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Efficacy of a new topical cyclosporine A formulation in the treatment of atopic dermatitis in dogs

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ABSTRACT

Topical treatment with cyclosporine A (CsA) has recently become possible with the development of novel nanotechnology pharmaceutical formulations of CsA able to penetrate through the epidermis providing good absorption and dermal action. The aim of this multicentre, blinded, parallel, randomized, placebo controlled trial was to evaluate the efficacy of a new topical CsA formulation in dogs with atopic dermatitis (AD). Dogs ($n = 32$) with severe and moderate clinical signs of non-seasonal AD, but few localized lesions, were randomly allocated to receive topical CsA (17 dogs) or placebo (15 dogs) and were treated twice a day for 6 weeks. Before and 21 and 45 days after starting the treatment, the severity of a previously selected skin lesion was evaluated according to a dermatological scoring system. Owners using a visual analogue scale also assessed pruritus weekly and effectiveness of the treatment was defined as a reduction of at least 50% in these variables after 45 days.

After 21 and 45 days the lesion severity score in animals treated with CsA was significantly lower than at baseline ($P < 0.01$, both times). In contrast, the animals on placebo showed no significant improvement at days 21 or 45. The percentage of dogs with an effective reduction in pruritus at the end of the trial was 87.5% and 28.6% in the CsA and placebo groups, respectively. These results suggest that topical administration of CsA is effective in reducing the severity of skin lesions and pruritus in dogs with moderate to severe AD as soon as 3 weeks after starting treatment.

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Introduction

The prevalence of atopic dermatitis (AD), the second most common form of allergic dermatitis in dogs, has risen in recent decades, and about 10% of dogs aged from 1 to 3 years are now thought to be affected with the condition (Bensignor, 2010). The pathogenesis of AD has not been fully elucidated and the treatment options available are limited. Although allergen-specific immunotherapy remains the treatment of first choice in some cases because it is the safest way to modulate the immune response (Olivry et al., 2010a), alternative treatments with immunosuppressive agents are also available and offer advantages, such as easy dosing protocols and faster response rates (Nuttall et al., 2009, 2012; Deboer

et al., 2002). For this reason, immunosuppressant drugs such as glucocorticoids or calcineurin inhibitors are also now considered well-established treatments for AD.

Cyclosporine A (CsA) was the first immunosuppressant found to act selectively on T cells (White et al., 1979). CsA forms a complex with cyclophilin, an intracellular immunophilin, and inhibits the activity of calcineurin phosphatase so depleting lymphocytes and macrophages and inhibiting the activation of T cells, natural killer cells, and antigen-presenting cells (Gupta et al., 1989; Stepkowski, 2000; Giese et al., 2004; Ferraccioli et al., 2005). CsA also inhibits keratinocyte proliferation (Won et al., 1994; Gottlieb et al., 1995), inhibits the release of histamine from mast cells (Amon, 1992; Sperr et al., 1996; García et al., 1998), and down-regulates the expression of cellular adhesion molecules on dermal capillary endothelium (Mrowietz and Ruzicka, 1999).

The potent immunosuppressive activity of oral CsA is responsible for its efficacy but also for toxic effects, which are

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dose-dependent and related to the duration of therapy but reversible. However, structural renal abnormalities and bacterial infections may persist (Radowicz and Power, 2005). Therefore, when a condition is chronic and CsA must be used long term, the potential risk of toxicity increases (Ring et al., 2008; Heinrich et al., 2011).

CsA was approved by the United States Food and Drug Administration for the treatment of psoriasis in humans in 1997. In Europe, CsA is the only immunosuppressant drug approved for the short-term treatment of severe human AD that cannot be controlled with topical agents (Busmann et al., 2009). In dogs, oral CsA has been widely used to control AD (Olivry et al., 2002a), but adverse effects have been reported in up to 81% of treated dogs (Nuttall et al., 2012). Another problem of CsA is its high cost, especially in large-breed dogs, so its use when there are only localized lesions is questionable. Although topical administration of CsA would seem to offer a more rational use of this drug, such use has not been feasible to date because only a lipophobic conformation has been available (CsA log P = 2.92).

