

Cyclosporine decreases skin lesions and pruritus in dogs with atopic dermatitis: a blinded randomized prednisolone-controlled trial*

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Abstract During the last decade, oral cyclosporin (CsA) has proven to be effective, in randomized controlled trials, for the treatment of atopic dermatitis (AD) in human patients. The purpose of this blinded randomized controlled trial was to test the hypothesis that CsA was successful in reducing the gravity of clinical signs of AD in dogs. Thirty dogs with nonseasonal AD were randomly allocated to receive an oral solution of either NEORAL CsA (5 mg kg⁻¹) or prednisolone (0.5 mg kg⁻¹) once daily for 6 weeks. Before, and 3 and 6 weeks after therapy, skin lesions were graded by clinicians using the Canine AD Extent and Severity Index (CADESI). Pruritus was assessed by the owners using a visual analog scale (PVAS). In both groups, CADESI and PVAS values were significantly lower at 6 weeks post treatment than before the initiation of therapy (Friedman test, $P < 0.0004$). The percentage reductions in CADESI and PVAS values from baseline were not statistically different between groups (Mann–Whitney test, $P > 0.3$). In this experiment, the tolerability and safety of oral CsA and prednisolone appeared similar. One-fifth of dogs given oral CsA occasionally developed diarrhoea or soft stools. One dog that was given CsA developed a generalized papillomatous skin eruption during the second half of the trial. Our study provides randomized controlled trial evidence that CsA reduces the severity of clinical signs in dogs with nonseasonal AD. Moreover, the anti-allergic efficacy of CsA appears comparable with that of prednisolone. We propose that oral CsA should be considered as a valuable alternative to glucocorticoid therapy in dogs with AD.

Keywords: allergy, atopic dermatitis, atopy, canine, ciclosporine, corticosteroids, cyclosporin A, cyclosporin, glucocorticoids, randomized controlled trial.

INTRODUCTION

Cyclosporin A (CsA, cyclosporin, ciclosporine) is a lipophilic macrolide cyclic oligopeptide that exhibits potent immunosuppressive properties stemming from its ability to block the transcription of cytokine genes in activated T cells.¹ Because of its unique mechanism of action as a gene repressor, CsA inhibits the activation of many different immune cells. For example, CsA prevents mast cell survival,² mast cell histamine release,^{3,4} prostaglandin production³ and cytokine secretion.^{5–8} In addition, CsA decreases eosinophil survival,^{9,10} release

of toxic granules,^{11,12} cytokine secretion¹³ and recruitment to the sites of allergic inflammation.¹⁴ The activation of epidermal antigen-presenting cells (i.e. Langerhans cell) also is decreased by CsA.^{15–18} Therefore, CsA demonstrates strong potential as an anti-allergic drug because it inhibits both the functions of cells that initiate the immune response (i.e. Langerhans cells and lymphocytes) as well as those of effector cells of the allergic reaction (i.e. mast cell and eosinophils).

The clinical benefit of oral CsA for the treatment of atopic dermatitis (AD) in humans, first documented in 1987,¹⁹ has now been confirmed in more than a dozen clinical trials (reviewed in Naeyaert *et al.*²⁰). A meta-analysis, comparing these studies using criteria of evidence-based medicine, confirmed that there was reasonable randomized-controlled trial evidence to support the use of oral CsA for treatment of human AD.²¹

In the dog, CsA also has been shown to suppress lymphocyte activation²² and mast cell histamine release.²³ These studies provided mechanistic data to support the evaluation of CsA for treatment of canine AD. Indeed, a recent study reported results of a small-sized open uncontrolled trial in which 14 dogs with AD were treated with 5 mg kg⁻¹ day⁻¹ of micro-emulsified CsA.²⁴ In all

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but one subject, pruritus and lesional scores decreased markedly during this trial. The median reductions from baseline of pruritus and lesional scores were 100 and 60%, respectively. Moreover, the dogs' owners perceived the efficacy of CsA to be good to excellent in 12/14 dogs (86%).

Our blinded randomized controlled trial (RCT) was designed to test the hypothesis that oral administration of CsA leads to a decrease in skin lesions and pruritus in dogs with nonseasonal AD. Considering the strong anti-allergic potency of CsA in humans and dogs, and because of the perceived unethical aspect of using placebo in a multiweek experiment, it was decided to control this trial with prednisolone. Indeed, oral glucocorticoids have been reported to be effective for the treatment of canine AD in prospective clinical trials^{25–27} or a case study.²⁸ Our study provides RCT evidence that, in dogs with AD, CsA does reduce the severity of clinical manifestations of canine AD, and that its degree of efficacy is similar to that of prednisolone. Hence, we propose that micro-emulsified CsA should be considered a valuable alternative to oral glucocorticoid therapy in the management of clinical signs of AD in dogs.

METHODS

Study design

This trial was designed as a prospective, double-blinded, multicentre, multi-investigator, randomized, controlled, parallel experiment of 6 weeks' duration. The length of this trial was selected from an unpublished pilot study performed at our institution. This protocol was reviewed and approved by the Institutional Animal Care and Use Committee at NC State University, Raleigh, North Carolina.

Study subjects

Sample size estimation. Fifteen subjects per treatment group were selected to enable a study with a 95% power to detect a 50% improvement from baseline in lesional scores after 6 weeks of therapy ($SD = 30\%$; $P = 0.05$) (INSTAT 2.01, GraphPad Software, San Diego, CA, USA). This improvement of 50% in lesional scores was estimated to be representative of a good clinical efficacy of the tested drug.

