A multicentre placebo-controlled clinical trial on the efficacy of oral ciclosporin A in the treatment of canine idiopathic sebaceous adenitis in comparison with conventional topical treatment

Lortz, Jutta

Abstract: Canine idiopathic sebaceous adenitis (ISA) is an inflammatory reaction of sebaceous glands, potentially resulting in their complete loss. It is considered a T-cell mediated disease, but its precise pathogenesis is still unknown. Topical treatment is an effective but laborious treatment. Ciclosporin A (CsA) has recently been shown to ameliorate the clinical picture of ISA. It is, however, an expensive treatment option. The objective of this multicentre, partially double-blinded, randomized controlled study was to evaluate the efficacy of CsA, either alone or with topical therapy, in comparison to topical treatment alone, as measured by the primary end-points alopecia and scaling, and multiple histological secondary objectives. 34 dogs with an established diagnosis were treated for 4-6 months and were evaluated before, during and after therapy. Both CsA and topical therapy demonstrated efficacy in this study. Differences between the treatment protocols were marginal. Topical treatment, both alone and in combination with CsA, appeared to reduce scaling more effectively than CsA alone. Both therapies reduced alopecia. There is evidence of a synergistic benefit on both scaling and alopecia, if both treatment options are combined. Inflammation of the sebaceous glands is also best reduced by a combination of both CsA and topical therapy. There is evidence that regeneration of sebaceous glands is best achieved by CsA, either given alone or in combination with topical treatment. Die idiopathische Sebadenitis des Hundes (ISA) ist eine gegen die Talgdrüsen gerichtete Entzündungsreaktion, die zu deren kompletten Verlust führen kann. Die exakte Pathogenese ist noch unklar, man geht von einer T-Zell-vermittelten Immunreaktion aus. Die topische Behandlung ist effektiv, aber zeit- und arbeitsaufwendig. Ciclosporin A (CsA) führte in einer Pilotstudie zu einer deutlichen klinischen Verbesserung von ISA. Es handelt sich jedoch um eine teure Behandlung. Das Ziel dieser Multizenter, Plazebo-kontrollierten, teilweise Doppelblindstudie war die Untersuchung von CsA auf die ISA im Vergleich zur topischen Therapie und der Kombination von beiden. Alopezie und Hyperkeratose dienten als primäre Parameter, während die histologischen Befunde sekundäre Parameter waren. 34 Hunde mit der Diagnose ISA wurden über 4 bis 6 Monate behandelt und vor, während und nach der Therapie untersucht. Sowohl CsA als auch die topische Therapie zeigten eine klinische Verbesserung. Die Unterschiede im Erfolg waren marginal. Die topische Therapie alleine als auch in Kombination mit CsA, zeigte einen besseren Rückgang der Schuppen als CsA alleine. Die Kombination beider Therapien zeigte einen synergistischen Effekt auf Schuppenbildung und Alopezie. Die Entzündung der Talgdrüsen ließ sich ebenfalls am besten durch die Kombination beider reduzieren. Es konnte gezeigt werden, dass eine Behandlung mit CsA sowohl alleine als auch in Kombination zu einer Regeneration der Talgdrüsen führte.

Other titles: Eine Multizenter, Plazebo-kontrollierte, teilweise Doppelblindstudie über die Untersuchung des Einflusses von Ciclosporin A auf die idiopathische Sebadenitis des Hundes im Vergleich zur topischen Therapie

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A multicentre placebo-controlled clinical trial on the efficacy of oral ciclosporin A in the treatment of canine idiopathic sebaceous adenitis in comparison with conventional topical treatment

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Canine idiopathic sebaceous adenitis (ISA) is an inflammatory reaction of sebaceous glands, potentially resulting in their complete loss. It is considered a T-cell mediated disease, but its precise pathogenesis is still unknown. Topical treatment is an effective but laborious treatment. Ciclosporin A (CsA) has recently been shown to ameliorate the clinical picture of ISA. It is, however, an expensive treatment option.
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A multicentre placebo-controlled clinical trial on the efficacy of oral ciclosporin A in the treatment of canine idiopathic sebaceous adenitis in comparison with conventional topical treatment

Introduction

Idiopathic sebaceous adenitis (ISA) is an inflammatory reaction of sebaceous glands that can lead to their complete destruction.[1] ISA is an uncommon dermatosis in dogs, and has been rarely reported in other mammals.[2-5] Although it can affect any breed, a strong breed predisposition has been proposed for the akita, standard poodle, vizsla, samoyed, chow chow, hovawart and recently the springer spaniel.[6] An autosomal recessive mode of inheritance has been suggested in the akita and standard poodle. Clinical signs of ISA usually first appear in young to middle-aged dogs. There is no apparent sex predilection.[4,5] The clinical features of ISA vary between different breeds, but they always comprise silvery-white or yellow-brown epidermal scales, and scales that are adherent to the hair shafts (follicular casts).