Nanotechnology, a field devoted to the manipulation of matter at a scale <1 μ M (i.e. at the level of atoms and molecules), has enabled the development of a lipophilic CsA emulsion. We prepared nanocapsules of chitosan, a mucoadhesive compound chosen to protect CsA from degradation and create a film on the skin surface to enhance penetration. These nanocapsules were incorporated into an oil-in-water emulsion (CsA concentration, 2.5%) for use in the present study; the excipients were poloxamer 188, castor oil and soy lecithin. This CsA nanocapsule preparation was designed to be sprayed on the skin, where it would quickly penetrate the epidermis and act within the dermis. Preliminary pharmacokinetic studies performed in our laboratory confirmed that the CsA nanocapsules accumulate in the skin without reaching the bloodstream, thus avoiding systemic immunosuppression, gastrointestinal disorders and other adverse effects (unpublished results). The aim of this pilot trial was to evaluate the efficacy of this formulation in the treatment of localized AD in 17 non-seasonal atopic dogs.

Materials and methods

Study design and subjects

This 45-day, double-blinded multicentre, multi-investigator, randomized placebo-controlled parallel-group (1:1) trial was reviewed and approved by the Spanish Agency for Medicines and Health Care Products (AEMPS) (protocol number 233/ECV).

The trial was carried out in two veterinary teaching hospitals and four private dermatology clinics in Spain. Thirty-two dogs were enrolled: 15 received placebo and 17 the active treatment. Inclusion criteria were a clinical diagnosis of non-seasonal AD according to the criteria of Willemse (1986) and exclusion of other allergic dermatoses based on absence of response to treatment for flea infestation (at least 8 weeks) and to an elimination diet (at least 6 weeks) before the start of the trial. Dogs were older than 12 months of age and presented with AD lesions on the front or hind limbs, including axilla or groin. Exclusion criteria included pregnancy or lactation, seasonal signs of AD involving flea infestation or food allergens, presence of bacterial or *Malassezia* skin infections or other infestations causing pruritus (e.g. mites), and being treated with one or more of the following: topical glucocorticoids, oral antihistamines or fatty acids within 2 weeks of enrolment, oral glucocorticoids, oral CsA or allergen-specific immunotherapy within 4 weeks of enrolment and injectable glucocorticoids within 8 weeks of enrolment. Concomitant treatments that could have an effect on AD and that would affect the assessment of the effectiveness of the products under investigation (topical CsA or placebo) were not allowed during the trial.

Blinding was guaranteed using identical emulsion spray containers and formulations with similar appearance for CsA and placebo. Each product was coded (labelled T02 and T01, respectively) to ensure that the researchers and staff involved in the evaluations would work under the same blinded conditions as the owners. Allocation to one of the two treatments was based on a random number generation system. The treatment codes were available to the trial's sponsor and the monitor but could only be consulted in cases of emergency. Such consultation never became necessary.

Study procedures and data collection

The animals underwent physical examination and if it was established that they met the criteria for inclusion, the investigator recorded weight, age, breed, sex and other relevant data. A complete dermatological examination was carried out by a veterinarian and the areas affected by AD were recorded. The intensity of pruritus was assessed on a visual analogue scale (VAS) comprising a 10 cm long line (oriented horizontally) on which owners indicated the intensity of pruritus by crossing the line at the point that corresponded to the severity of the dog's pruritus. Descriptors were indicated at the ends (0 and 10 cm), but the line was not graded to facilitate a more realistic measure of the symptom that would not be influenced by previous measurements. Assessments were converted to a number and noted.

The researcher evaluated and recorded baseline severity levels of erythema, lichenification, and excoriations for the lesional area to be treated. These clinical signs were evaluated on a scale of 0–3 points (Table 1) for a maximum of 9 points per lesion and patient. At this time one lesion scored ≥ 6 was selected for later assessment of outcome and its location, surface area (cm²), and severity score were recorded. The allocated product was then applied by a member of the clinic's staff to limb, axilla and/or groin lesions; this application served to show the owner how to use the spray device, which delivered 0.8 mL with each spray. The affected areas were sprayed once or twice, depending on the size of the lesion; the nozzle was held at a distance of approximately 20 cm from the skin. Afterwards at home, the owner applied the treatment twice a day (approximately every 12 h) for a period of 45 ± 2 days. Owners were told to wear gloves when spraying the product and to distract the dog for a few minutes afterwards until the product was completely absorbed. Baths were not permitted 3 h before or 3 h after treatment.

Each owner was given a form to record the dose applied (one or two sprays) to be completed every day and to show to the researcher at every visit in order to evaluate compliance. For animal welfare reasons, other lesions on the animal could be treated even though they were not included in the outcome evaluation.