Inclusion criteria. After informed consent had been obtained from the owners, we enrolled 30 adult dogs with nonseasonal AD, defined as 'a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features that is associated most commonly with IgE antibodies to environmental allergens'.²⁹ In these dogs, the diagnosis of AD was made by fulfilment of three major and three minor clinical criteria, as proposed by Willemse.³⁰ Moreover, resembling pruritic diseases (e.g. adverse food reactions, sarcoptic acariasis and flea allergy dermatitis) were ruled out using standard diagnostic or therapeutic

methods. Finally, to be included in this study, dogs could not exhibit clinical signs suggestive of overt surface, superficial or deep microbial skin infection (i.e. bacterial pyoderma and *Malassezia* dermatitis) at the time of entry.

Most dogs were enrolled by investigators practising at a veterinary teaching hospital (NC State University, four investigators, 22 dogs). Other cases were seen at two private dermatology specialty practices (Animal Dermatology Clinic, Sacramento, CA; one investigator, seven dogs and Animal Allergy & Dermatology, Chesapeake, VA, one investigator, one dog).

Medications administered before the study. Anti-inflammatory drugs had to be discontinued for variable lengths of time, depending upon the drug category, so that they would not interfere with the evaluation of clinical signs during this RCT (e.g. 2 weeks for anti-histamines, 3 weeks for oral corticosteroids and 6 weeks for repository glucocorticoid formulations).

For this experiment, previous allergen-specific immunotherapy (ASIT) was allowed provided that the following criteria were met: (i) ASIT had been continued for at least 1 year; (ii) pruritus and skin lesions had failed to respond, even partially, to ASIT; and (iii) ASIT had been discontinued for a minimum of 3 months before the beginning of the study.

Medications allowed during the study. During this RCT, dogs could be maintained on antibiotics and fatty acid supplementation in the following situations: (i) if changes in antibiotics/fatty acids had not been made during the preceding month; (ii) antibiotic and fatty acid therapy had not resulted in $> 50\%$ improvement in skin lesions/pruritus at the time of enrolment; and (iii) changes in the antibiotic or fatty acid intake, either dietary or from supplement, would not be made during the trial. Even though it is theoretically possible that dogs given fatty acids prior to the trial would improve during the course of this study, we did not believe that this was a major confounding issue because of the lack of reliable evidence of efficacy of essential fatty acids for treatment of canine AD.³¹ Finally, application of nonmedicated shampoos and emollients/humectants was allowed, but at a frequency of no more than once a week.

Exclusion criteria. Signs of active cutaneous microbial infection during the course of the trial led to exclusion of the study subject. The use of anti-inflammatory drugs other than those allowed above also warranted immediate exclusion from this study.

Intervention

A pharmacist generated a computer randomization code of two groups of 15 dogs. The code was not known by the investigators, and could be broken only in cases of suspected drug events leading to the withdrawal of study subjects.

In the PRED group, dogs were administered prednisolone at the dosage of 0.5 mg kg^{-1} once a day orally

(prednisolone 21-hemisuccinate USP, in a solution with sodium salt and simple syrup). This prednisolone solution was prepared extemporaneously at the time of dispensing, and was expected to remain stable for at least 6 weeks. The dosage of prednisolone was selected from previously published trials in which it had been proven efficacious for treatment of canine AD.^{25,26}

In the CYCLO group, dogs were given micro-emulsified CsA at the dosage of 5 mg kg⁻¹ once a day orally (NEORAL oral solution 100 mg mL⁻¹, Novartis-Pharma, Basel, Switzerland). During this study, the dose of CsA was not adjusted from blood trough levels because of the reliable dose-dependent cyclosporin-mias achieved with this formulation (Novartis Animal Health: data on file). Furthermore, there are no studies demonstrating, in either humans or dogs, that improvement in the clinical signs of AD depends upon the achievement of specific trough levels. Both medications were given without food and not within 2 h of a meal, as per the manufacturer's recommendation.

Maintenance of blinding

To guarantee adequate blinding, both CsA and prednisolone solutions were made similar in regard to consistency and transparency, and were dispensed in identically labelled 50-mL stained glass bottles. At the time of prescription, neither clinicians nor clients knew of the randomization code and the identity of the bottle's content. Moreover, the prednisolone was formulated as a solution containing 10 mg mL⁻¹ to ensure that the same volume of solution was given to dogs in both groups (1 mL 20 kg⁻¹). Finally, to prevent the clinicians guessing the nature of the treatment based on known side effects, questioning of owners regarding adverse drug effects was made only after recording of lesional and pruritus scores (see below).

Outcome measures

Over the time course of the intervention, the primary end point variables were: (i) improvement or clearance of AD lesions, as measured by a reduction from baseline of clinician's assessed scores; and (ii) improvement or disappearance of pruritus, as determined by a decrease from baseline of owner's assessed scores. Both of these variables, establishing the amelioration of clinical signs and symptoms of AD, were considered relevant for the characterization of the treatment effect of the evaluated drugs. Moreover, the minimum clinically important difference in outcome measures was estimated to be an improvement of 50% in lesional or pruritus scores.

Other recorded variables were the assessment of drug tolerability and changes in blood count and serum chemistry panel parameters.

Assessment of lesional scores. To estimate the severity of the clinical signs of AD in the study subjects, clinician investigators used, at 3- and 6-week re-evaluation visits, a lesional scoring system modified from the original Canine Atopic Dermatitis Extent and Severity Index (CADESI).³² In this revised version of the

CADESI, the number of body sites to be evaluated was increased compared with the original template. The CADESI now consists of an assessment of the severity (0, none; 1, mild; 2, moderate; and 3, severe) of three cardinal signs of AD at 40 sites spanning the whole body surface. Clinical signs evaluated included erythema as a marker of acute skin inflammation, lichenification as a sign of chronic cutaneous inflammation and excoriations as an objective lesion of underlying pruritus. The maximum CADESI score achievable therefore was 360 (i.e. 3 × 3 × 40).

