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Clinical evaluation of pimecrolimus eye drops for treatment of canine keratoconjunctivitis sicca: A comparison with cyclosporine A

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Abstract

The aim of this study was to evaluate the efficacy of pimecrolimus oil-based eye drops in alleviating the clinical signs of keratoconjunctivitis sicca (KCS) in dogs and to compare the efficacy with that of cyclosporine A (CsA) ointment. An open-label, multicenter study enrolling 44 dogs previously untreated with CsA was conducted. Dogs were randomly assigned to a treatment group and medicated twice daily for 8 weeks. After that time the mean increase (±SEM) in the Schirmer tear test was 9.2 ± 1.6 mm/min in the pimecrolimus group and 5.8 ± 1.1 mm/min in the CsA group ($P = 0.085$). The improvement in clinical signs of inflammation in eyes treated with pimecrolimus was significantly greater than in eyes treated with CsA ($P = 0.02$). The results show that 1% pimecrolimus oily eye drops are as safe as and more effective than CsA ointment in controlling KCS in dogs.

Keywords: Dog; Keratoconjunctivitis sicca; Dry eye; Cyclosporine A; Pimecrolimus

Introduction

Tears play an important role in maintaining the health and normal function of the cornea and conjunctiva; they provide essential nutrients to the cornea, help remove foreign matter and waste products, and contain immunoglobulins, lysozymes and other components of the ocular defense mechanisms. Therefore, it is not surprising that tear deficiency is a major cause of corneal and conjunctival inflammation. Millions of people worldwide are afflicted with keratoconjunctivitis sicca (KCS), or dry eye, and symptoms of the disease are reported by 17–25% of patients visiting ophthalmic clinics (McCarty et al., 1998; Moss et al., 2000; Schaumberg et al., 2003). The disease is also prevalent in dogs, with a diagnosis of KCS made in 1–1.5% of all dogs visiting veterinary teaching hospitals in North America (Kaswan et al., 1991; Helper, 1996).

In both humans and dogs, the most common form of dry eye is a quantitative deficiency in the middle, aqueous layer of the tear film. This deficiency can cause a large range of clinical signs, depending on the severity and duration of the disease. Acute cases may present with severe pain and corneal ulceration which may rapidly progress to corneal perforation and iris prolapse (Moore, 1999). Chronic cases present with classic signs of keratitis (including infiltration of inflammatory cells, vascularization, pigmentation and thickening) and conjunctivitis (including

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congestion, pigmentation and thickening) (Moore, 1999). Mucoid to mucopurulent discharge is present, and secondary bacterial infection is a common complication. Visual acuity is affected due to loss of the contribution of tears to the overall refractive power of the eye (Montes-Mico et al., 2004) and because of deterioration in corneal transparency.

There are numerous causes of KCS in the dog. The leading cause is most likely immune-mediated dacryoadenitis (Moore, 1999). Histopathological studies of lacrimal tissue of affected dogs show lymphocytic–plasmacytic infiltration associated with acinar atrophy, suggesting an immune-mediated basis for the disease in this species (Kaswan et al., 1984; Bounous et al., 1995). Circulating antibodies to the lacrimal gland and the nictitating gland have been found in significant numbers of affected dogs (Kaswan et al., 1985). The disease may also be associated with systemic canine autoimmune conditions, including systemic lupus erythematosus (SLE), rheumatoid arthritis and pemphigus. A high percentage of dogs with KCS are also positive for rheumatoid factor (34%), antinucleolar antibodies (40%), or have high levels of gamma globulins (90%) (Kaswan et al., 1983, 1985). In humans it is also suspected that an autoimmune inflammation of the lacrimal glands, mediated by T cells, similarly plays an important role in the disease pathogenesis (Pflugfelder et al., 1986, 1999).

Further indication of the immune-mediated inflammatory etiology of the disease comes from studies in dogs and humans demonstrating that topical cyclosporine A (CsA) is an efficacious treatment for KCS. This immunosuppressive therapy results in a significant increase in tear production and improvement in clinical signs of inflammation in dogs (Olivero et al., 1991; Morgan and Abrams, 1991; Sansom et al., 1995), and for the past 15 years veterinarians have been using CsA ointment (Optimmune; Schering-Plough) or solution for treatment of canine KCS. Successful trials in humans (Sall et al., 2000; Stevenson et al., 2000) have recently led to the approval of a CsA ophthalmic emulsion (Restasis; Allergan) for the treatment of human patients.