The clinical disease differs in long-coated versus short-coated dog breeds.[4,5] In the former, the lesions tend to be symmetric multifocal alopecia[2] (probably induced by broken hair shafts) associated with scaling and a dull and brittle hair coat.[7-9] Lesions start at the head, the pinnae, the dorsal neck and the tail and can extend to the dorsal midline. In short-coated dogs, lesions tend to be more arciform,[5] with coalescing areas of alopecia and fine non-adherent scaling that is present predominately on the trunk.[10-12] ISA in dogs is non-pruritic unless there is a secondary bacterial pyoderma. Histopathological findings in ISA are variable in terms of severity, and their character changes in association with chronicity of the disease.[7,13,14,15]

ISA is commonly unresponsive to treatment with anti-inflammatory doses of glucocorticoids.[2,10] Synthetic retinoids have been used for their anti-inflammatory properties, their effects on keratinocyte differentiation, and their inhibitory effect on sebaceous gland activity.[11,12,16,17] Isotretinoin has been reported to be effective in vizslas.[11] A more than 50% reduction in clinical signs was seen in 47% and 52% of dogs treated with isotretinoin or etretinate respectively, but outcome was unpredictable.[12] Since etretinate is no longer commercially available, acitretin is being considered as an alternative. Various nutritional supplements with essential fatty acids at high dosages seem to ameliorate clinical signs.[3,18,19] Treatment is currently largely restricted to antiseborrheic shampoos, emollient rinses and humectants. They have been useful for partial control of the scaling, but full recovery is often not obtained, and the treatment protocol is very labour intensive for the owner.[4, 6, 9, 10, 18] Ciclosporin A (CsA) has shown promising results in the therapy of ISA.[9,10,18,20] In a pilot study, sole therapy with CsA significantly reduced the inflammation and improved the clinical presentation by 60%.[20]

The objective of this clinical study was to compare the efficacy of oral ciclosporin A in the treatment of ISA in the dog when used alone and when used in combination with conventional topical therapy, as measured by the primary end-points alopecia and scaling, and histopathological secondary objectives.

Materials and methods

Study design

The study was designed as a multicentre, randomized, partly double-blinded, placebo-controlled clinical trial. Owners and investigators were aware of the group of dogs that received ciclosporin A as a sole therapy. In the groups where topical treatment was added either to ciclosporin A or placebo, neither was aware of the treatment. Therapy was dispensed by mail by a person who randomized the dogs. All owners gave written informed consent for their dogs to be entered in this trial.

This clinical study was not designed as a pivotal regulatory trial and was not conducted under "Good Clinical Practice" (GCP). Nevertheless, it followed many of the GCP principles, including definition of primary and secondary end-points, and written owner consent. The study followed a previous pilot study.[20] The primary end-point was reached when an improvement of at least 46% (moderate effect) in alopecia or scaling was observed after a treatment period of 4 months. Sample sizes were ten or 12 dogs per group, and were sufficient to detect the above-mentioned differences in primary objectives between study groups.

Cases

Before entering the study, ISA was confirmed by clinical examination and histopathology, fulfilling the widely published criteria for the diagnosis of ISA.[2,9,14,21] Briefly, the clinical picture of ISA is characterized by a general dull and brittle hair coat often associated with hypotrichosis and alopecia.[4,5,21] Typically, scales adhere firmly to the hair shafts and are called "follicular casts". A secondary pyoderma may lead to folliculitis and furunculosis accompanied by pruritus and occurs mostly in akitas and springer spaniels.[6,9] Skin lesions in the vizsla tend to be more arciform with coalescing areas of alopecia and fine non-adherent scaling.[11] Histopathological findings in ISA are variable in terms of severity, and their character changes in association with chronicity of the disease.[14,15] The most common finding is a nodular, granulomatous to pyogranulomatous inflammatory reaction at the isthmus level, obscuring the sebaceous glands. Sebocytes are destroyed and may no longer be detectable in histological sections. Often a marked orthokeratotic hyperkeratosis is detectable within the follicular infundibulum. In advanced stages of the disease, sebaceous glands are completely destroyed and inflammatory reactions become sparse.

Varying dermatological procedures to exclude differential diagnoses such as dermatophytosis, demodicosis, infectious pyogranulomas, and leishmaniasis were conducted individually by each clinician as necessary for the corresponding case. Dogs with a secondary bacterial folliculitis were treated with cefalexin (25 mg/kg, twice daily) until cytologically and clinically cured, before entering the study.

Treatment

Dogs were randomly assigned to one of three treatment groups (Figure 1), as follows.

1) The group "*CsA*" was treated with 5 mg/kg/day of ciclosporin A (Atopica®, Novartis Animal Health, Basel, Switzerland).
2) The group "*CsA/Top*" was treated with 5 mg/kg/day of ciclosporin A (Atopica®) and was simultaneously washed with sulphur/salicylic acid (Sebolytic®, Virbac Animal Health, Carros, France) or ethyl lactate (Etiderm®, Virbac Animal Health) and treated with baby oil soaks and 70% propylene glycol in water.
3) The group "*Placebo/Top*" was treated with placebo capsules identical to CsA once a day and was simultaneously washed with sulphur/salicyclic acid (Sebolytic®, Virbac Animal

Health) or ethyl lactate (Etiderm®, Virbac Animal Health) and treated with baby oil soaks and 70% propylene glycol in water.