Preliminary analyses, performed at our institution, have allowed us to determine that this revised CADESI was associated with low variability for repeated assessments by the same clinician (average coefficient of variation: 9%; *n* = 10 dogs with AD; data not shown). This coefficient of variation was deemed acceptable to ensure intra-assessor reliability over time. Moreover, in unpublished clinical trials involving dogs with AD, a reduction of > 50% in the CADESI score was highly correlated with overall assessment of treatment efficacy by both owners and clinician (Novartis Animal Health: data on file). Altogether, these data suggest that the determination of CADESI values during treatment does represent a relevant and reliable assessment of clinical severity of canine AD.

Assessment of pruritus scores. At 3- and 6-week re-evaluation visits, owners indicated the perceived degree of pruritus present within the past 24 h, by pointing to a position on a Pruritus Visual Analog Scale (PVAS).³² This scale consisted of a 200-mm line, with 25 mm major and 5 mm minor graduations, labelled from 'not itchy' to 'extremely itchy'.

Assessment of tolerability scores. At each re-evaluation visit, adverse drug events were recorded and the owners were asked to estimate the tolerability of the drug administered on a 5-point VAS. This 200-mm line was labelled from 0 (poor tolerability, many side effects) to 5 (excellent tolerability, no side effects).

Blood count and chemistry panel. Before and after 6 weeks of treatment, blood was drawn to determine a complete blood count and serum chemistry panel. Values outside the normal range were assessed and compared for each dog before and after therapy, and between the two treatment groups at the end of the trial.

Analysis of data set

'Per protocol' data set. The per protocol (PP) set of animals comprises study subjects that received the medication for the entire duration of the trial and that reasonably complied with the various aspects of the protocol. Three dogs had originally been enrolled in the study but were later excluded because of perceived adverse drug effects or development of superficial pyoderma (see section on adverse effects); these dogs thus were excluded from this section. Data from 14 dogs in

the PRED group and 13 dogs in the CYCLO group remained for per protocol analysis.

'All randomized study animals' data set. To prevent over-estimation of treatment benefit because of the drop-out of study subjects, we included an 'intent-to-treat' analysis. We created an 'all randomized study animals' (ARSA) data set, in which we included all study subjects enrolled in the trial and that had received the medication for at least 1 week. Of the three dogs that did not complete this RCT, data from two of these dogs (one in the PRED group, no. 29, and one in the CYCLO group, no. 13) were thus added to the ARSA list, and the lesional and pruritus scores last observed were carried forward throughout the remainder of the study. We excluded from the ARSA series only the scores from dog no. 8, which had only received two doses of CsA and had been excluded from the study for potentially unrelated reactions. More details on these excluded cases are provided below (see section on adverse drug events).

Statistical analyses

Within each group, lesional (CADESI) and pruritus (PVAS) values recorded at baseline and at 3- and 6-week re-evaluation visits were compared using a two-sided nonparametric repeated-measures analysis of variance (ANOVA; Friedman's test) and Dunn's multiple comparison post-test. Comparisons of values between prednisolone and CsA groups were also determined with a two-tailed nonparametric Mann-Whitney *t*-test. Analyses of randomization data between groups were made with two-sided Fisher's exact tests or Mann-Whitney *t*-test. For all statistical analyses, the level of significance chosen was 5% ($P < 0.05$). All statistical comparisons were made using the computer software PRISM 3.0 (GraphPad Software). Whenever possible, all data were expressed as median (95% confidence intervals).

Role of the funding source

This study was funded in 1997 by a generous gift of 50 vials of NEORAL CsA as part of a material transfer agreement between NC State University and Sandoz (now Novartis)-Pharma division (Basel, Switzerland). The pharmaceutical company had no influence in the study design, collection of samples, analysis and interpretation of data, or in the publication of study results.

According to this agreement, the sponsoring company was provided with the manuscript 30 days prior to submission of the study for patent protection. The authors have no direct financial conflict of interest related to this study, even though the leading author (TO) began university-approved consultancy activities with Novartis Animal Health 1 year after the onset of this trial.

RESULTS

Verification of randomization effectiveness

To verify that the randomization of study subjects within the two treatment groups did not lead to treatment selection bias, multiple parameters of signalment and disease severity were compared (Table 1). We consider, *a posteriori*, that our computer randomization was homogeneous because there were no significant differences between treatment groups in regard to the following parameters: number of pure-bred dogs, sex distribution, age at study onset, weight at study onset, CADESI and PVAS at study onset and previous ASIT (Table 1).

Treatment effect on lesional scores

Per protocol data set. In dogs from PRED and CYCLO groups, CADESI values were significantly lower after 3 and 6 weeks of treatment than at baseline (Fig. 1) (see figure for level of significance).

All randomized study animals. Using this data set, CADESI values from dogs of PRED and CYCLO groups also decreased significantly during the 6 weeks of this trial ($P = 0.0004$ and < 0.0001 , respectively).

Treatment effect on pruritus scores

Per protocol data set. Dogs from both PRED and CYCLO groups exhibited significantly lower PVAS values at the end than at the beginning of the study (Fig. 2) ($P = 0.0002$ and 0.0003 , respectively).

All randomized study animals. Using this data set, PVAS values from dogs of PRED and CYCLO groups also declined markedly and significantly during the trial ($P = 0.0002$ and 0.0003 , respectively).