However, a significant number of canine KCS patients do not respond to CsA treatment (Berdoulay et al., 2005), and patent and supply issues affect availability in some markets. Pimecrolimus (SDZ ASM 981; Novartis Institute for BioMedical Research) is a new ascomycin derivative which interferes selectively with the activation of T cells and mast cells and inhibits the production of inflammatory cytokines. The drug has been demonstrated to be more than 10-fold more effective than CsA in inhibiting cytokine production by T cells in vitro and has proven also to be superior to CsA in animal models of skin inflammation (Meingassner et al., 1997, 2003; Grassberger et al., 1999; Kalthoff et al., 2002). In a previous exploratory study, pimecrolimus has shown significant activity in animal models of immune-mediated inflammatory eye diseases, in particular in canine KCS (Nell et al., 2005). The aim of the present study was to conduct a large, multicenter, outpatient clinical dog trial to confirm the efficacy of topical pimecrolimus in alleviating clinical signs of KCS in dogs and compare it with the veterinary form of CsA (Optimmune).

Materials and methods

Animals

An open-label, multicenter, randomized, 8-week outpatient clinical dog study was conducted by four Diplomates of the European College of Veterinary Ophthalmologists. The study was approved by the respective Institutional Animal Care and Use Committees and all investigations adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The dog owners read an information sheet and signed an informed consent form prior to participation.

Dogs of either gender and of any breed or age were enrolled in the study following diagnosis of uni- or bilateral KCS. KCS was diagnosed based on medical history, a Schirmer tear test (STT) value $\leqslant$10 mm/min *and* a total score $\geqslant$4 in grading clinical signs of conjunctival and/or corneal inflammation (for details see below). Dogs were excluded from the study if they had ever been treated previously with topical or systemic CsA or with any of the following drugs within 14 days before the study: topical or systemic corticosteroids, atropine, antihistamines, pilocarpine, or sulfa-containing drugs; phenazopyridine; essential fatty acids; or general anesthetics. Other exclusion criteria included the presence of any systemic disease other than dermatological disorders or of any ocular diseases affecting the ocular surface other than those related to orbital conformation in brachycephalic breeds. Dogs in which KCS was determined to be congenital, secondary to neuroparalysis, to surgery of the nictitans gland, to distemper or to the use of lacrimotoxic drugs, were not included in the trial. Cases of KCS that had undergone parotid duct transposition or lacrimal duct occlusion were also excluded.

Treatments

The test material was 1% pimecrolimus experimental corn oil-based eye drops. The 1% pimecrolimus ophthalmic formulation was sterilized by filtration, and then aseptically filled into 5 mL polypropylene bottles with polypropylene droppers and high density polyethylene closures. Storage and use conditions were also mentioned on the bottle. Stability of the formulation was guaranteed for the duration of the study, if required storage conditions were observed.

Optimmune (Schering-Plough), the commercial ophthalmic ointment approved for veterinary use containing 0.2% CsA, was used as the comparative product. In severe cases, when considered necessary by the investigator, the dogs were treated t.i.d. with commercial artificial tears (Oculotect Fluid, Novartis). In cases of corneal ulceration, topical treatment (TID) with chloramphenicol solution was also permitted. It was allowed to clean secretions from the lids 15 min prior to drug administration. When several medications were administered, the order of treatment was as follows: artificial tears, followed by chloramphenicol solution, followed by the KCS medication. Owners were instructed to wait 15 min between medications.

Dogs that met the inclusion criteria were randomly assigned to either the CsA or the pimecrolimus group. Both treatments were administered twice daily for 8 weeks in both eyes. In cases of unilateral disease, only the data of the affected eye were analyzed in this study.

