2 ($P = 0.031$), and in the vehicle group at treatment weeks 8 and 12 ($P \leq 0.025$). There were no significant among-group differences in the change from baseline in nasal conjunctival rose bengal staining.

At baseline, mean scores for superficial punctate keratitis ranged from 1.6 to 1.9 in all treatment groups. Cyclosporin A 0.1% produced the greatest improvement from baseline in superficial punctate keratitis scores throughout the treatment and posttreatment periods (range, −0.9 to −1.4 units) (Fig 2). With the exception of the 0.05% concentration at treatment week 12 and posttreatment week 4, significant improvements from baseline in superficial punctate keratitis were seen in all cyclosporin A treat-

Table 2. Patient Demographics

	Moderate-to-Severe Dry Eye Disease (n = 90)	Intent-to-Treat Population (n = 162)
Age		
Mean (range)	58 (31–88)	59 (31–88)
Gender (%)		
Male	17 (18.9)	26 (16.0)
Female	73 (81.1)	136 (84.0)
Race (%)		
White	82 (91.1)	145 (89.5)
Black	4 (4.4)	12 (7.4)
Asian	0 (0.0)	1 (0.6)
Hispanic	4 (4.4)	4 (2.5)
Sjögren's syndrome (%)	29 (32.2)	43 (26.5)

CsA = cyclosporin A; NA = data not available.

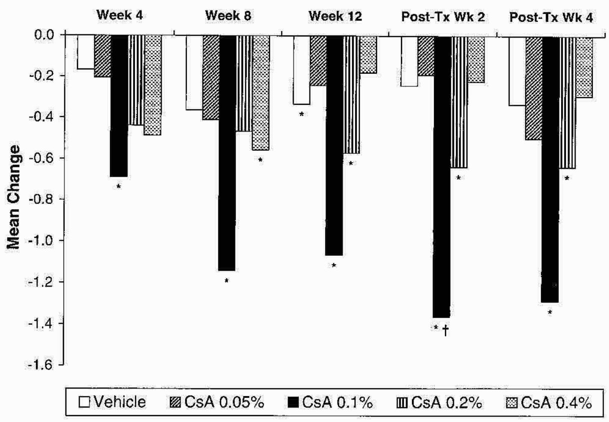

Figure 1. Change from baseline in temporal rose bengal staining. CsA, Cyclosporin A. *, Significantly different from baseline ($P \leq 0.047$); †, Significantly different from vehicle, cyclosporin A 0.05%, and cyclosporin A 0.4% ($P \leq 0.022$).

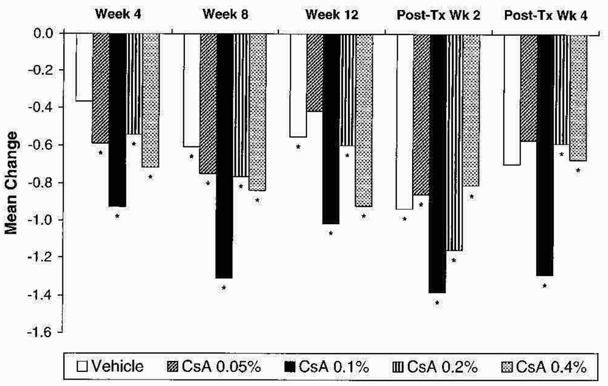

Figure 2. Change from baseline in superficial punctate keratitis. CsA, Cyclosporin A. *, Significantly different from baseline ($P \leq 0.018$).

ment groups at all time points during the 12-week treatment phase ($P \leq 0.012$) and 4-week posttreatment period ($P \leq 0.018$). Significant improvement in superficial punctate keratitis was also observed in patients treated with vehicle at treatment weeks 8 and 12 and posttreatment week 2 ($P \leq 0.041$). No statistically significant among-group differences in superficial punctate keratitis values were observed.

Baseline values for Schirmer tear test wetting scores ranged from 2.4 to 3.1 in all treatment groups. The most consistent improvements were in the cyclosporin A 0.1% group, with mean increases in wetting length of 4.3 mm at week 8 and 2.8 mm at week 12, but these increases only approached statistical significance (week 8, $P = 0.051$; week 12, $P = 0.055$). The only statistically significant improvement from baseline occurred in the cyclosporin A 0.4% group at treatment week 4 ($P = 0.008$) and posttreatment week 4 ($P = 0.023$), whereas a significant worsening occurred in the vehicle group at week 4 (3.0 mm, $P = 0.047$). Cyclosporin A 0.4% produced significantly ($P \leq 0.025$) greater improvements from baseline in Schirmer tear test results than either vehicle or cyclosporin A 0.2% at posttreatment week 4.