The topical treatment (Top) consisted of a shampoo, containing either a combination of sulphur and salicylic acid or ethyl lactate. The shampoo was left on for a minimum of 10 min to remove scales and was then rinsed off. Thereafter, generic baby oil (e.g. BeBe baby oil, Nestle, Frankfurt, Germany) was applied on the whole body and left on for up to 2 h before it was washed off with one of the aforementioned shampoos. After the shampoo was rinsed off, a mixture off 70% propylene glycol in water was used topically as a spray on affected areas. In the early weeks of therapy, this procedure was applied as often as three times a week, but not less than once a week. The 70% propylene glycol mixture was sprayed onto affected areas, as needed. As soon as an improvement was achieved, the interval between the baby oil soaks was increased.

No other treatment was allowed. If clinical improvement was seen after 4 months, the dose of CsA or the placebo was reduced to every other day for another 2 months. If no clinical improvement was noted, therapy was continued daily for another 2 months.

Randomization
All dogs were allocated to one of the three treatment groups according to an independently established randomization list. Randomization was balanced every three cases. Five dogs were not randomized, since no placebo was available at that point in time. Within this group of five dogs, three were allocated to the *CsA/Top* group and two dogs to the *CsA* group (Figure 1).

Clinical evaluation
To determine clinical improvement under therapy, scaling, alopecia and folliculitis (the latter being characterized by focal erythema and papules) were subjectively evaluated and graded (0, absent; 1, mild; 2, moderate; and 3, severe) at 22 different body regions. Scaling, alopecia and folliculitis were considered mild if these clinical features were visible only with close inspection and not easily noticed by the owner. Scaling, alopecia and folliculitis were considered moderate if these clinical features were visible by the investigator and possibly noticed by the owner. Scaling, alopecia and folliculitis were considered severe if these clinical features were easily noticed by the owner. The sum of scores at 22 body regions resulted in a total alopecia score, a total scaling score and a total folliculitis score for each patient. The maximum final total score was a possible 66 points.

A clinical evaluation was performed at three time points in the study: before treatment, after 4 months of treatment and after 6 months of treatment. Skin cytology was performed whenever a skin infection, either by bacteria or malassezia was suspected.

Histopathological evaluation
In addition to the above-mentioned clinical investigation, treatment-related changes were also determined by histopathological examination of skin biopsy specimens. At least two 6-8 mm punch biopsies were taken from clinically affected areas of the body before treatment. After 4 months of treatment, skin biopsies (same size as before) were obtained from each patient at the same initial body area site. Biopsy specimens from patient no. 3 before treatment and from patient no. 18 after 4 months of therapy were subsequently lost and could not be included in further analysis.

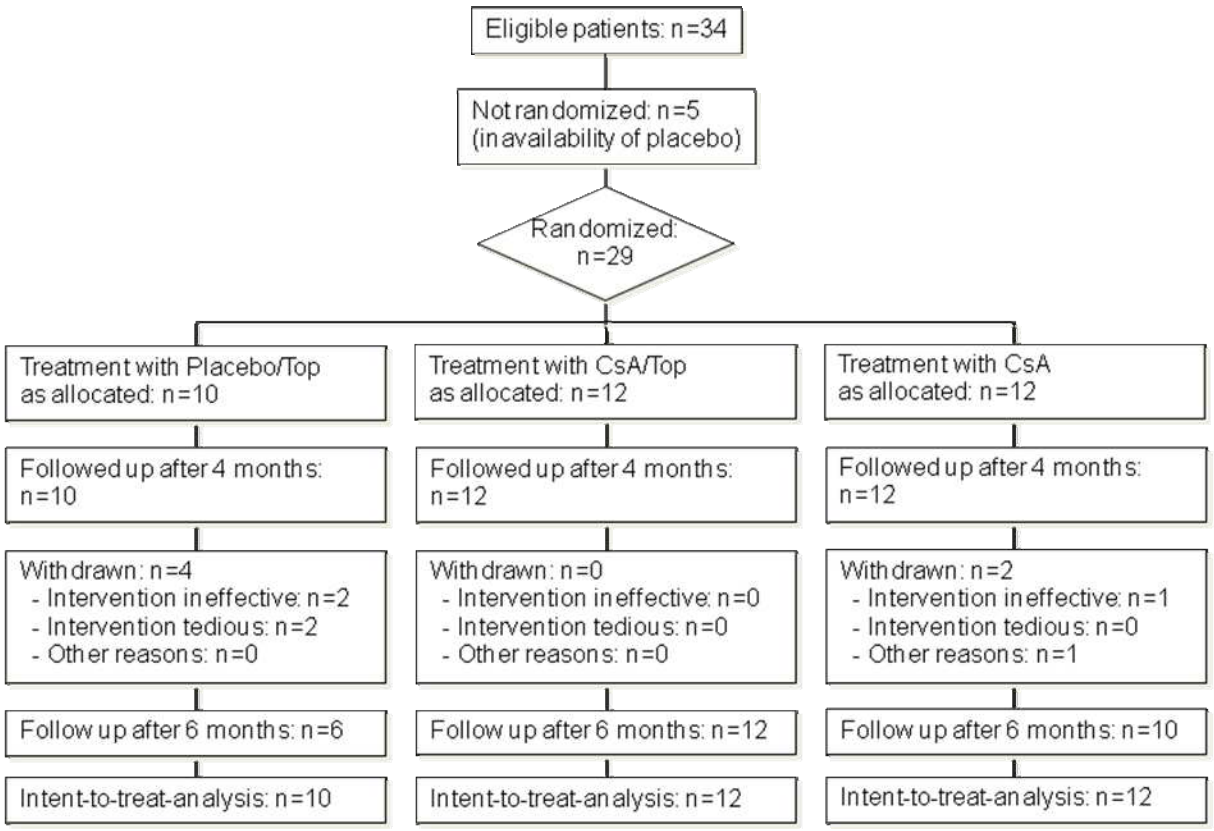

Figure 1 Flow chart describing progress of patients through the trial.