Evaluation of clinical efficacy

Upon enrollment and at weeks 2, 4 and 8, all dogs underwent complete physical and ophthalmic examinations, including slit-lamp biomicroscopy, STT and indirect ophthalmoscopy. The STT was performed using commercial tear test strips from the same lot (Schering-Plough). A complete medical history was recorded at each visit, with special attention paid to

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owner's impression of clinical improvement and/or side-effects. All examinations of a given animal were conducted by the same (enrolling) practitioner.

The following clinical signs of KCS were evaluated: blepharospasm, conjunctival thickening, conjunctival hyperemia, conjunctival pigmentation, corneal pigmentation, corneal edema, corneal vascularization, corneal infiltration and amount of ocular discharge. These parameters were graded on a 5-point rating scale (0 = normal; 1 = light/early; 2 = moderate; 3 = advanced; 4 = severe). The absence or presence of stained ulcers (corneal fluorescein staining) and the character of discharge (serous, mucoid or purulent and their intermediate grades) were also monitored. Results of these examinations were recorded on appropriate case report forms.

Statistical analysis

Efficacy analyses were performed on the evaluable population, which included all randomized dogs who received a treatment and who completed the trial. Categorical variables (e.g. gender, neutered status, breed of dog) were analyzed by Fisher's exact test, whereas the continuous variable (i.e. age) was analyzed by the Wilcoxon rank sum test. Changes from baseline in STT values were analyzed by ANOVA. Subgroups of dogs, based on baseline KCS severity, were analyzed by descriptive statistics. Subgroups examined were those dogs with severe KCS (STT = 0–2 mm/min), moderate KCS (STT = 3–6 mm/min) or mild KCS (STT $\geqslant$ 7 mm/min). The proportion of dogs responding to therapy (response being defined as a STT value >10 mm/min) was analyzed by Fisher's exact test. Between-treatment differences in changes from baseline in the total and individual ocular sign scores were analyzed by the Wilcoxon rank sum test. Within-treatment differences from baseline were tested using a paired *t* test. All statistical tests in this trial were two-sided and those with a corresponding *P* value $\leqslant$0.05 were considered statistically significant.

As there were no substantial differences between the worst-eye and best-eye analyses, the results for only the worst-eye (with the lowest STT value at baseline) are summarized.

Results

Study population

Forty-seven dogs were enrolled into the study, of which 22 dogs were randomly assigned to treatment with pimecrolimus and 25 to treatment with CsA. Three dogs did not complete the study; two dogs enrolled in the pimecrolimus group withdrew from the study due to severe but transient local irritation, and one dog in the CsA group was euthanised for unrelated reasons (traumatic paralysis of the hind legs). Of the 44 dogs that completed the study, 11 were diagnosed with unilateral disease. Therefore, the total number of eyes evaluated in this study was 77, of which 40 (24 dogs) were treated with CsA and 37 (20 dogs) were treated with pimecrolimus.

The protocol of the comparative study was well executed with minimal protocol deviations; all together, patients only missed 3/132 visits (=three rechecks for each of the 44 dogs). There was no significant difference between the two groups in the number of dogs receiving supplemental treatment with chloramphenicol solution (five dogs in the CsA group, seven in the pimecrolimus group) and/or artificial tears (21 dogs in the CsA group, 19 dogs in the pimecrolimus group).

Fifty-five percent of the subjects in the study population were female and the majority of dogs (77%) were not neutered (Table 1). Terriers ($n = 14$; Sealyham, West Highland White, Yorkshire) were the largest group of dogs enrolled, followed by Spaniels ($n = 9$; American Cocker, English Cocker, Cavalier King Charles) and Shi Tzus ($n = 7$). The mean age of the dogs in this study was 7.2 ± 3.2 (SD) years (range 2–13 years). There were no statistically significant differences between the treatment groups in patient signalment. Patient signalment, including breed sensitivity and female disposition, is in agreement with previous reports of prevalence of KCS in dogs (Kaswan et al., 1991; Sansom et al., 1995; Moore, 1999).