Parameter	Prednisolone group	Cyclosporine group	Test (<i>P</i> -value)
Number of pure-bred dogs	15	12	FET (0.23)
Sex distribution			
(M/MC)	3/2	1/7	FET (0.22)
(F/FS)	2/8	1/6	FET (1.00)
Age at study onset (years)	3 (3–5)	4 (3–5)	MWT (0.82)
Weight at study onset (kg)	21 (14–31)	30 (22–34)	MWT (0.29)
CADESI at study onset	19 (16–36)	19 (16–38)	MWT (0.77)
PVAS at study onset	150 (127–168)	130 (110–163)	MWT (0.42)
Previous immunotherapy	4	2	FET (0.67)

FET, Fischer's exact test; MWT, Mann-Whitney test. Values are reported as median (95% confidence interval).

Table 1. Verification of randomization effectiveness

Figure 1. Lesional scores (CADESI) decreased in dogs receiving either prednisolone (●) or cyclosporin (○) (per protocol data set; each dog is represented by one plot, and the bar denotes medians of each group at each time point).

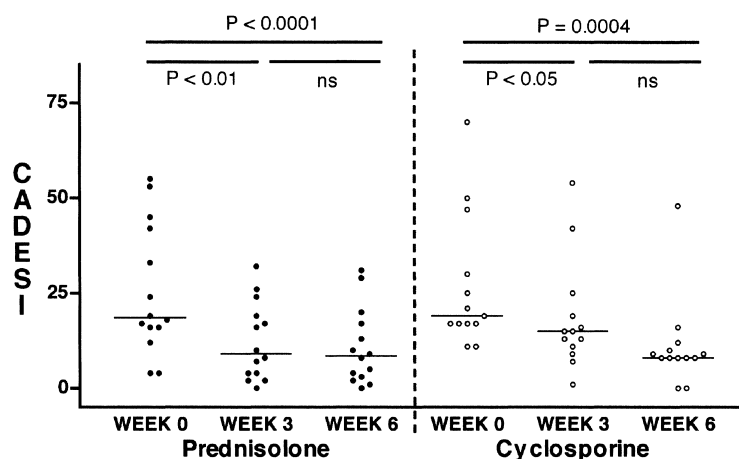


Figure 2. Pruritus scores (PVAS) declined in dogs given prednisolone (●) or cyclosporin (○) (per protocol data set; each dog is represented by one plot, and the bar denotes medians of each group at each time point).

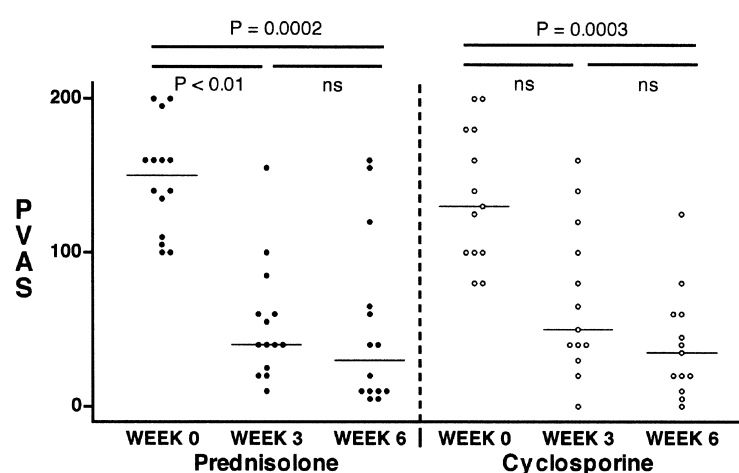


Table 2. Percentage reduction from baseline of lesional and pruritus scores (per protocol analysis)

Treatment group	% reduction CADESI	% reduction PVAS
Prednisolone	69 (30–84)*	81 (45–86)†
Cyclosporine	58 (43–74)*	78 (52–87)†

Median (95% confidence interval). Mann–Whitney test: * $P = 0.38$, † $P = 0.87$.

Percentage reduction of lesional and pruritus scores during treatment

Per protocol data set. During this trial, the percentage reduction from baseline of CADESI and PVAS values scores was almost identical between dogs allocated to PRED and CYCLO treatment groups. There was no significant difference between the two groups (Mann–Whitney test, $P = 0.38$ for CADESI; $P = 0.87$ for PVAS) (Table 2, Fig. 3).

In the PRED group, lesional and pruritus scores improved by $> 50\%$ in 12/14 (86%) and 10/14 dogs (71%), respectively. Similarly, in the CYCLO group, such degree of improvement ($> 50\%$) was seen in 9/13 (69%) and 10/13 (77%) of patients, respectively. Thus, both tested drugs yielded clinically important differences in outcome measures (i.e. were clinically effective) in $\approx 70\%$ or more of the study subjects.

All randomized study animals data set. Using this data set, percentage reductions from baseline of CADESI and PVAS values also were not significantly different between PRED and CYCLO treatment groups (Mann–Whitney test, $P > 0.44$ for CADESI; $P = 0.88$ for PVAS).

Adverse drug events

Tolerability scores. In subjects that completed this RCT, tolerability scores after 6 weeks were excellent (median value of 5 in each group). Statistical analysis did not reveal any significant differences between tolerability scores from both groups (Mann–Whitney test; $P = 0.76$).

Adverse drug events in subjects completing the trial. Adverse drug events were not reported, by owners or clinicians, in 8/14 (57%) dogs receiving prednisolone and 9/13 (69%) given cyclosporin.

In the PRED group, mild side effects were observed in six subjects, and consisted of polydipsia (two dogs), polyphagia (two), loose stools (one) and a behaviour change (one).