Symptoms of ocular discomfort were evaluated from scheduled visit queries from the clinical investigator and from self-administered, weekly patient diaries. Baseline symptom results suggest that patients may have consistently underreported the severity of their symptoms when responding to the query from the health professional compared with what they recorded in their diaries. Therefore, only symptom data from patient diaries (using the entries immediately before each scheduled visit) are presented.

At baseline, the mean score for sandy or gritty feeling ranged from 1.7 to 2.2 (mild to moderate) in all treatment groups. There was a significant improvement from baseline in sandy or gritty feeling in the cyclosporin A 0.05% and 0.4% groups at several visits ($P \leq 0.039$) (Fig 3). At treatment week 12, all cyclosporin A treatment groups had significantly greater improvements in sandy or gritty feeling than the vehicle group ($P \leq 0.04$). This significant difference from vehicle was also seen at posttreatment week 2 in the cyclosporin A 0.05% and 0.4% groups ($P \leq 0.006$) and at posttreatment week 4 in the cyclosporin A 0.05%, 0.2%, and 0.4% groups ($P \leq 0.027$). At posttreatment week 2, the cyclosporin A 0.4% and 0.05% groups also demonstrated a significantly greater improvement than the cyclosporin A 0.2% group ($P \leq 0.037$).

At baseline, the mean score for ocular dryness ranged from 2.3 to 2.7 (moderate to severe) in all treatment groups. Significant improvements from baseline in ocular dryness were seen at two or more time points in all cyclosporin A groups except the cyclosporin A 0.1% group ($P \leq 0.036$) (Fig 4). At posttreatment week

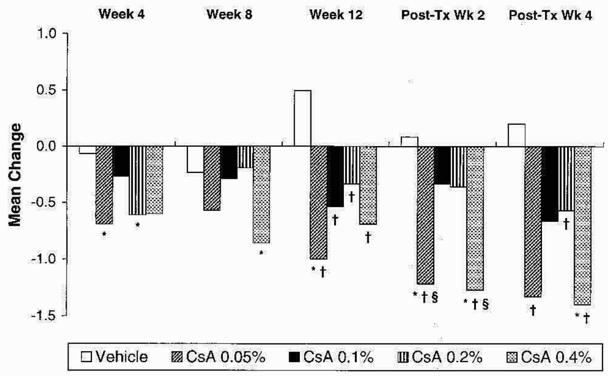

Figure 3. Change from baseline in sandy or gritty feeling. CsA, Cyclosporin A. *, Significantly different from baseline ($P \leq 0.039$); †, significantly different from vehicle ($P \leq 0.027$); §, significantly different from cyclosporin A 0.2% ($P \leq 0.037$).

4, cyclosporin A 0.05%, 0.2%, and 0.4% groups all demonstrated significantly greater improvements in ocular dryness than did the vehicle group ($P \leq 0.010$).

At baseline, the mean score for ocular itching ranged from 1.4 to 1.9 (mild to moderate) in all treatment groups. Significant improvements from baseline in ocular itching were seen at one or more time points in all of the cyclosporin A groups ($P \leq 0.031$) but not in the vehicle group. The magnitude of improvement in the cyclosporin A groups was larger than that in the vehicle group at all time points, but there were no statistically significant differences among any of the groups at any time point.

There were no significant within-group or between-group differences in photophobia, pain, or burning and stinging at any time point. The mean scores at baseline for all these parameters ranged from 1 to 2 (mild to moderate) in all treatment groups.

Baseline OSDI scores ranged from 33 to 42 (on a scale from 0 to 100, where 0 indicates no disability and 100 indicates complete disability) in all treatment groups. At both treatment week 12 and posttreatment week 4, there was at least a trend toward improvement in the OSDI score in the cyclosporin A 0.1%, 0.2%, and 0.4% groups, whereas there was either no change or worsening in the vehicle group (Fig 5). At week 12, cyclosporin A 0.1% and 0.2% significantly reduced OSDI scores ($P \leq 0.008$). This decrease was significantly greater with cyclosporin A 0.1% than with cyclosporin A 0.05% or 0.2% ($P \leq 0.032$). This improvement in OSDI scores persisted into the posttreatment period, with a significant

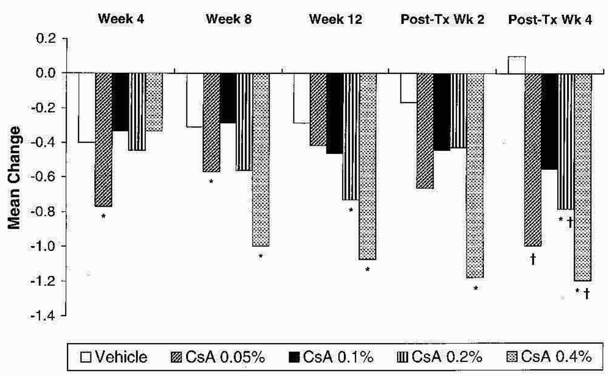

Figure 4. Change from baseline in ocular dryness. CsA, Cyclosporin A. *, Significantly different from baseline ($P \leq 0.036$); †, significantly different from vehicle ($P \leq 0.010$).