Increase in tear production

At baseline, the mean STT values in the pimecrolimus and the CsA groups were 3.8 ± 0.7 mm/min ($n = 20$) and 4.6 ± 0.7 mm/min ($n = 24$), respectively ($P = 0.413$). Statistically significant improvements from baseline were observed within both groups ($P < 0.001$) at all follow-up visits (Fig. 1). In the pimecrolimus group, mean increases of 7.4 ± 1.4, 7.8 ± 1.5 and 9.2 ± 1.6 mm/min were observed at weeks 2, 4 and 8, respectively, compared to 6.1 ± 1.2, 6.8 ± 1.0 and 5.8 ± 1.1 mm/min in the CsA group. Though at every recheck the improvement in STT in the pimecrolimus group was higher than in the CsA group, the difference did not reach statistical significance ($P = 0.085$; by-visit ANOVA).

As shown in Table 2, there were equal numbers of dogs between treatment groups within each subgroup (categorized on the basis of the initial KCS severity) upon enrollment in the study, with the exception of the subgroup of dogs with mild KCS, which had twice as many dogs in the CsA treatment group. In both the mild KCS and severe KCS subgroups, a significant increase in tear secretion was observed throughout the study after administration of either treatment. For unknown reasons, the smallest improvement in STT results (for both drugs) was recorded in the subgroup with moderate KCS, especially after 2 weeks of treatment.

Dogs responding to treatment were defined as those in which STT values increased to more than 10 mm/min. About 60% and 50% of dogs responded to pimecrolimus and CsA, respectively (Table 3). No statistically significant differences in response rates were detected between the treatment groups ($P = 0.36$; Fisher's exact test). In the pimecrolimus group, 24% of the responsive cases were from the severe KCS group, 24% were from the moderate KCS group, and 52% were from the mild KCS group. In the CsA group, the respective numbers of responsive cases were 26%, 4% and 70%.

Improvement of clinical signs of inflammation

At baseline, the mean total scores for signs of corneal and conjunctival inflammation (nine signs evaluated;

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Table 1
Patient signalment (study animals)

	Pimecrolimus 1% ($n = 20$)	CsA 0.2% ($n = 24$)	Total ($n = 44$)	P value
Female	10 (50%)	14 (58%)	24 (55%)	0.762
Male	10 (50%)	10 (42%)	20 (45%)	
Neutered				
Yes	5 (25%)	4 (17%)	9 (20%)	0.711
No	15 (75%)	19 (79%)	34 (77%)	
Unknown	0 (0%)	1 (4%)	1 (2%)	
Breed of dog				
American Cocker Spaniel	2 (10%)	1 (4%)	3 (7%)	0.689
Boxer	1 (5%)	0 (0%)	1 (2%)	
Bullterrier	0 (0%)	1 (4%)	1 (2%)	
Cavalier King Charles	0 (0%)	2 (8%)	2 (5%)	
Spaniel	0 (0%)	1 (4%)	1 (2%)	
Japan Chin	0 (0%)	2 (8%)	2 (5%)	
Dachshund	2 (10%)	2 (8%)	4 (9%)	
English Cocker Spaniel	0 (0%)	1 (4%)	1 (2%)	
Maltese	0 (0%)	1 (4%)	1 (2%)	
Miniature Poodle	2 (10%)	3 (13%)	5 (11%)	
Mixed Breed	1 (5%)	1 (4%)	2 (5%)	
Pekingese	1 (5%)	0 (0%)	1 (2%)	
Sealyham Terrier	3 (15%)	4 (17%)	7 (16%)	
Shi Tzu	1 (5%)	0 (0%)	1 (2%)	
Small Muensterlaender	0 (0%)	1 (4%)	1 (2%)	
Terrier cross	6 (30%)	2 (8%)	8 (18%)	
West Highland White Terrier	1 (5%)	2 (8%)	3 (7%)	
Yorkshire Terrier				
Age (years)				
<2	0 (0%)	0 (0%)	0 (0%)	0.547
2–4	3 (15%)	7 (29%)	10 (23%)	
5–6	7 (35%)	5 (21%)	12 (27%)	
7–8	3 (15%)	4 (17%)	7 (16%)	
9–10	2 (10%)	5 (21%)	7 (16%)	
11–12	3 (15%)	0 (0%)	3 (7%)	
>12	2 (10%)	3 (13%)	5 (11%)	
Mean age	7.5	6.9	7.2	
SD of age	3.0	3.3	3.2	
Median age	6.8	6.5	6.8	
Minimum age	2.0	2.0	2.0	
Maximum age	13.0	13.0	13.0	

CsA, 0.2% cyclosporine A ointment.