Tissues were processed routinely, embedded in paraffin and sectioned at approximately 5 µm thickness. Sections were stained with haematoxylin and eosin and were evaluated under a light microscope. All biopsy specimens were independently assessed in a blinded manner by three of the authors (J.L., M.L., and L.M.). Discrepancies in the histopathological evaluation were resolved in a pathology working group consisting of these three evaluators. Biopsies were considered diagnostic for ISA based on published morphological criteria.[2,9,14,21]

In each tissue section, the number of hair follicles with and without sebaceous glands and the number of hair follicles with and without perifollicular inflammation at the isthmus level was assessed, resulting in a sebaceous gland score (i.e. percent of hair follicles with sebaceous glands) and an inflammation score (i.e. percent of hair follicles with perifollicular inflammation). On average, six hair follicles were present in each section. All sections showed mostly longitudinal cuts of hair follicles and were sufficient to evaluate the presence of sebaceous glands.

Definition of primary and secondary end-points, and statistical analyses

Primary objectives were alopecia and scaling. These objectives had already been used in a pilot study, where they had been shown to be suitable to evaluate the clinical efficacy of therapeutic regiments in canine ISA.[20] The primary end-point was reached, when an improvement of at least 46% (defined as a moderate effect by clinical relevance) in alopecia or scaling were observed after a treatment period of 4 months. The folliculitis score was not included in further analysis of treatment efficacy, since its standard deviation was extremely high (based on the fact that it only occurred in a few dogs) and the sample size did not provide sufficient power to detect differences in this parameter.

Secondary objectives were evaluated by histopathology. The percentage of hair follicles with sebaceous glands (sebaceous gland score) and the percentage of hair follicles with perifollicular inflammation (inflammation score) were evaluated as mentioned above.

Demographic and baseline data were compared between groups using nonparametric statistics. Baseline clinical and histopathological data was analyzed with an analysis of variance (ANOVA). Clinical data, measured at two different treatment time points, were analyzed using a repeated-measurements analysis of covariance (RMANCOVA) with the parameters treatment group, baseline value, time, and treatment*time interaction. Histopathological data, measured at one treatment time point only, was analyzed using an analysis of covariance (ANCOVA) model, with treatment group and baseline value as parameters. Pairwise comparison of treatment groups was conducted as linear contrasts. Since the scale for clinical scores is not numeric but rather ordinal, this data was analyzed using a generalized linear model, in which the log odds of having a higher score are modelled as linear functions of the model parameters, i.e. treatment group, time, treatment*time interaction, body region, baseline value and a random effect for each subject. To assess a possible relationship between the change over time for different variables, rank correlations were calculated for the log change of the two clinical and the two histopathological scores. To avoid division by zero or calculating the log of zero, "1" was added to all values before dividing and calculating the log. Values of $P \leq 0.05$ were considered significant. A comparison to baseline (month 0) was not possible as the baseline data entered the model only as covariates.

The statistical software program SAS® (Institute Inc., Carry, NC, USA), version 9.1.3. was used for ANOVA, ANCOVA and RMANCOVA. For the generalized linear model the SAS procedure GLIMMIX was used.

Results

Four investigators from Germany and three investigators from Switzerland recruited a total of 34 dogs with ISA over a period of 3 years (Figure 1). The 34 dogs belonged to the following breeds: hovawart (n=6), akita inu (n=3), Bernese mountain dog (n=3), German shepherd dog (n=2), vizsla (n=2), elo (n=2), mixed-breed dogs (n=5) and one each of the following: Australian shepherd, Pekinese, cuvac, Tibetan terrier, Labrador retriever, golden retriever, Berger blanc, fox terrier, shih tzu, West Highland white terrier and English springer spaniel. The mean age was 7 years, with a range from 3 to 12 years. The sex distribution was ten intact male dogs, six castrated males, 13 intact females and five spayed female dogs. The mean duration of clinical signs before entering the study was 14 months (2 months to 4 years). Of these 34 dogs, 12 were assigned to the *CsA* group, 12 to the *CsA/Top* group and ten to the *Placebo/Top* group.

Before treatment, there were no significant differences between groups regarding duration of the disease, breed, sex, or age. There was a significantly higher number of neutered male dogs in the *CsA/Top* group (P=0.0474), which was considered to have no relevance for the study.

Analysis of the 22 different body regions that were examined for alopecia and scaling revealed that locations such as head, ears, neck, dorsal rump/back, flank/thighs and tail were most frequently affected, whereas locations such as thorax, abdomen, limbs and paws were not or rarely affected.