Outcome measures, endpoints, and withdrawals

Effectiveness of treatment was assessed 21 and 45 days (endpoint) after the baseline treatment. As at baseline, the researcher evaluated the severity of erythema, lichenification, and excoriations on a scale of 0–3 points. The owner also estimated the severity of pruritus on the VAS at each visit and a score (0–10) was noted. For purposes of the intention-to-treat analysis (including all initially enrolled dogs), animals withdrawn early for reasons of severe pruritus were assigned the same VAS score the owner reported at the time the animal left the study.

Animals with a higher lesion severity score on day 21 than at baseline were removed from the trial and the treatment was considered to have failed. A higher lesion score on day 45 (endpoint) than at baseline also indicated therapeutic failure. Once failure had been recorded at either visit, the animal could be treated with what the researcher considered appropriate. Animals could also be withdrawn for other reasons, such as use of prescribed concomitant treatments, concomitant disease that could interfere with the assessment of CsA efficacy (e.g., bacterial pyoderma, *Malassezia* dermatitis, etc.), or the owner's decision to discontinue participation.

Efficacy within groups was defined by the rates of lesion improvement from baseline according to the dermatological scoring system for lesion severity or by associated pruritus on days 21 and 45 for each treatment group. The mean lesion severity scores on days 21 and 45 were then compared between treatments. For pruritus control, efficacy was defined as a reduction from baseline in the VAS score of $\geq 50\%$ on days 21 and 45; the percentages of animals meeting that target were compared between groups.

For efficacy comparisons, data for the enrolled dogs were divided into two groups for analysis per treatment protocol and per intention to treat. The per-protocol analysis included data for all animals reaching the end of the treatment protocol (45 days); this dataset comprised 23 cases (14 in the CsA group and 9 in the placebo group). The intention-to-treat analysis was based on all 32 cases (17 in the CsA group and 15 in the placebo group). The per-protocol analysis included only dogs whose owners adhered to the protocol and continued in the trial.

Statistical analysis

The statistical analysis was performed using SAS software (v.9.1, SAS Institute). Results are expressed as group means ($\pm$ standard deviations). Statistical significance was set at a level of $P \leq 0.05$. The between-group comparisons of quantitative response variables were performed with ANOVA and the Mann-Whitney-Wilcoxon and Kruskal-Wallis tests. Compliance with conditions for applying these tests was checked with the Shapiro-Wilk and Kolmogorov-Smirnov normality tests and the Levene test for homogeneity of variances. Within-group comparisons of quantitative response variables were accomplished with ANOVA for paired measures and the Wilcoxon test for paired measures. Compliance with conditions for these tests was also checked with the Shapiro-Wilk and Kolmogorov-Smirnov normality tests.

Table 1
Scoring of dermatological lesions.

<i>Erythema</i>	
0	Absence
1	Presence of a few red macules of low intensity
2	Presence of several red macules of low intensity over a wider area or a few stains of higher intensity
3	Large areas of intense reddish colour
<i>Excoriation</i>	
0	Absence, normal skin colour
1	Presence of erosion with or without haemorrhagic crust on the surface, measuring <0.5 cm in diameter
2	Several erosions with or without haemorrhagic crusts on the surface measuring <0.5 cm in diameter
3	One or more erosions with or without haemorrhagic crusts on the surface measuring >0.5 cm in diameter
<i>Lichenification</i>	
0	Absence
1	Presence of increased thickness of the epidermis with barely visible erythema
2	Presence of increased thickness of the epidermis with noticeable erythema
3	Presence of increased thickness of the epidermis with marked erythema and hyperpigmentation

Results

Randomization

Randomization was effective in dividing the population into two similar groups (Table 2).

Withdrawals

Six of the 15 dogs treated with placebo (40%) had left the trial before day 45, whereas only 3/17 treated with CsA (17.6%) were withdrawn. Thus, only 9/15 (60%) of placebo-treated dogs completed the trial whereas 14/17 CsA-treated dogs (82.4%) continued treatment until the 45-day visit. Of the three CsA-treated animals removed from the study, one was withdrawn by the owner (unexplained decision) and two were withdrawn due to therapeutic failure. Of the six placebo-treated dogs removed early, four were withdrawn for severely increased pruritus and two because of the owner's unexplained decision.