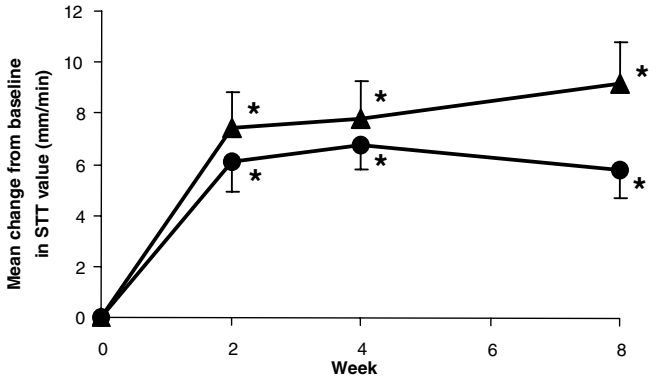

Fig. 1. Effects of 1% pimecrolimus eye drops (▲) and 0.2% cyclosporine A (CsA) ointment (●) on tear production in dogs with keratoconjunctivitis sicca (KCS). Each point represents the mean ± SEM of 20 and 24 eyes, respectively. (*) Statistically significant difference from baseline ($P < 0.001$; paired t test).

maximum total score = 36) in the pimecrolimus and the CsA groups were 16.0 ± 1.1 ($n = 20$) and 13.9 ± 1.4 ($n = 24$), respectively. Statistically significant improvements from baseline were observed within both groups ($P < 0.001$) at all follow-up visits (Fig. 2). In the pimecrolimus group, mean decreases of 5.0 ± 0.4, 7.9 ± 0.6 and 10.3 ± 0.8 were observed at weeks 2, 4 and 8, respectively. During the same time period, mean decreases of 4.7 ± 0.8, 6.5 ± 1.0 and 7.6 ± 1.2 were seen in the CsA group.

Though the reduction in clinical scores was higher in the pimecrolimus group, in rechecks conducted at 2 and 4 weeks there was no significant difference in the overall improvement of clinical signs between the groups ($P = 0.39$ and $P = 0.07$, respectively). However, after 8 weeks of treatment, there was a significantly larger reduction in the total score for signs of inflammation in dogs treated with pimecrolimus as compared to dogs treated

Table 2
Effect of treatment on STT

Initial KCS severity	Treatment group	Mean change from baseline (mm/min)				
		n	Baseline	Week 2	Week 4	Week 8
Severe (STT = 0–2 mm/min)	Pimecrolimus	10	0.9 ± 0.3	8.2 ± 2.4*	9.5 ± 2.6*	10.3 ± 2.6*
	CsA	10	1.2 ± 0.3	6.4 ± 2.3*	7.0 ± 2.0*	7.3 ± 2.2*
Moderate (STT = 3–6 mm/min)	Pimecrolimus	5	5.0 ± 0.6	5.2 ± 1.7*	4.2 ± 2.3	8.0 ± 4.0
	CsA	4	4.5 ± 0.7	2.3 ± 0.9	3.0 ± 1.2	1.3 ± 0.8
Mild (STT ⩾ 7 mm/min)	Pimecrolimus	5	8.2 ± 0.4	7.8 ± 2.1*	8.0 ± 1.7*	8.2 ± 1.7*
	CsA	10	8.0 ± 0.4	7.4 ± 1.5*	8.0 ± 1.2*	6.3 ± 1.3*

Effects of 1% pimecrolimus eye drops and 0.2% cyclosporine A (CsA) ophthalmic ointment on tear production in subgroups of dogs with keratoconjunctivitis sicca (KCS) of different severity based on Schirmer tear test (STT) values at baseline. Each point represents the mean ± SEM of *n* eyes.
* $P < 0.05$ compared to baseline (paired *t* test).