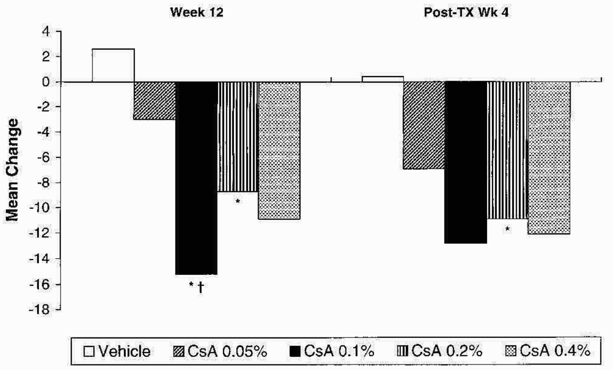

Figure 5. Change from baseline in the OSDI. CsA, Cyclosporin A. *, Significantly different from baseline ($P \leq 0.026$); †, significantly different from cyclosporin A 0.05% and cyclosporin A 0.2% ($P \leq 0.032$).

improvement seen in the cyclosporin A 0.2% group (P = 0.026) and a trend toward significance seen in the cyclosporin A 0.1% group (P = 0.055).

Only cyclosporin A 0.1% produced significant improvements from baseline values in tear film debris at any time point (week 8, P = 0.005; week 12, P = 0.031). There were no significant differences in tear break up time among the treatment groups.

At treatment week 12 and both posttreatment visits, patients in the vehicle group used a greater number of REFRESH units per day (mean values, week 12, 4.3 units; posttreatment week 2, 5.3 units; posttreatment week 4, 5.8 units) than those in any of the cyclosporin A treatment groups (mean values, week 12, 2.5 to 4.1 units; posttreatment week 2, 2.8 to 3.9 units; posttreatment week 4, 3.0 to 3.7 units), but this difference was not statistically significant.

Safety Analysis

Analysis of blood samples revealed that peak cyclosporin A levels were ≤0.16 ng/ml in all dosage groups. Only five patients had cyclosporin A blood levels at trough that were greater than 0.1 ng/ml (range, 0.1 ng/ml to 0.16 ng/ml), the limit of detection for the assay method. No significant differences were noted in blood concentrations among any dosage treatment group at any visit. Comparison of trough whole blood cyclosporin A concentrations between the beginning and end of the treatment period suggested no substantial accumulation of cyclosporin A after multiple ocular instillations for 12 weeks.

No ocular infections occurred in any of the treatment groups at any time during the treatment or posttreatment periods, and there was no overgrowth of ocular microorganisms with any of the treatments. The microbial analysis found that the cyclosporin A groups generally had fewer ocular microorganisms than the vehicle group. At week 12 in the vehicle group, 9 of 11 patients (82%) were culture positive, whereas 24 of 47 patients (51%) were culture positive in the cyclosporin A groups. There was also a trend for a decrease in bacterial species (from 19 to 10) and total strains of organisms recovered (from 40 to 31) after 12 weeks of cyclosporin A treatment, whereas there was a trend for an increased number of bacterial species (from 4 to 11) and total organisms (from 7 to 19) after vehicle treatment. Staphylococcus epidermidis was the organism most frequently isolated both at baseline and at week 12. Although there were changes in microbial flora during the treatment period in all patients analyzed (changes in the amount or type of bacteria detected), these changes were comparable in all treatment groups, suggesting that transient changes in ocular flora may be normal in patients with dry eye disease (Table 3).

Table 3. Change in Ocular Microflora From Baseline to Week 12

Treatment Group	n	Number of Patients: *No Growth to Any Growth*	*Any Growth to No Growth*	*Change in Gram's Stain*
Vehicle	14	4	1	1
CsA 0.05%	15	3	3	0
CsA 0.1%	14	3	2	1
CsA 0.2%	15	1	4	1
CsA 0.4%	16	5	0	2

CsA = cyclosporin A.