Scaling

Before treatment, mean total scores for scaling were similar between the three study groups (*CsA* versus *CsA/Top,* P=0.3080; *CsA* versus *Placebo/Top,* P=0.5704; *CsA/Top* versus *Placebo/Top,* P=0.1285). After 4 months of treatment the mean scaling score at baseline in the groups *CsA*, *CsA/Top* and *Placebo/Top* (24.75, 29.83, and 21.80, respectively) decreased to 17.67, 8.92, and 11.40, respectively (Figure 2). Improvement in scaling ranged from 29% and 48% in the *CsA* group and in the *Placebo/Top* group, respectively, to 70% improvement in the *CsA/Top* group. Thus, only the *Placebo/Top* and the *CsA/Top* group reached the primary end-point.

A statistical evaluation of this obvious decline was not conducted, since the baseline data could only be used as covariates in the employed general linear model. A statistical comparison between treatment groups demonstrated that the *CsA/Top* group improved more than the *CsA* group at month 4 (P=0.0014) and month 6 (P=0.0016), while there were no statistical differences when comparing *CsA* versus *Placebo/Top* and *CsA/Top* versus *Placebo/Top* (Table 1). The overall estimated odds ratio for comparison of the *CsA/Top* group and the *CsA* group was 0.18, which means that the odds of having any particular scaling score was 0.18 times lower in the *CsA/Top* group than in the *CsA* group (Table 1). In the *CsA/Top* group, a statistically significant improvement was found comparing scaling at month 6 versus scaling at month 4 (P=0.0476), while this was not the case for the other two groups (Table 2).

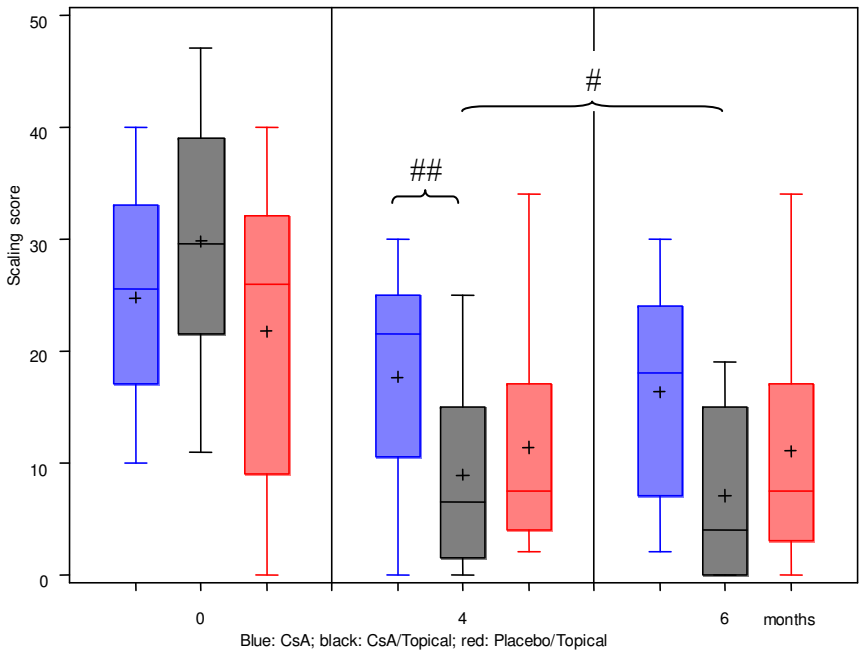

Figure 2

Box plots comparison of the scaling score over time in the three treatment groups. Each box goes from the lower quartile to the upper quartile, with a horizontal line at the median; whiskers extend from the minimum to the maximum. A plus sign marks the mean value. # = p≤0.05; ## = p≤0.01.

Response	Month	Odds Ratios (S.E.)			P-values for comparison		
		CsA/Top vs CsA	Placebo/Top vs CsA	Placebo/Top vs CsA/Top	CsA/Top vs CsA	Placebo/Top vs CsA	Placebo/Top vs CsA/Top
Scaling	overall	0.18 (0.10)	0.47 (0.26)	2.56 (1.45)	**0.0014 (B)**	0.1755	0.0990
	4	0.20 (0.11)	0.39 (0.22)	1.98 (1.15)	**0.0031 (B)**	0.1000	0.2429
	6	0.17 (0.10)	0.56 (0.35)	3.31 (2.07)	**0.0016 (B)**	0.3513	0.0566
Alopecia	overall	0.33 (0.26)	0.21 (0.19)	0.65 (0.60)	0.1610	0.0886	0.6371
	4	0.33 (0.27)	0.55 (0.47)	1.65 (1.45)	0.1738	0.4810	0.5668
	6	0.33 (0.27)	0.08 (0.09)	0.25 (0.29)	0.1769	**0.0268 (T)**	0.2283

Table 1
Generalized linear models for clinical data show estimates and standard errors for odds ratios between each pair of groups, and p-values for the pairwise comparison of groups, overall (i.e. averaged over months four and six) and separately for each month. Next to each significant p-value, the group (B=CsA/Top, T=Placebo/Top) with the smaller scores is printed.

Response	Treatment	Odds Ratios (S.E.) 6 vs 4 month	P-values for comparison 6 vs 4 month
Scaling	all	0.79 (0.12)	0.1227
	CsA	0.73 (0.16)	0.1464
	CsA/Top	0.63 (0.15)	**0.0476 (6)**
	Placebo/Top	1.05 (0.36)	0.8804
Alopecia	all	0.37 (0.11)	**0.0008 (6)**
	CsA	0.70 (0.19)	0.1934
	CsA/Top	0.69 (0.22)	0.2343
	Placebo/Top	0.11 (0.08)	**0.0042 (6)**

Table 2
Generalized linear models for clinical data show estimates and standard errors for odds ratios between each pair of groups, and p-values for comparison of month six with month four. Next to each p-value, the month with the smaller score is printed.