In the CYCLO group, mild side effects were reported in five subjects, and included diarrhoea/soft stools (three dogs), sleepiness (one) and a papillomatous cutaneous eruption that appeared during the second half of the

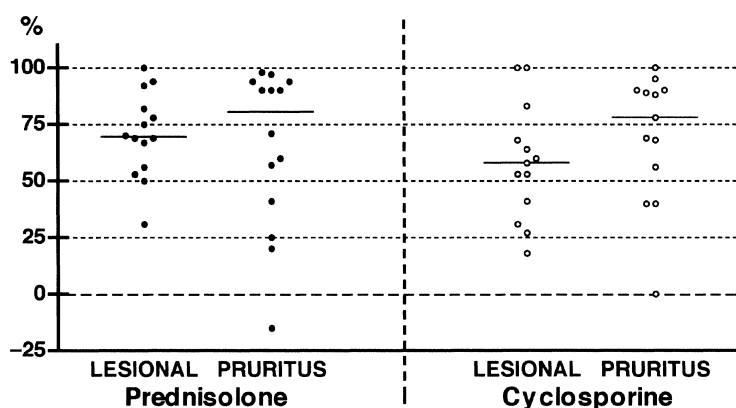


Figure 3. Percentage reduction from baseline of lesional (CADESI) and pruritus (PVAS) scores after 6 weeks of treatment with prednisolone or cyclosporin (per protocol data set; each dog is represented by one plot, and the bar denotes medians of each group at each time point).



Figure 4. Flat-topped depigmented verrucous lesions appeared, during the second half of the trial, on the skin of a subject (dog no. 8) given cyclosporin (area photographed is the back of one hock).

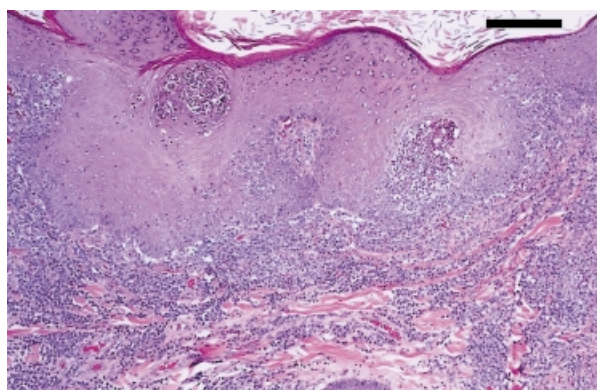


Figure 5. Histopathological examination of skin biopsy specimens from the verrucous lesions revealed a pustular, hyperplastic and mononuclear superficial dermatitis (dog no. 8; haematoxylin & eosin, bar = 180 μ m).

trial (dog no. 4; a 4-year-old male neutered Doberman). These lesions were described as 3–5 mm, whitish, slightly raised scaly verrucous plaques (Fig. 4). These plaques began to appear on the hocks and on the hind legs, and extended subsequently to elbows, axillae and along flank folds. Numerous lesions also were found around the eyes, trunk and convex aspect of the right ear pinna. All lesions underwent remission following

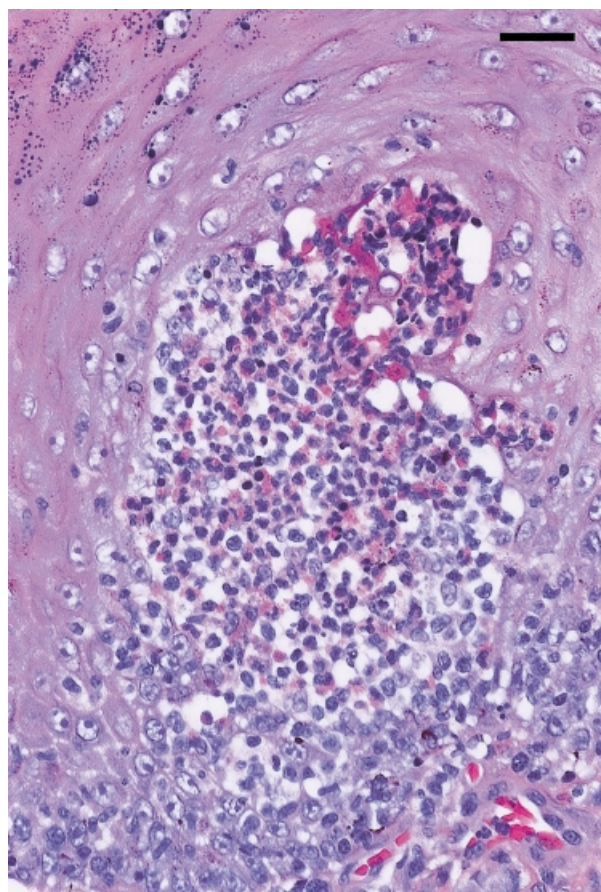


Figure 6. A suprabasal neutrophilic and eosinophilic pustule can be seen (higher magnification of Fig. 5) (dog no. 8; haematoxylin & eosin, bar = 30 μ m).

discontinuation of the drug and the administration of oral cephalexin at 23 mg kg⁻¹, twice daily for 30 days.

