

Research Article

An Investigation Comparing the Efficacy of Topical Ocular Application of Tacrolimus and Cyclosporine in Dogs

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The purpose of this paper was to determine the efficacy and safety of topical tacrolimus, compared to cyclosporine, for treating keratoconjunctivitis sicca (KCS) in dogs. This study was a two-phase, randomized, controlled, masked clinical trial. Phase 1 evaluated ophthalmic 0.03% tacrolimus in normal dogs. Ocular examinations were performed daily. Phase 2 evaluated the efficacy of tacrolimus in treating KCS. Half the dogs received 2% cyclosporine A; the others received 0.03% tacrolimus, both diluted in olive oil. Four ophthalmic examinations were done over 12 weeks. There was no significant difference between groups in phase I. In phase 2, there was no significant difference in Schirmer tear test I (STT) results between the two groups, and both groups had a significant increase in STT over time. Both drugs were effective in increasing the STT in dogs naïve to lacrimostimulants. Tacrolimus was effective in increasing the STT in 4 dogs currently nonresponsive to cyclosporine.

1. Introduction

Keratoconjunctivitis sicca (KCS) is a deficiency of the aqueous layer of the precorneal tear film. Clinical signs in dogs with KCS include mucopurulent ocular discharge, conjunctivitis, keratitis, and blepharospasm. Dogs with KCS have a Schirmer tear test I (STT) result of <10 mm/minute, and severely affected dogs often have an STT result of 0 mm/min. While many causes of KCS in dogs have been described [1–10], KCS in dogs is most often considered to be immune-mediated based on studies that showed increased numbers of lymphocytes and plasma cells with acinar atrophy in the lacrimal glands [11, 12]. Immune-mediated KCS is typically bilateral and affects many breeds.

Treatment of immune-mediated KCS is aimed at decreasing the immune response in the lacrimal gland and restoring aqueous tear production. Cyclosporine A (CsA) is a commonly used and effective treatment for canine KCS [13–16]. Formulations typically used are previously commercially available as 0.2% CsA ophthalmic ointment (Optimmune),

and 1% and 2% CsA in olive or corn oil [13–19]. In addition to increasing tear production by inhibiting T-helper lymphocyte proliferation and infiltration of lacrimal gland acini, CsA restores conjunctival goblet cell mucin production [12, 13, 17, 18]. Previous studies have shown that dogs treated with ophthalmic CsA treatment have a decreased lymphocyte stimulation index compared to control dogs and that there are measurable levels of CsA in peripheral blood of treated dogs [20, 21]. However, a more recent study did not show a change in the lymphocyte stimulation index or significant blood levels with use of topical cyclosporine [22]. Unfortunately, approximately 25% of dogs with KCS do not respond to treatment with 1% or 2% CsA [15–17].

Tacrolimus is a newer immunosuppressant agent that has been used for the treatment of immune-mediated KCS in dogs [23]. Tacrolimus is a macrolide antibiotic that has a similar immunopharmacological profile as CsA but is approximately 100-times more potent than CsA [24–26]. In 2005, the United States Federal Drug Administration issued an advisory for the potential carcinogenic effects of

the dermal tacrolimus when used on humans. This advisory recommended that tacrolimus only be used as a second-line agent for short-term and intermittent use [27]. This advisory was based on a small number of patients, and the ramifications for dogs being treated with tacrolimus for KCS are not known.

Both CsA and tacrolimus are calcineurin inhibitors that reversibly inhibit T-cell proliferation and prevent the release of proinflammatory cytokines. Calcineurin inhibitors bind to intracellular immunophilins and form complexes that subsequently bind to and inhibit calcineurin. In blocking calcineurin, translocation of the cytoplasmic component of the nuclear factor of activated T cells to the nucleus is prevented. This prevention of translocation impairs transcription of the genes encoding IL-2 and other cytokines, thereby suppressing T-cell proliferation and normal immune function [28].

The first purpose of this study was to determine the safety of topical tacrolimus in normal dogs. The second purpose was to compare the efficacy of topical ophthalmic application of 0.03% tacrolimus with that of 2% CsA in olive oil in a double-masked study.