Alopecia
Before treatment, mean total scores for alopecia were similar between the three study groups (*CsA* versus *CsA/Top,* P=0.7034; *CsA* versus *Placebo/Top,* P=0.7647; *CsA/Top* versus *Placebo/Top,* P=0.9490). In the groups *CsA*, *CsA/Top* and *Placebo/Top*, the mean alopecia score at baseline (16.25, 16.75, and 17.90, respectively) decreased after 4 months of treatment to 7.17, 3.25, and 7.70, respectively (Figure 3). A statistical evaluation of this obvious decline in alopecia, which was in the range of 57% to 81% (i.e. primary end-point reached in all three groups), was not conducted, since the baseline data could only be used as covariates in the employed general linear model. A statistical comparison between treatment groups demonstrated that none of the treatment groups was superior to another after 4 months of therapy (Table 1). At month 6, the mean alopecia score of the *Placebo/Top* group was statistically significantly lower than the mean alopecia score of the *CsA* group (P=0.0268). However, there was no statistical difference between the *CsA* group and the *CsA/Top* group and none between the *CsA/Top* group and the *Placebo/Top* group (Table 1). Only in the *Placebo/Top* group was a statistically significant improvement found between month 6 and month 4 (P=0.0042) (Table 2).

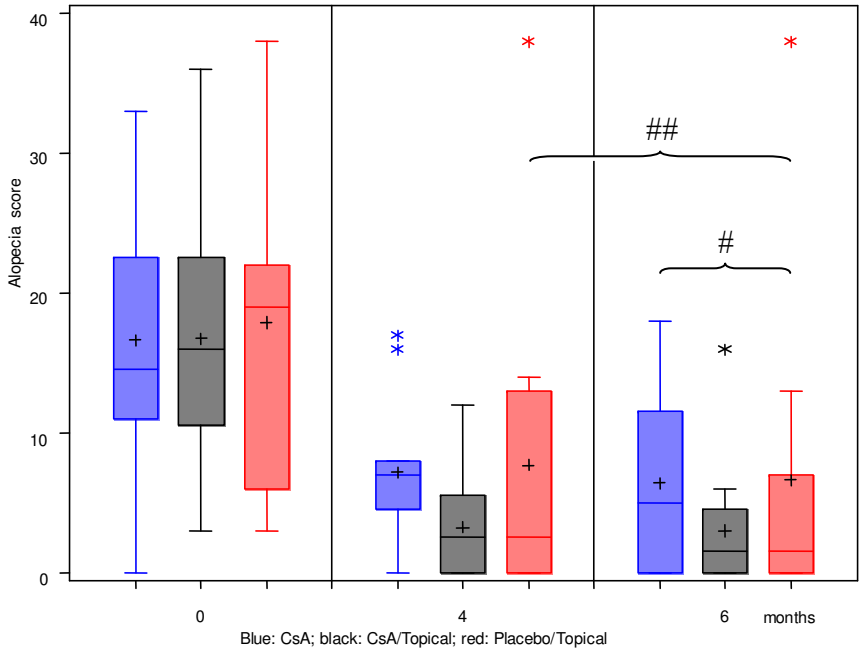

Figure 3
Box plots comparison of the alopecia score over time in the three treatment groups. Each box goes from the lower quartile to the upper quartile, with a horizontal line at the median; whiskers extend from the minimum to the maximum. Values lying more than 1.5 times the interquartile distance (which is upper quartile minus lower quartile) away from the box are displayed as asterisks and are not included in the whiskers. A plus sign marks the mean value. . # = p≤0.05; ## = p≤0.01.

Inflammation
On histopathological examination of biopsy specimens prior to treatment, the number of hair follicles with inflammation was similar among all groups (*CsA* versus *CsA/Top,* P=0.7460; *CsA* versus *Placebo/Top,* P=0.5054; *CsA/Top* versus *Placebo/Top,* P=0.7250). After 4 months of treatment, the inflammation score decreased in all treatment groups (Figure 4). Although the *CsA/Top* group showed the lowest inflammation score, no statistical difference was found between the treatment groups (*CsA* versus *CsA/Top,* P=0.0690; *CsA* versus *Placebo/Top,* P=0.8612; *CsA/Top* versus *Placebo/Top,* P=0.1066).