Table 3
Proportion of responders to treatment

Treatment group	Responders (%)		
	Week 2	Week 4	Week 8
Pimecrolimus	65%	60%	63%
Cyclosporine A (CsA)	48%	54%	48%

Response to treatment defined as an eye achieving a Schirmer tear test (STT) value >10 mm/min.

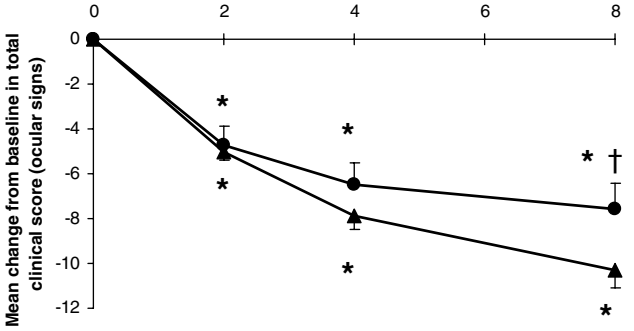

Fig. 2. Effects of 1% pimecrolimus eye drops (▲) and 0.2% cyclosporine A (CsA) ointment (●) on total clinical score for signs of ocular surface inflammation in dogs with keratoconjunctivitis sicca (KCS). Each point represents the mean ± SEM of 20 and 24 eyes, respectively. A negative value indicates an improvement in ocular signs. (*) Statistically significant difference from baseline ($P < 0.001$; paired *t* test). (†) Statistically significant difference compared to pimecrolimus ($P < 0.05$; Wilcoxon rank sum test).

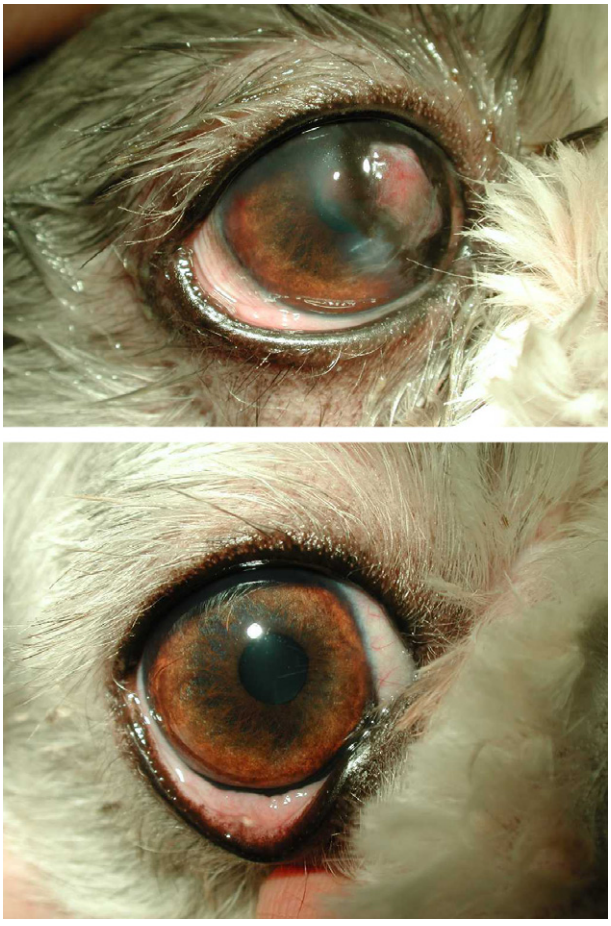

Fig. 3. Right eye of a 4.5 year old male Shi Tzu treated with pimecrolimus. Top: At presentation. Schirmer tear test (STT) = 3, clinical score = 20. Bottom: After 8 weeks treatment with pimecrolimus. STT = 9, clinical score = 8.

with CsA ($P = 0.02$). Fig. 3 illustrates the efficacy of pimecrolimus in alleviating clinical signs of KCS.

Analysis of the data by subgroup made on the basis of the initial KCS severity upon enrollment in the study showed that important improvements, albeit not statistically significant on some occasions, were observed in all subgroups after administration of pimecrolimus or CsA (Table 4).