Lesion severity scores

In the per-protocol analysis, animals on topical CsA treatment had significantly lower lesion severity scores at days 21 (4.6 ± 1.9) and 45 (2.8 ± 2.9) compared to baseline (6.9 ± 1.2) ($P = 0.0020$ and $P = 0.0020$, respectively); the difference between days 21 and 45 was also significant ($P = 0.0020$). In contrast, in the placebo group, scores were similar at baseline (7.2 ± 1.1), 21 days (5.8 ± 2.7) and 45 days (5.2 ± 3.1). In the comparison between groups, at day 45 the scores tended to be lower in topical CsA-treated dogs (2.8 ± 2.9) than in placebo-treated dogs (5.2 ± 3.1), although the difference was not statistically significant given the small number completing this pilot trial ($P = 0.0748$). Fig. 1 shows changes in a topical CsA-treated lesion between baseline and after 21 and 45 days.

Table 2
Dog characteristics.

	Cyclosporine group	Placebo group	P
Number of pure-breed dogs	15 (88.2%)	14 (93.3%)	0.621
Sex distribution			
Male	6 (35.3%)	7 (46.7%)	0.513
Female	11 (64.7%)	8 (53.3%)	
Age at study onset (years)	5.3 ± 3.0	4.4 ± 3.0	0.388
Weight at study onset (kg)	13.7 ± 9.5	17.0 ± 15.1	0.489
Lesion score (day 0)	6.7 ± 1.1	7.1 ± 1.0	0.262
Pruritus score (day 0)	7.0 ± 1.5	6.5 ± 1.4	0.418

Data are number (%) or mean $\pm$ standard deviation (SD).

The results of the intention-to-treat analysis of data for all animals in this trial were similar to those of the per-protocol analysis. Thus, the within-group analysis revealed significant decreases in the lesion scores of animals treated with topical CsA between baseline and days 21 and 45 ($P = 0.0024$ and $P = 0.0015$, respectively); however, the comparison between treatments on day 45, showed no difference between the groups ($P = 0.0894$). The placebo-treated dogs were similar at baseline and days 21 and 45 (Fig. 2).

Pruritus

In the per-protocol comparison of pruritus scores (VAS), dogs in the CsA-treated group experienced a significant improvement over the course of the full study period, with significant reductions in pruritus intensity on day 21 (5.6 ± 1.7) compared with baseline (7.1 ± 1.3) ($P = 0.0134$) and on day 45 (3.5 ± 2.6) compared with day 21 ($P = 0.0034$) or baseline ($P = 0.0012$). However, no significant reductions were seen in the placebo group ($P = 0.4258$, $P = 0.7344$ and $P = 0.4375$ for day 21 vs. day 0, day 45 vs. day 0 and day 45 vs. day 21, respectively). The intention-to-treat comparison (CsA group, mean VAS score of 3.4 ± 2.8 after 45 days of treatment vs. 5.0 ± 3.1 in the placebo group) gave similar results ($P = 0.2591$) (Fig. 3).

Neither per-protocol, nor intention-to-treat analysis revealed significant differences in intensity of pruritus between groups, even though the mean pruritus score in the placebo group was nearly double the score in the topical CsA group at day 45. However, on analysis of the effectiveness of topical CsA on pruritus, based on the percentages of dogs with $\geq 50\%$ reduction on the last visit, we did detect a significant effect between treatments ($P = 0.0072$): 64% of topical CsA-treated animals and 11% of placebo-treated animals achieved the targeted response according to per-protocol analysis (Table 3).

Tolerance to treatment

Safety assessment of topical CsA was conducted on all animals enrolled regardless of their level of fulfilment of the experimental protocol (intention-to-treat). Four adverse events (two in each group) occurred. None of the events was classified as serious. One of the topical CsA-treated animals developed mild bicipital tendonitis and the other had an interdigital verruca. Neither of these events was likely to have been related to topical CsA treatment. One placebo-treated dog was removed for vomiting and another placebo-treated dog developed irritation on the legs, the last being the only event that was possibly related to topical application of placebo.