No growth to any growth = no bacteria at baseline but the presence of bacteria at week 12.

Any growth to no growth = presence of bacteria at baseline but no bacteria at week 12.

Change in Gram's stain = from gram-negative to gram-positive, or conversely.

A total of 15 treatment-related adverse events were reported by the 162 patients in the intent-to-treat group (Table 4). The greatest incidence of treatment-related adverse events occurred in patients in the vehicle group (3 of 33; 9.1%), and the most frequently reported adverse event was superficial punctate keratitis (5 of 162; 3%). No deaths or serious treatment-related adverse events occurred in any patient during the study.

No clinically significant changes in visual acuity or blood chemistry and hematology values (including liver and renal function parameters) were observed in any treatment group, and no patients experienced adverse events related to blood chemistry or hematology. Only one patient had a clinically significant increase in intraocular pressure from baseline (defined as an intraocular pressure that was within the normal range at baseline and increased to >25 mmHg) and that patient was in the vehicle group. There were no statistically or clinically significant within-group or among-group changes in any biomicroscopic variables, except at week 8 in the vehicle group in which there was a statistically significant increase from baseline in lid erythema (P = 0.016).

Discussion

The most important results of this study were that treatment with topical cyclosporin A 0.05% to 0.4% ophthalmic emulsions significantly improved the ocular signs and symptoms of moderate-to-severe dry eye disease and that these improvements resulted in a significant decrease in the effect of the disease on vision-related functioning (as measured by the OSDI).

These findings support the results of earlier studies that demonstrated a beneficial effect of topical cyclosporin A on dry eye disease[15] or dry eye in Sjögren's syndrome[14] (Foulks et al, Invest Ophthalmol Vis Sci 1996;37(Suppl): S646; Helms et al, Invest Ophthalmol Vis Sci 1996;37 (Suppl):S646) and expands the range of patients who may potentially benefit from cyclosporin A treatment to those with moderate-to-severe dry eye disease with or without Sjögren's syndrome. In addition, these findings may provide further insight into the pathophysiology of dry eye disease.

The mechanisms contributing to dry eye disease are still being explained, but several lines of evidence suggest that

Table 4. Treatment-Related Adverse Events

	Vehicle (n = 33)	CsA 0.05% (n = 31)	CsA 0.1% (n = 32)	CsA 0.2% (n = 34)	CsA 0.4% (n = 32)	Total Events (n = 162)
Adverse event						
Superficial punctate keratitis	2	1	0	0	2	5
Conjunctivitis	1	0	0	0	0	1
Conjunctival hyperemia	0	0	0	1	0	1
Burning eye	1	0	0	1	0	2
Pain in the eye	1	0	0	0	0	1
Foreign-body sensation	0	0	0	0	1	1
Photophobia	0	0	0	0	1	1
Visual disturbance	1	0	0	0	0	1
Contact dermatitis	1	0	0	0	0	1
Headache	0	0	1	0	0	1
Total patients	3	1	1	1	2	8

CsA = cyclosporin A.

Total patients = The number of patients who had any treatment-related adverse event.

dry eye disease is the result of a cytokine and receptor-mediated inflammatory process. This process affects the lacrimal gland acini and ducts, leading to abnormalities in the tear film and ultimately disrupting the homeostasis of the ocular surface. To date, much of the evidence for this hypothesis has come from the observation of lymphocytic infiltrates,[9,19] proinflammatory cytokines,[20] and autoantibodies[9] in the lacrimal glands of patients with dry eye disease associated with immune-mediated systemic disease (Sjögren's syndrome). The ability of the immunomodulatory agent cyclosporine to improve the signs and symptoms of moderate-to-severe dry eye disease unrelated to Sjögren's syndrome lends further support to the hypothesis that localized, cell-mediated inflammatory processes may contribute to the development of dry eye disease regardless of the cause.[8]

The contribution of inflammatory processes to dry eye disease unrelated to systemic inflammatory disease has also been demonstrated in histologic studies.[10,11] These studies demonstrated that evidence of inflammatory processes was associated with abnormal lacrimal gland histologic findings, suggesting that inflammation in dry eye may contribute to the progression of the disease by causing permanent damage to the lacrimal gland.

It is important to note that the vehicle emulsion used in this study performed well on its own, producing significant improvement from baseline in several parameters. This suggests that the nature of the formulation contributed to the therapeutic benefits observed in all treatment groups in this study. One of the factors contributing to the beneficial effects of the vehicle may be its sustained residence time on the ocular surface (3 to 4 hours; data not shown). This long residence time may help reduce evaporation of the limited volume of natural tears present in patients with dry eyes.