Histopathological examination of lesional skin biopsy specimens revealed a hyperplastic superficial diffuse dermatitis with intraepidermal pustule formation (Fig. 5). Epithelial microscopic lesions consisted of irregular epidermal hyperplasia with follicular hyperkeratosis, focal neutrophilic superficial luminal folliculitis and intraepidermal neutrophilic and eosinophilic microabscesses at various levels of the epidermis (Fig. 6) and hair follicle infundibulum wall. In addition, there was mild to moderate lymphocyte exocytosis interspersed

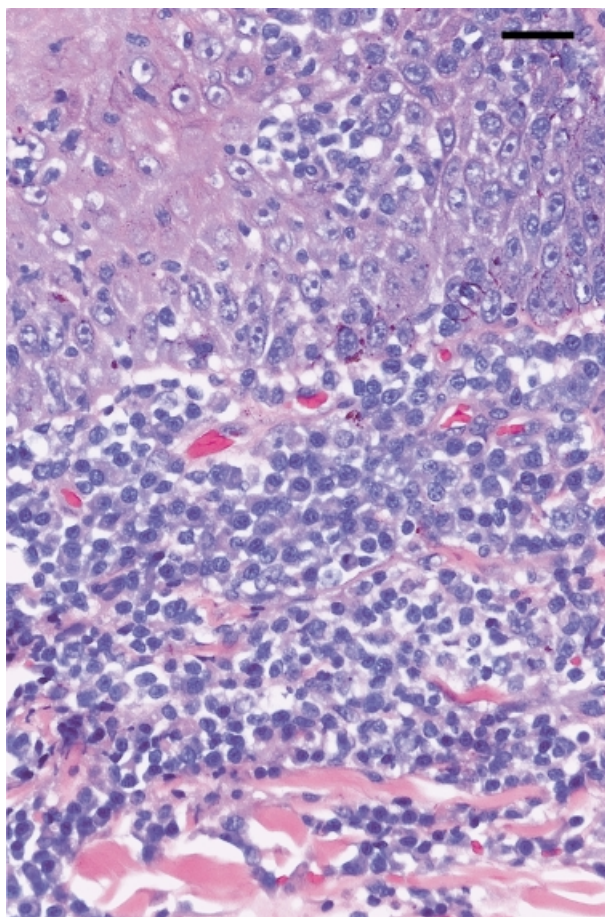


Figure 7. A lichenoid lymphocyte and plasma cell-rich infiltrate occupies the superficial dermis. Lymphocyte exocytosis to the lower epidermis also is present (higher magnification of Fig. 5) (dog no. 8; haematoxylin & eosin, bar = 30 µm).

between lower epidermal cells. An interface dermatitis *sensu stricto* was not seen. Dermal inflammation was superficial and often appeared as a lichenoid band of mononuclear cells composed primarily of plasma cells, lymphocytes, histiocytes or dendritic cells with rare eosinophils (Fig. 7).

Immunohistochemical staining, performed with a polyclonal antiserum specific for papillomavirus group specific antigen (AR087-5R; Biogenex, San Ramon, CA, USA), did not reveal any papillomavirus inclusions in the nuclei of epidermal or follicular keratinocytes.

Adverse drug events in subjects not completing the trial.

Three dogs did not complete this RCT. In the PRED group, one dog (no. 29) developed superficial pyoderma and was excluded from the study at the 3-week re-evaluation visit.

In the CYCLO group, two dogs were withdrawn from the trial. One dog (no. 8) exhibited urticaria, angioedema and diarrhoea after the second dose of CsA and the clinician elected to remove the dog from the study. In dog no. 13, an exacerbation of pre-existing regurgitations was observed during the first week of the study. These clinical signs prompted the clinician to withdraw this dog on the ninth day of the trial.

Changes in blood parameters

Treatment effect on complete blood count parameters.

In the PRED group, changes in blood count parameters were seen in 11/14 dogs (79%). These changes, which included neutrophilia (three dogs), lymphopenia (four) and eosinopenia (five), were consistent with the stress leukogram usually seen after glucocorticoid oral therapy.

Complete blood count values outside the normal ranges were rarely noticed in dogs of the CYCLO group. Mild leukopenia was found in two dogs, eosinophilia in two others and eosinopenia in three. Of note is that two of three dogs with eosinopenia after therapy exhibited eosinophilia at the trial's onset.

Treatment effect on plasma chemistry parameters.

In dogs of the PRED group, the changes most commonly observed at the end of the trial were elevation of the plasma activity of alanine aminotransferase (three dogs), alkaline phosphatases (five) and lipase (three) enzymes. Changes of ionogram, albumin and globulin concentrations were occasionally seen, however, most of these also were observed before treatment was initiated.

In the CYCLO group, abnormal values most commonly seen after treatment consisted of mild to moderate elevations of plasma total proteins (four dogs) and globulins (seven). In most cases, however, these changes were also observed before CsA administration.

DISCUSSION

Results of this RCT provide high-quality evidence that oral CsA reduces skin lesions and pruritus in dogs with AD (grade I of five; US Preventive Services Task Force Evidence Ratings).³³ Albeit encompassing a modest number of animals, this study supports the recommendation of oral CsA for treatment of canine AD, as proposed for human patients with this affection.²¹

During the course of this RCT, a rapid reduction in lesional and pruritus scores was observed within the first 3 weeks of CsA administration. A more modest improvement was seen during the second half of the study. In most dogs given CsA for this trial, the improvement in lesional scores was usually lower than that of pruritus scores. This finding is not surprising when considering that lichenification is one of the three cardinal signs of AD whose assessment of severity make up the CADESI scoring system. Indeed, this lesion, which reflects chronic inflammation and rubbing of the skin, is likely to take longer than 6 weeks to resolve and will probably not contribute to the reduction of CADESI values in a short clinical trial. Results of this RCT also confirm the magnitude of reduction of skin lesions and pruritus that was reported in the small open pilot experimental study of 14 dogs with AD.²⁴ Remarkably, the degree of improvement in lesional and pruritus scores seen during CsA monotherapy in our atopic dogs is in the same range as that reported for

human patients with AD treated with similar dosages ($5 \text{ mg kg}^{-1} \text{ day}^{-1}$) and for the same length of time (6 weeks).^{34–37} Taken together, these results confirm the remarkable anti-allergic effectiveness of this dosage of oral CsA for treatment of signs and symptoms exhibited by humans and dogs with AD.