Statistically significant improvements from baseline were seen in the majority of the individual ocular signs of inflammation within both treatment groups (Table 5). However, significant differences between the two treatment groups were noted only in one sign of inflammation. Dogs treated with pimecrolimus had a significantly larger reduction in conjunctival thickening than dogs in the CsA group after 4 weeks ($P = 0.003$) and 8 weeks ($P < 0.001$) of treatment.

Effect of treatment on corneal ulcers

Three dogs (one in the pimecrolimus group and two in the CsA group) entered the study with ulcers. Of these, two ulcers (CsA group) had completely resolved by week

Table 4
Effect of treatment on clinical signs (overall score)

Initial KCS severity	Treatment group	Mean change from baseline				
		n	Baseline	Week 2	Week 4	Week 8
Severe (STT = 0–2 mm/min)	Pimecrolimus	10	16.6 ± 1.8	–4.5 ± 0.5*	–7.7 ± 0.9*	–9.4 ± 1.6*
	CsA	10	15.1 ± 2.6	–5.1 ± 1.3*	–5.3 ± 1.8*	–6.9 ± 2.0*
Moderate (STT = 3–6 mm/min)	Pimecrolimus	5	17.2 ± 2.1	–6.4 ± 1.0*	–9.0 ± 0.9*	–12.0 ± 0.7*
	CsA	4	16.5 ± 3.9	–3.5 ± 0.9*	–7.5 ± 2.9	–9.0 ± 4.0
Mild (STT ⩾ 7 mm/min)	Pimecrolimus	5	13.4 ± 2.1	–4.6 ± 0.8*	–7.0 ± 0.4*	–10.2 ± 1.1*
	CsA	10	11.6 ± 1.8	–4.9 ± 1.4*	–7.2 ± 1.4*	–7.6 ± 1.7*

Effects of 1% pimecrolimus eye drops and 0.2% cyclosporine A (CsA) ophthalmic ointment on total score for signs of ocular surface inflammation in subgroups of dogs with keratoconjunctivitis sicca (KCS) of different severity based on Schirmer tear test (STT) values at baseline. Each point represents the mean ± SEM of *n* eyes. Negative values represent improvement in severity of 9 signs of inflammation, compared to the clinical score at enrollment (maximum possible score of 36).

* $P < 0.05$ compared to baseline (paired *t* test).

Table 5
Effect of treatment on clinical signs (individual scores of signs)

Ocular sign	Treatment group	Mean change from baseline		
		Week 2	Week 4	Week 8
Blepharospasm	Pimecrolimus	–1.2 ± 0.2*	–1.4 ± 0.2*	–1.5 ± 0.3*
	CsA	–0.8 ± 0.2*	–0.8 ± 0.2*	–0.9 ± 0.2*
Conjunctival hyperemia	Pimecrolimus	–0.7 ± 0.1*	–1.5 ± 0.2*	–1.8 ± 0.3*
	CsA	–0.7 ± 0.1*	–1.1 ± 0.2*	–1.3 ± 0.2*
Conjunctival pigmentation	Pimecrolimus	0.0 ± 0.1	−0.2 ± 0.1	–0.4 ± 0.1*
	CsA	0.0 ± 0.0	−0.1 ± 0.1	–0.2 ± 0.1*
Conjunctival thickening	Pimecrolimus	–0.8 ± 0.1*	–1.6 ± 0.2**	–2.1 ± 0.2**
	CsA	–0.5 ± 0.1*	–0.8 ± 0.2*	–1.0 ± 0.2*
Corneal edema	Pimecrolimus	–0.4 ± 0.1*	–0.4 ± 0.1*	–0.3 ± 0.1*
	CsA	−0.2 ± 0.1	−0.3 ± 0.2	–0.4 ± 0.2*
Corneal infiltrates	Pimecrolimus	–0.5 ± 0.1*	–0.8 ± 0.2*	–1.3 ± 0.2*
	CsA	–0.4 ± 0.1*	–0.7 ± 0.2*	–0.8 ± 0.2*
Corneal pigmentation	Pimecrolimus	−0.1 ± 0.3	–0.4 ± 0.5*	–0.5 ± 0.5*
	CsA	−0.2 ± 0.7	–0.4 ± 0.9*	–0.4 ± 0.9*
Corneal vascularization	Pimecrolimus	–0.4 ± 0.6*	–0.6 ± 0.7*	–0.9 ± 0.8*
	CsA	–0.5 ± 0.7*	–0.8 ± 1.0*	–1.0 ± 1.1*
Amount of discharge	Pimecrolimus	–1.1 ± 0.7*	–1.3 ± 0.8*	–1.4 ± 1.1*
	CsA	–1.3 ± 0.9*	–1.5 ± 0.8*	–1.5 ± 1.2*