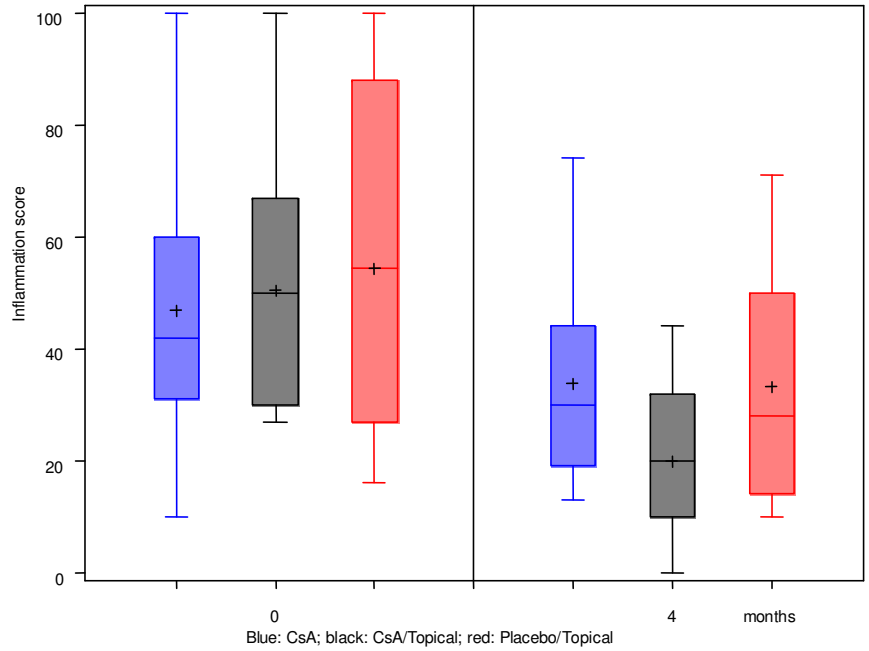

Figure 4
Box plots comparison of the inflammation score over time in the three treatment groups. Each box goes from the lower quartile to the upper quartile, with a horizontal line at the median; whiskers extend from the minimum to the maximum. A plus sign marks the mean value.

Sebaceous glands
All groups had a very low sebaceous gland score, reflecting the absence of sebaceous glands from most hair follicles (Figure 5). Prior to treatment, the *CsA/Top* group showed a statistically significantly lower sebaceous gland score than the *CsA* group (P=0.0206) and was also lower than the *Placebo/Top* group (although not statistically different; P=0.0834). There was no statistical difference between the CsA group and the Placebo/Top group (P=0.5552). In light of the obvious increase in the sebaceous gland score in all groups, this difference prior to treatment is not considered to impair interpretation of the sebaceous gland score. Any association with the comparatively high number of neutered male dogs in the *CsA/Top* group and the influence of testosterone on sebaceous glands can only be speculated on.

In the groups *CsA*, *CsA/Top* and *Placebo/Top*, the mean sebaceous gland score at baseline (9.03, 1.19, and 9.43, respectively) markedly increased after 4 months of treatment to 39.88, 20.84, and 19.35, respectively (Figure 5). Statistical evaluation demonstrated that the *CsA* group and the *CsA/Top* group showed higher levels of sebaceous glands than the *Placebo/Top* group (P=0.0203 and P=0.0290, respectively), while there was no statistical difference between the *CsA* group and *CsA/Top* group (P=0.9629).

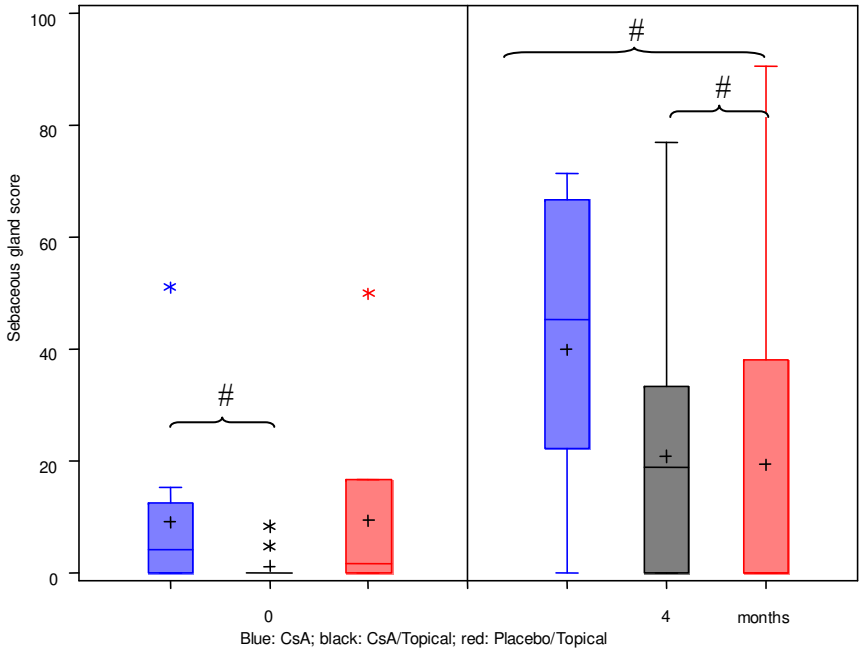

Figure 5
Box plots comparison of sebaceous gland score over the time in the three treatment group. Each box goes from the lower quartile to the upper quartile, with a horizontal line at the median; whiskers extend from the minimum to the maximum. Values lying more than 1.5 times the interquartile distance (which is upper quartile minus lower quartile) away from the box are displayed as asterisks and are not included in the whiskers. A plus sign marks the mean value. # = $p \leq 0.05$.

Correlations between clinical and histolopathological scores
Pairwise rank correlation revealed a statistically significant correlation between the two primary objectives scaling and alopecia (P=0.0028). A correlation was also found between alopecia and inflammation (P=0.0484).