2. Materials and Methods

This study was designed as a two-phase, controlled, masked, randomized clinical trial. Phase 1 was a toxicity study, and phase 2 was an efficacy study. This study was approved by the University of Tennessee Animal Care and Use Committee. Thirteen clinically normal research beagles were treated in the toxicity study. Prior to inclusion in the study, all dogs received complete ophthalmic examinations including STT, fluorescein staining, tonometry, slit-lamp biomicroscopy, and indirect ophthalmoscopy. Each dog was randomly assigned to either the tacrolimus or control group. Seven dogs were treated twice daily topically with the injectable form of tacrolimus (5 mg/mL; Fujisawa Inc., Japan) formulated into a 0.03% concentration in an olive oil vehicle. Six dogs were treated twice daily with the olive oil vehicle alone. All dogs were treated for 14 days. All dogs received daily ophthalmic examinations including STT, fluorescein staining, and slit lamp biomicroscopy during the treatment period. Daily clinical scores were assigned to each dog grading the severity of conjunctival hyperemia, blepharospasm, and epiphora by a masked observer (EA). The grading scale consisted of grades 0 through 3 for each of the criteria evaluated. Grade 0 was considered normal, grade 1 mildly affected, grade 2 moderately affected, and grade 3 severely affected. Serum biochemistry profiles were evaluated on days 0 and 15 to determine if the treatment caused any alterations in these values.

For the phase 2 efficacy study, patients were enrolled for 12 weeks. Inclusion criteria consisted of an STT result less than 10 mm/minute in both eyes (OU) even with current CsA treatment, clinical signs of KCS OU, and written consent of the owner. Exclusion criteria included corneal ulceration, suspected bacterial keratitis, systemic neoplasia, immunosuppressive therapy for a systemic disease, and previous treatment with sulfonamides or etodolac.

Dogs were randomly assigned to 1 of 2 groups. One group was treated with 2% CsA in olive oil OU BID, and the other group was treated with 0.03% tacrolimus in olive oil OU BID. Any medications that the patient was currently receiving to treat KCS such as antibiotic ointment and ocular lubricants were continued during the course of the study. Five dogs in the CsA group were already being treated with CsA (three with 2% solution and two with 0.2% ointment). Six dogs in the tacrolimus group were already being treated with CsA (four with 2% solution and two with 0.2% ointment). Upon enrollment, the current CsA formulation was discontinued and the CsA or tacrolimus treatment was initiated without having a washout period. Criteria used to terminate participation in the study included corneal ulceration, development of bacterial keratitis, or signs of excessive ocular irritation secondary to drug administration.

Each dog was evaluated on day 0 and at weeks 4, 8, and 12 of the study by a masked observer (EA, DH, DW, or BS). Each dog had a complete ophthalmic examination at each evaluation, including STT, fluorescein staining, slit lamp biomicroscopy, and indirect ophthalmoscopy. Serum biochemistry profiles were evaluated at weeks 0 and 4 of the study.

Both eyes of each dog in the study were photographed at each visit and were graded by a masked observer (DH). Clinical signs were evaluated as follows: conjunctival hyperemia (score 0: no conjunctival hyperemia, 1: mild hyperemia, 2: moderate hyperemia), ocular discharge (score 0: no ocular discharge, 1: slight ocular discharge at medial canthus, 2: discharge across cornea or on eyelid margins), area of corneal pigmentation (score 0: no corneal pigmentation, 1: pigment over less than 25% of the cornea, 2: pigment over 25–50% of the cornea, 3: pigment over 50–75% of the cornea, 4: pigment over >75% of the cornea), corneal pigment density (score 0: no pigment, 1: iris easily visualized through the pigment, 2: iris partially visualized through the pigment, 3: iris not visible through the pigment), corneal vessel length (Score 0-no corneal vessels, 1: limbal corneal vessels, 2: vessels halfway towards the corneal axis, 3: vessels extending to axial cornea), corneal vessel density (Score 0: no vessels, 1: vessels extending to cornea from <25% of the circumference of the limbus, 2: vessels extending to cornea from 25–50% of the circumference of the limbus, 3: vessels extending to cornea from 50–75% of the circumference of the limbus, 4: vessels extending to cornea from >75% of the circumference of the limbus), and corneal keratinization (score 0: none, 1: mild opacity, 2: moderate opacity).