In this study, the most important safety findings were that few adverse effects were reported (none serious) and that no ocular infections occurred in any of the treatment groups. Microbial analysis of conjunctival bacterial isolates demonstrated that there was not only no bacterial overgrowth in the cyclosporin A treatment groups but that the number of bacterial species and total strains of organisms actually tended to decrease after cyclosporin A treatment. This finding is supported by the observation of similar decreases in ocular microflora in dogs treated with topical cyclosporin A for chronic idiopathic keratoconjunctivitis sicca.[21] If such decreases in ocular microflora are commonly seen in subsequent clinical studies, it would suggest that treatment with topical cyclosporin A may actually help diminish the increased risk of ocular infection associated with dry eye disease.

In addition, blood analysis demonstrated that even the greatest concentrations of topical cyclosporin A used resulted in minimal systemic absorption. This study used a highly sensitive assay (LC-MS/MS) that was specifically designed to detect low levels of cyclosporine in the blood (as low as 0.1 ng/ml). This assay was developed because the more common tests used to monitor blood levels in patients receiving systemic cyclosporine after organ transplantation were designed to detect much greater drug concentrations. Although the recommended safe therapeutic range of blood levels for systemic cyclosporine therapy varies widely depending on the indication and the assay method used,[22] it is clear that the maximum level reported here (0.16 ng/ml) is many orders of magnitude less than both mean levels at trough (75 to 361 ng/ml) and C_{max} values (655 to 1802 ng/ml) reported for patients receiving recommended systemic doses of cyclosporine for a wide range of indications.[23] Consequently, it is unlikely that topical use of cyclosporin A in the treatment of dry eye will exert any systemic effects.

In further support of the favorable safety profile of topical cyclosporin A, this study demonstrated that this particular ophthalmic cyclosporin A emulsion formulation was well tolerated by patients. There were no complaints of ocular discomfort, burning, or itching such as has been reported by dry eye patients administered cyclosporine 1% ointment[15] or cyclosporine 2% in olive oil.[12,14]

Although no clear dose-response relationship emerged among the different cyclosporin A concentrations tested, cyclosporin A 0.1% produced the most consistent improvement in objective and subjective end points (such as superficial punctate keratitis and rose bengal staining), and cyclosporin A 0.05% produced the most consistent improvements in patient symptoms (such as sandy/gritty feeling and ocular dryness). Although even the greatest concentrations of cyclosporin A were demonstrated to be safe, the lack of any additional therapeutic benefit with increasing concentration suggests that subsequent clinical studies should focus on the cyclosporin 0.05% and 0.1% formulations.

The lack of a dose-response relationship may be because the vehicle for each concentration of cyclosporin A ophthalmic emulsion was formulated slightly differently, with the higher concentrations of cyclosporin A being accompanied by a greater oil content in the formulation. Therefore, properties of the formulation other than cyclosporin A content may be affecting the clinical efficacy observed in this study.

In conclusion, the findings of this study support the continued investigation of the use of topical cyclosporin A as a safe and effective treatment for dry eye disease. The formulations used in this study were demonstrated to be free of the patient-tolerability problems associated with other topical cyclosporin A formulations and may provide additional therapeutic benefits associated with the properties of the vehicle. In addition, this dose-ranging study established that the most appropriate concentrations for future investigations are cyclosporin A 0.05% and 0.1%. The mechanisms by which topical cyclosporin A affects the signs and symptoms of moderate-to-severe dry eye disease cannot be discerned from this study. However, the results of previous studies suggest that an investigation of the effects of topical cyclosporin A on conjunctival and lacrimal immune cell populations in future clinical trials could not only provide valuable information on the mechanism of action of cyclosporin A in dry eye disease but may also expand our understanding of the pathogenesis of the disease itself.

Acknowledgments. The Cyclosporin Phase 2 Study Group: Peter Donshik, MD, FACS (University of Connecticut Health Center, West Hartford, CT), Gary N. Foulks, MD (Duke University Medical Center, Durham, NC); Harold A. Helms, MD (Birmingham, AL); Robert A. Laibovitz (Clinical Research in Ophthalmology, Austin, TX); Marta Loatynsky, MD (Contemporary Eye Associates, Bayonne, NJ); Earl Nelson, MD, (Eye Surgery Center of Louisiana, New Orleans, LA); Peter Rapoza, MD (Cornea Consultants, Boston, MA); and Caroline T Burk, PharmD, Katherine L Stern, MS, and Allan Rosenthal, PhD. (Allergan, Inc., Irvine, CA).

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