It is of interest to practitioners to determine how the anti-allergic efficacy of oral CsA compares with that of drugs investigated previously in RCT for canine AD. In this study, we observed that the degree of score reduction in dogs given CsA was similar to that of subjects treated with prednisolone. Importantly, prednisolone is a drug that belongs to a class of glucocorticoids known to exhibit highest efficacy for treatment of clinical signs of canine AD^{25–27} and oral glucocorticoids presently constitute the 'standard-of-care' for the pharmacotherapy of canine AD.³⁸ Moreover, our observations that oral CsA provides 'good-to-excellent' (i.e. > 50% decrease from baseline) reduction of pruritus in $\approx 75\%$ of subjects establish that this drug is one of the most potent anti-allergic drugs available to treat dogs with AD. Indeed, other drugs investigated in RCT (e.g. clemastine, misoprostol and pentoxifylline) exhibit antipruritic effects that appear to be of much lower magnitude than those of CsA.^{25,39,40}

In this experiment, the tolerability and safety of oral CsA and prednisolone were comparable. Indeed, approximately two-thirds of subjects of either group did not exhibit any adverse drug event. Although some dogs receiving prednisolone were affected with side effects classically seen during glucocorticoid therapy, one-fifth of the dogs given oral CsA occasionally developed diarrhoea or soft stools. This adverse drug event is commonly seen in humans⁴¹ or dogs⁴² treated with cyclosporin therapy for cutaneous diseases. Unfortunately, the mechanisms underlying this common side effect remain unknown.

Of particular interest was the observation of generalized papillomatous eruption, during the second half of the trial, in a subject receiving CsA therapy. In dogs, this phenomenon has been reported previously in a 1-year high dosage ($45 \text{ mg kg}^{-1} \text{ day}^{-1}$) toxicological study,⁴³ in a 6-month high dosage ($30 \text{ mg kg}^{-1} \text{ day}^{-1}$) experiment,⁴⁵ in a 98-days mid-dosage ($10 \text{ mg kg}^{-1} \text{ day}^{-1}$) experiment⁴⁴ and in one case study in which dogs were given $15 \text{ mg kg}^{-1} \text{ day}^{-1}$ for a few months.⁴² In these cases of generalized verrucous dermatoses, including the one described here, skin lesions resolved within 2 months of discontinuation of the drug. In previous reports, the histopathology of these papillomatous lesions was described as an abrupt change from normal skin, with epidermal hyperplasia, moderate to marked orthokeratotic hyperkeratosis with focal to extensive parakeratosis, subcorneal pustules and a band of lymphocytes, plasma cells and fewer inflammatory cells of other types.^{43,45} In the case study, the lymphoplasmacytoid superficial dermal infiltrate was characterized as 'exhibiting malignant features' because some cells had increased mitotic activity and occasional bizarre mitotic figures were seen.⁴² In the case described

here, the macroscopic and microscopic findings closely resemble those seen in Springer Spaniel dogs with psoriasiform lichenoid dermatosis,⁴⁶ a benign verrucous skin disease. This resemblance needs to be considered in future observations of this adverse drug event. In none of the reported cases, including our single observation, were viral inclusions or cytopathic effects detected within epidermal keratinocytes or dermal cells.^{43–45}

The mechanism of lesion formation of this cutaneous papillomatosis during CsA therapy remains elusive. One of the hypotheses is infection with a novel canine papillomavirus of low pathogenicity. However, all attempts to uncover papillomavirus using electron microscopy, immunohistochemistry and DNA analyses have remained unsuccessful.^{43,45}

Two dogs of this study were withdrawn because of the observation of clinical signs perceived related to the administration of oral CsA. One dog was excluded because of the rapid development of clinical signs of immediate type hypersensitivity after the second dose of medication. Because this dog had not been prescribed this medication prior to this RCT, it is improbable that CsA-specific reaginic antibodies would have been generated in 1 day. Thus, it remains debatable whether this adverse event should be imputed to the active drug, its vehicle or to another fortuitous event. In the second dog, CsA administration was noticed to exacerbate previously existing regurgitations. Whether or not these clinical signs are imputable to the active medication or to the bitter tasting vehicle unfortunately remains unknown.

In this RCT, although changes in laboratory parameters reflected glucocorticoid administration in many prednisolone-receiving dogs, uniform anomalies of complete blood counts and serum chemistry were not seen commonly in dogs given CsA. Of particular interest is that increases in blood urea nitrogen and creatinin were not seen in our dogs, in contrast to human patients given oral CsA for many weeks.⁴⁷

In conclusion, this study, designed as a double-blinded RCT, provides evidence that oral CsA is highly effective in reducing clinical signs and symptoms in dogs with AD. This anti-allergic efficacy was shown to be of similar potency to that of prednisolone. Moreover, the administration of CsA or prednisolone led to the occurrence of adverse drug events of comparable severity. It is our belief therefore that oral CsA should be considered a potent alternative to oral glucocorticoids in the pharmacotherapy of canine AD.