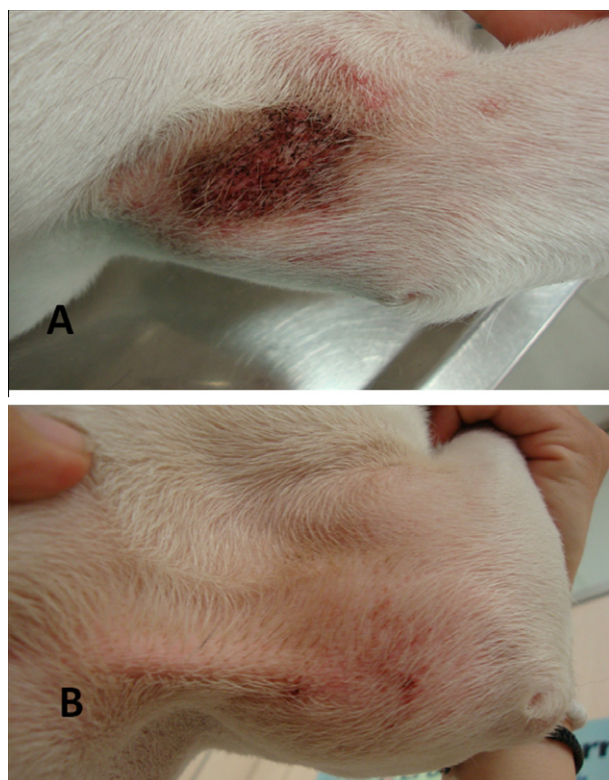


Fig. 1. One of the topical cyclosporine A-treated lesions (left groin) at baseline (A) and after 45 days of topical spray application (B).

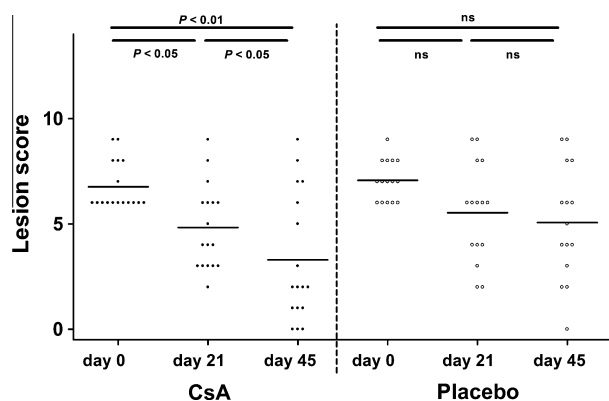


Fig. 2. Lesion severity score changes in topical cyclosporine A (CsA)- and placebo-treated dogs. *P* values are for the intention-to-treat analysis for all 32 dogs enrolled. ns, not significant.

Discussion

After 45 days of topical treatment with CsA, lesion severity and pruritus were significantly reduced in comparison with scores on baseline and on day 21. The most interesting result, however, was the rapid onset of effect, which was significant even after only 21 days of treatment, in both lesional and pruritus scores. In contrast, no significant improvement from baseline was observed after placebo treatment on day 21 or on day 45. Topical application of CsA was able to control the main clinical manifestations of AD (erythema, excoriation and lichenification).

The present pilot trial was designed on the basis of reports that oral CsA treatment led to slow improvement in AD, with onset of effect after 4–6 weeks of treatment (Steffan et al., 2006; Nuttall

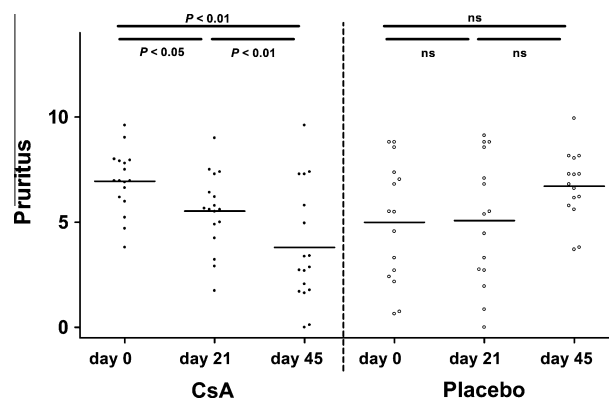


Fig. 3. Pruritus score changes reported by owners for topical cyclosporine A (CsA)- and placebo-treated dogs. *P* values are for the intention-to-treat analysis of all 32 dogs enrolled. ns, not significant.

Table 3

Pruritus and lesion score improvement on day 45 compared to day 0, in per-protocol (PP) and per intention-to-treat (ITT) populations.