Effects of 1% pimecrolimus eye drops and 0.2% cyclosporine A (CsA) ophthalmic ointment on scores of individual signs of ocular surface inflammation in dogs with KCS. Each point represents the mean ± SEM of 20 and 24 eyes, respectively. Negative values represent improvement in severity of each clinical sign, compared to the clinical score at enrollment (maximum possible score of 4).

* $P < 0.05$ compared to baseline (paired *t* test).

** $P < 0.01$ compared to CsA (Wilcoxon rank sum test).

2. The third dog (treated with pimecrolimus) presented with a deeper ulcer which resolved by week 8.

Adverse events

Transient irritation following treatment was noted in six dogs treated with CsA and in three treated with pimecrolimus. Two dogs (one in each group) experienced "excessive lacrimation" attributed to the treatment. Corneal lipidosis was noted in one CsA-treated eye.

Discussion

The results demonstrate that 1% pimecrolimus is highly effective in alleviating the clinical signs of KCS in dogs with regard to both tear secretion and ocular surface inflammation, fully confirming the observations of a preceding exploratory study (Nell et al., 2005). In particular, a significant increase in tear secretion, as well as a significant decrease in clinical signs of inflammation, was already observed at the first recheck after two weeks of treatment. In addition, the response to pimecrolimus was as good in dogs with low initial STT values (0–2 mm/min) as in animals with less severe KCS.

Pimecrolimus showed superiority over CsA in the control of clinical signs of conjunctival and corneal inflammation in canine KCS after 8 weeks of treatment ($P = 0.02$). Approximately two-thirds of the dogs treated with pimecrolimus and half the dogs treated with CsA attained STT values higher than 10 mm/min ($P = 0.36$). In the present study, the clinical efficacy of 0.2% cyclosporine ophthalmic ointment in controlling the clinical signs of KCS

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in dogs was similar to that previously described (Sansom et al., 1995). However, unlike previous observations, the response to CsA was independent of initial KCS severity. The better efficacy of pimecrolimus may result from the higher drug concentration used in this study, a higher intrinsic potency, a better penetration of the compound into injured ocular tissues or a combination of these factors.

These observations were not unexpected because pimecrolimus and CsA have in common the same molecular mechanism of action, namely inhibition of calcineurin. At the molecular level, pimecrolimus binds to the cytoplasmic receptor macrophilin 12 and inhibits calcineurin, an enzyme required for the dephosphorylation of the cytosolic form of the nuclear factor of activated T cells. As a consequence, it prevents the transcription and release of both T helper type 1 cell (Th1) and T helper type 2 cell (Th2) inflammatory cytokines as well as T cell proliferation (Grassberger et al., 1999). Inhibition of co-stimulatory molecules and expression of interleukin-2 receptor in T cells may contribute to impaired activation and proliferation of T cells (Kalthoff et al., 2002). In vivo, pimecrolimus has been shown to inhibit T cell mediated allergic contact dermatitis in rodents and domestic pigs (Meingassner et al., 1997). These findings have led to the development of the drug for treatment of immune-mediated skin diseases such as atopic dermatitis and psoriasis (Rappersberger et al., 2002; Eichenfield and Beck, 2003; Wolff and Stuetz, 2004).

Conclusions

The results of the present study suggest that the efficacy of pimecrolimus is not limited to dermatological diseases. Our findings confirm the interest to develop pimecrolimus as a promising new therapy for the topical treatment of dry eye in dogs and humans. Additional investigations to determine the minimally effective dose in particular should be considered.

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