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Résumé Depuis 10 ans, la cyclosporine (CsA) a prouvé son efficacité dans des études randomisées et contrôlées pour le traitement de la dermatite atopique (AD) chez l'homme. Le but de cet essai randomisé, contrôlé, en aveugle, était de vérifier l'hypothèse selon laquelle la cyclosporine pouvait diminuer la gravité des signes cliniques de DA chez le chien. Trente chiens présentant une dermatite atopique non saisonnière ont reçu au hasard soit du NEORAL CsA (5 mg kg⁻¹) ou de la prednisolone (0.5 mg kg⁻¹), une fois par jour pendant 6 semaines. Avant, et 3 et 6 semaines après le traitement, les lésions cutanées ont été gradées par des cliniciens, à l'aide du Canine AD Extent and Severity Index (CADESI). Le prurit était évalué par les propriétaires grâce à une échelle analogue de prurit (PVAS). Dans les deux groupes, les valeurs de CADESI et de PVAS étaient significativement diminuées 6 semaines après traitement par rapport à avant la mise en place de la thérapeutique (Friedman test, $P < 0.0004$). Les pourcentages de réduction des valeurs de CADESI et de PVAS par rapport à l'inclusion n'étaient pas significativement différents entre les deux groupes (Mann-Whitney test, $P > 0.3$). Dans cette étude, la tolérance et la sécurité d'utilisation de la cyclosporine et de la prednisolone étaient semblables. Un cinquième des chiens recevant la CsA a occasionnellement présenté de la diarrhée ou des selles molles. Un chien traité par la CsA a présenté une éruption papillomateuse généralisée pendant la seconde moitié de l'essai. Notre étude démontre par un essai contrôlé randomisé, que la CsA diminue la gravité des signes cliniques chez les chiens souffrant d'AD non saisonnière. En outre, l'effet anti-allergique de la CsA semble comparable à celui de la prednisolone. Nous proposons que la CsA soit considérée comme un traitement alternatif intéressant aux traitements corticostéroïdes chez les chiens présentant une AD.

Resumen Durante la década pasada, se ha probado la efectividad de la ciclosporina oral (CsA) para el tratamiento de la dermatitis atópica (AD) en pacientes humanos, en pruebas al azar controladas. El propósito de esta prueba ciega al azar controlada fue probar la hipótesis de que la CsA era efectiva en reducir la gravedad de los síntomas clínicos de la AD en perros. Treinta perros con AD no-estacional fueron escogidos al azar para recibir una solución oral de NEORAL CsA (5 mg kg⁻¹) o de prednisolona (0.5 mg kg⁻¹), una vez al día durante 6 semanas. Anteriormente, y 3 y 6 semanas después de la terapia, los clínicos puntuaron las lesiones cutáneas utilizando el Índice de Severidad y Extensión de la AD Canina (CADESI). El prurito fue evaluado por los propietarios utilizando la escala análoga visual (PVAS). En ambos grupos, los valores de CADESI y PVAS fueron significativamente inferiores a las 6 semanas después del tratamiento que antes del inicio de la terapia (test de Friedman, $P < 0.0004$). El porcentaje de las reducciones en los valores de CADESI y PVAS desde el nivel base no fue significativamente diferente entre los grupos (test de Mann-Whitney, $P > 0.3$). En este experimento, la tolerabilidad y seguridad de la CsA oral y la prednisolona parecieron similares. Una sexta parte de los perros que recibieron CsA oral desarrollaron ocasionalmente diarrea o heces blandas. Un perro que había recibido CsA desarrolló una

erupción cutánea papilomatosa generalizada durante la segunda mitad de la prueba. Nuestro estudio aporta una prueba controlada al azar que la CsA reduce la severidad de los síntomas clínicos en perros con AD no-estacional. Además, la eficacia antialérgica de la CsA parece comparable a la de la prednisolona. Proponemos que la CsA oral debería considerarse como una alternativa válida a la terapia con glucocorticoides en perros con AD.

Zusammenfassung Orales Zyklosporin (CsA) hat sich im letzten Jahrzehnt beim Menschen in randomisierten Kontrollstudien als wirksam in der Behandlung der atopischen Dermatitis (AD) erwiesen. Der Zweck dieser randomisierten und kontrollierten Blindstudie war die Überprüfung der Hypothese, dass CsA die Schwere der klinischen Symptomatik der AD beim Hund vermindert. Dreissig Hunde mit nicht-saisonalen AD erhielten einmal täglich für 6 Wochen randomisiert eine orale Lösung mit entweder NEORAL CsA (5 mg/kg^{-1}) oder Prednisolone (0.5 mg/kg^{-1}). Vor, nach 3 und nach 6 Wochen wurden die Hautläsionen nach dem Caninen AD Extend and Severity Index (CADESI) durch Kliniker beurteilt. Der Juckreiz wurde von Besitzern mittels einer visuellen Analoggradierung (PVAS) beurteilt. In beiden Gruppen waren die CADESI und PVAS Werte 6 Wochen nach Beginn der Behandlung signifikant niedriger als zu Beginn der Behandlung (Friedman test, $P < 0.0004$). Zwischen der prozentualen Reduktion der CADESI und PVAS Werte der Gruppen bestand kein signifikanter Unterschied (Mann-Whitney Test, $P > 0.3$). In dieser Studie schienen CsA und Prednisolon gleich gut toleriert zu werden und gleich sicher zu sein. Ein Fünftel der mit CsA behandelten Hunde zeigte gelegentlich Durchfall oder weichen Stuhl. Ein Hund entwickelte während der zweiten Hälfte der Behandlung eine generalisierte papulöse Hauterkrankung. Unsere randomisierte Kontrollstudie lässt darauf schliessen, dass CsA die Schwere der klinischen Symptomatik bei Hunden mit nicht-saisonalen AD reduziert. Die anti-allergische Wirksamkeit scheint mit der von Prednisolon vergleichbar zu sein. Wir schlagen vor, dass CsA als wertvolle Alternative zur Glukokortikoidtherapie betrachtet werden sollte.