Data from both eyes from each dog were combined for statistical analysis. For phase 1, the daily clinical scores and STT results between the two groups were compared by Student's *t*-test. Within each group the effect of treatment on STT over time was evaluated by repeated measures analysis of variance (RM-ANOVA).

For the clinical study, the data from both eyes from each dog was combined. Within each group the effect of the treatments on STT and clinical scores over time was evaluated by one-way RM-ANOVA. When normality failed, the Friedman repeated measures ANOVA on ranks was used. The STT and clinical scores comparing the CsA and tacrolimus groups on day 0, and at weeks 4, 8, and 12 were evaluated by Student's

t-test. Statistical significance was set at $P \leq .05$. Statistical analyses were done with SPSS software (SPSS Inc., Chicago, Illinois).

3. Results

For all dogs in phase 1, the serum biochemistries were within reference ranges on day 0 and day 15. There was no difference between the two groups in the daily clinical scores (data not shown). Mean STT on day 0 was 19.08 (± 2.7) for the control group and 20.64 (± 3.2) in the tacrolimus group. On day 15, the mean STT was 21.08 (± 2.3) for the control group and 24.29 (± 3.2) for the tacrolimus group. There was no statistical difference in clinical scores between groups on day 0 or day 15 or between STT on day 0 and day 15 over time in either group.

Of the 29 dogs enrolled in the efficacy study, 18 dogs completed the study, with 10 dogs in the CsA group and eight dogs in the tacrolimus group. Of the 18 dogs, 16 were pure breeds, representing 14 different breeds, and two were mixed-breed dogs. Nine were spayed females, one was an intact female, four were neutered males, and four were intact males. Mean age in the CsA group was 8 years, and mean age in the tacrolimus group was 9 years. Five dogs in the CsA group and six dogs in the tacrolimus group had been treated with CsA prior to enrolling in the study.

Six dogs in the CsA group and eight dogs in the tacrolimus group had photographic images from all visits available for scoring. There was no significant difference in combined clinical scores for conjunctival hyperemia, ocular discharge, corneal pigmentation, corneal vascularization, or keratinization over time for the CsA or tacrolimus groups. There was no statistical difference in clinical scores between the CsA group and tacrolimus group at day 0, week 4, or week 8. There was a significant difference in the clinical scores between the CsA group and the tacrolimus group at week 12 ($P = .040$). The CsA group had lower clinical scores than the tacrolimus group at all time points, although this was not significant except at week 12.

Both groups had an increase in mean tear production over time. In both groups, the STT was higher at the fourth visit (CsA group: median 11 mm/minute, range 1.5–21.5; tacrolimus group: median 9.75 mm/min, range 7–20 mm/minute) compared to the first visit (CsA group: median 5 mm/minute, range 0–8.5 mm/minute; tacrolimus group: median 4.5 mm/minute, range 0–6.5). This difference between the STT at time 0 and at week 12 in both groups was significant (CsA, $P < .001$, tacrolimus $P = .001$; Table 1).

There was no significant difference in mean STT between the groups at time 0, 4 weeks, 8 weeks, or 12 weeks (Table 2).

No dogs had any signs of irritation from either drug. Serum biochemistry values were within normal limits for all patients.

Four of five dogs in the CsA group not previously treated with lacrimostimulants responded with an increase in tear production >5 mm/minute when treated with CsA. There was no change in the tear production in the one dog that did not respond. Of the five dogs in the CsA group that were already being treated with CsA (three with 2% solution

TABLE 1: Median tear production (STT) in mm/min at baseline and week 12 for the cyclosporine group and the tacrolimus group.