Reduction	CsA <i>n</i> (%)			Placebo <i>n</i> (%)		
	>50%	≥90%		>50%	≥90%	
<i>ITT</i>						
Lesion score	11 (85%)	5 (38%)		4 (27%)	1 (6%)	
Pruritus	10 (85%)	2 (15%)		5 (33%)	1 (6%)	
<i>PP</i>						
Lesion score	10 (71%)	5 (36%)		1 (11%)	0	
Pruritus	9 (64%)	2 (14%)		1 (11%)	0	

The intention-to-treat (ITT) analysis included data for 32 dogs (CsA, 17; placebo, 15). The per-protocol (PP) analysis included 14 CsA-treated dogs and nine placebo-treated dogs.

et al., 2012). In our trial, however, an effect was evident by the first check-up after 21 days. Moreover, most owners of topical CsA-treated dogs reported good responses after only 2 weeks of treatment (results not recorded). A possible explanation for the rapid onset of effect would be the ability of this new CsA formulation to rapidly penetrate through the epidermis to the upper layers of the dermis. Consistent with this interpretation, our preliminary studies with tritium-labelled CsA have shown the presence of this compound within the epidermis and hair follicles within 24 h after lotion application (unpublished results).

Only animals with moderate to severe lesions (minimum score of 6) were included in this pilot trial to reproduce the clinical conditions for which this topical CsA spray is intended and also to allow us to quantify efficacy more easily. It was therefore interesting to see complete remission of AD signs in some animals despite the inclusion of severe cases. Of the 17 dogs that received topical CsA, three recovered fully, according to scoring of the lesion that had been prospectively designated in the protocol; thus, these three dogs all achieved a lesion severity score of 0 by day 45 and two of them also had a pruritus score of 0. These results are in marked contrast to those of a recent trial comparing oral CsA and hydrocortisone aceponate efficacy: none of the dogs in that trial achieved complete remission of clinical signs after 84 days of treatment (Nuttall et al., 2012).

An important advantage of topical CsA treatment is the absence of undesirable systemic effects. The sprayed drug does not reach the bloodstream and therefore cannot exert its immunosuppressive effects throughout the body. Preliminary studies performed in our laboratory showed no blood levels of CsA after 1 month of

topical treatment in six Beagle dogs (unpublished results). No serious adverse effects were reported for any of the animals in our trial, whereas trials of oral CsA have recorded one or more events in up to 81% of treated dogs (Olivry et al., 2002b; Steffan et al., 2003, 2005; Steffan et al., 2006; Diesel and Moriello, 2008; Olivry et al., 2010b; Nuttall et al., 2012). The side effects of oral CsA include mainly gastrointestinal disturbances, lethargy, gingival hyperplasia, squamous cell papillomas, hirsutism, and insulin resistance. Opportunistic infections have also been observed (Paul et al., 2010) and are probably complications of immunosuppressive CsA therapy. Usually the only way to partially resolve these problems has been through CsA dose reduction or discontinuation of treatment, although antibiotic therapy has helped in some cases (Olivry et al., 2002b; Radowicz and Power, 2005; Kovalik et al., 2011a,b).

As is strongly recommended for humans, oral immunosuppressant drugs should be reserved for short-term rescue treatment of AD only in appropriate patients and when topical formulations are not available (Amor et al., 2010; Ryan et al., 2010). Topical treatments are currently preferred to systemic ones for the localized lesions that characterize less severe clinical stages of hypersensitive skin disorders. They are also indicated to enhance the efficacy of systemic treatments by acting directly at the site of a lesion in selected cases (Olivry et al., 2010a).

Owners find topical treatment very acceptable: in a recent trial, no differences were reported between the scores for ease of administration of capsules or a spray formulation (Nuttall et al., 2012). Glucocorticoid creams and tacrolimus ointment are the topical therapies currently available for use in dogs with AD. Both are effective, although topical glucocorticoids are associated with adverse events, such as thinning of the skin (cutaneous atrophy), comedones and superficial follicular cysts (milia), and the relatively slow onset of the clinical benefit conferred by tacrolimus ointment suggests that this formulation is not suitable for treating the acute flares of canine AD (Olivry et al., 2010b).

Conclusions

The topical CsA formulation evaluated in this double-blinded randomized clinical trial effectively controlled pruritus and reduced moderate to severe AD lesion severity. Moreover, improvements in both outcome measures were observed after 21 days of topical CsA treatment, and by day 45 clinical signs had resolved completely in 3/17 CsA-treated dogs. Most importantly, no adverse events related to treatment were described in any of the dogs that received topical CsA. Therefore, the fast onset of effect of topical CsA, in comparison with oral CsA, together with the lack of side effects, encouraged owner compliance, which was high in the present study. Topical CsA seems to offer a promising and safe alternative to existing treatments for the management of AD in animals with few localized lesions.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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