Group	Baseline (range)	12 weeks (range)	<i>P</i> value
Cyclosporine	5 (0–8.5)	11 (1.5–21.5)	$P < .001$
Tacrolimus	4.5 (0–6.5)	9.75 (7–20)	$P = .001$

TABLE 2: Mean tear production (STT) in mm/min at baseline, 4 weeks, 8 weeks, and 12 weeks for the cyclosporine group and the tacrolimus group. There was no difference between groups at any time.

Time (weeks)	Cyclosporine	Tacrolimus	<i>P</i> value
Baseline	4.6	3.2	$P = .336$
4	9.3	7.0	$P = .302$
8	10.8	10.0	$P = .768$
12	11.7	11.1	$P = .850$

and two with 0.2% ointment), only one dog, which was being treated with 2% CsA prior to being enrolled in the study, had an increase in STT of >5 mm/minute. Only two dogs in the tacrolimus group were not already being treated with CSA at time of enrollment in the study, and they both responded with a tear production increase >5 mm/minute. Four of the six dogs in the tacrolimus group that were already being treated with CsA (two with 2% CsA in oil and two with 0.2% CsA ointment) had an increase in mean STT >5 mm/minute. Two dogs did not have a mean increase in STT >5 mm/minute. One of those had a tear production of 5 mm/minute in the right eye with no change during the study and the left eye increased from 8 mm/minute in to 12 mm/minute at 12 weeks. The other dog was similar and had no change in the right eye, but the STT increased from 0 mm/minute in the left eye upon enrollment to 9 mm/minute at 12 weeks.

4. Discussion

The results of this small, short-term study support tacrolimus ophthalmic solution as potentially successful in increasing tear production in dogs nonresponsive to treatment with CsA. As previously reported, both CsA and tacrolimus are useful in the treatment of dogs with KCS previously not treated with lacrimostimulants [13–16, 23].

A previous study investigating the effects of 2% CsA in olive oil administered TID in normal beagles showed a significant increase in STT after 7 days. Our study did not show a significant increase in tear production between the dogs treated with 0.03% tacrolimus in olive oil and control dogs treated with the olive oil alone after 14 days of BID treatment. However, a direct comparison between the two studies cannot be made because of the difference in treatment frequency.

While the numbers are small, having a response of >5 mm/minute STT in the majority of dogs naïve to CsA was expected, as was having most of the dogs that were already

being treated with CsA not show a response. The single dog in the CsA group that was already being treated with 2% CsA that responded to the study CsA with an increase in STT >5 mm/minute may have done so simply because of increased owner compliance. Of the eight dogs in the tacrolimus group, two that were naïve to lacrimostimulant therapy and four that were already being treated with CsA (two with 2% CsA in oil and two with 0.2% CsA ointment) responded to the tacrolimus. While the numbers are small in this study (using tacrolimus as a 0.03% solution in olive oil), these results support the findings by Berdoulay who used 0.02% aqueous solution that tacrolimus may be effective at increasing tear production in dogs nonresponsive to CsA [23].

Significant improvement in clinical signs over time was not seen in this study. This is in contrast to previous studies evaluating CsA and tacrolimus that found a decrease in severity of signs both with and without increased tear production [17, 23]. In contrast to those studies, which evaluated only ocular discharge and signs of conjunctivitis, the current study also assessed corneal pigmentation, corneal vascularization, and corneal keratinization. These signs develop with chronicity and are also expected to require more time to improve. The clinical signs of the dogs of this study may have improved significantly with a longer follow-up period.

This study used olive oil rather than a lactose cellulose base for the increased lubrication and contact time associated with an oil base. Similar efficacy of the tacrolimus in olive oil compared to the aqueous base was seen, and there were no complications seen with the olive oil base [23]. Therefore, it may be the formulation of choice in dogs with very low tear production or in dogs with increased corneal exposure secondary to lagophthalmos.

CsA was used as the control in this study to ensure that no eyes deteriorated due to lack of standard and proven treatment of KCS. To assure that differences between the two drugs could not be attributed to different vehicles, both the CsA and tacrolimus were diluted in olive oil. Unfortunately for this study, most dogs in our region are treated appropriately for KCS, and, therefore, we were not able to obtain large numbers of dogs that had not had CsA therapy initiated prior to the study.

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