Dose tapering and discontinuation
The study protocol allowed an optional discontinuation of therapy after 4 months of participation. All 34 patients completed 4 months of therapy and were re-evaluated clinically and histopathologically at this point of time. After 4 months of therapy, six owners elected to discontinue treatment. Data from these six dogs remained included for intent-to-treat analyses. From the *CsA* group two dogs were withdrawn from the study, one due to the development of spontaneous hyperadrenocorticism and one due to apparent inefficacy of therapy. In the *Placebo/Top* group, four dogs were withdrawn, two due to lack of efficacy of therapy and two because application of the topical treatment was not feasible for the owner (Figure 1).

If the primary end-point (i.e. at least 46% reduction in scaling or alopecia) was met after 4 months of treatment, the study protocol allowed a change in dosing from once daily to every other day. The dosing regimen of CsA and/or placebo was reduced in all dogs from all groups, except for two dogs (both from the *CsA* group), where there was insufficient response to therapy after 4 months.

Variations in individual dogs
In some dogs, the treatment response differed markedly from the mean response in the respective treatment group. One patient from each the *CsA* group and the *CsA/Top* group showed a temporary worsening of alopecia after 4 months of therapy, while alopecia improved 2 months later. Two dogs from the *CsA* group and one patient from the *CsA/Top* group showed a worsening of alopecia between month 4 and 6, despite continuation of treatment. With regard to scaling, three dogs (two of the *CsA* group and one of the *Placebo/Top* group) showed worsening during the first 4 months of therapy and improvement thereafter. In one patient of each group, scaling after 6 months of treatment was more severe than at baseline, although it had partially resolved after 4 months. One explanation for this might be the dose reduction of CsA and the reduced frequency of topical treatment.

Adverse drug events
Adverse drug events were not reported in the *Placebo/Top* group, but in ten out of 24 dogs receiving CsA (42%). The adverse drug events reported in the *CsA* group and *CsA/Top* group included vomiting and diarrhoea (n=2), gingival hyperplasia (n=3) and hypertrichosis (n=5).

Discussion

The 34 dogs allocated to the three treatment groups showed homogeneity in terms of age, sex, breed and duration of disease. The age of onset and duration of signs until final diagnosis was similar to what has been described in the literature.[4,9,12] Only 28 % of the dogs belonged to breeds that are reported to be predisposed to ISA (akita inu, n=3; vizsla, n=2; hovawart, n=6; and springer spaniel, n=1).[6,9,20] Therefore, no conclusions could be made regarding breed responses to therapy. Three cases were Bernese mountain dogs, a breed that is rather common in Germany and Switzerland and in which ISA has rarely been described. It is known that ISA can actually affect any breed. The lack of inclusion of standard poodle dogs (reported to be predisposed to ISA) in this study reflects the overall rarity of this breed in Germany and Switzerland.

Since there are no validated and published criteria to determine clinical features of canine ISA objectively, a scoring system was invented for the two main clinical features of ISA, namely alopecia and scaling.[20,21] After it had proven its usefulness in a pilot study,[20] the same scoring system was employed in the clinical trial reported here. Folliculitis, however, was not a useful parameter to evaluate treatment response, because it was inconsistently present in dogs of this study. It is known that folliculitis associated with ISA varies between breeds, with the akita and the springer spaniel being more seriously affected than other breeds.[6,14] These two breeds, however, were under-represented in our study.
One weakness of the study is that the conclusions on the efficacy of topical therapy may be limited by the variability in accurateness and frequency applied by owners. Owners were instructed to treat their dogs topically at least once a week, but were allowed up to three treatments a week. We did not collect data on frequency of topical therapy in individual cases, and there was no way to assess owner application techniques.

With regard to the primary end-points, all three treatment protocols were able to reduce alopecia and scaling. There was no clear difference between the *CsA* group and the *Placebo/Top* group, demonstrating that both CsA and topical treatment have largely the same clinical effect on ISA. The small statistical difference between the *CsA* group and the *Placebo/Top* group with regard to alopecia after 6 months of treatment is not considered to be meaningful in terms of superiority of treatment.

Interestingly, there is evidence of a synergistic benefit, if both treatment options are combined, since mean scores for alopecia and scaling of the *CsA/Top* group were always lower than those of the other groups. This is supported by a statistical difference in the scaling score after 4 months of therapy. The synergism of topical and systemic treatment on scaling could be explained by the different mode of action of these two therapeutic approaches. While topical treatment has been reported to reduce the keratinous material around the hair and within the hair follicle,[18] CsA might reduce scaling by an anagen induction of hair follicles and a subsequently increased removal of excessive keratin.[22-29] A precise anagen-telogen evaluation to support this hypothesis, however, was not performed in this study.

Comparing the scaling and alopecia scores from 4 and 6 months of treatment, a statistically significant further improvement was demonstrated for the *CsA/Top* group in terms of scaling and for the *Placebo/Top* group in terms of alopecia. No statistically significant increase was observed between month 4 and 6. It is therefore concluded that, in accordance to previous observations,[20] most dogs show a marked improvement in clinical signs within 4 months, irrespective of the treatment. Further improvement is possible with continuation of therapy, but its extent is limited. In most dogs, after 4 months of therapy the disease can be controlled by a dose reduction of CsA to every other day and/or a reduction in the frequency of topical therapy.

The improvement in primary objectives was confirmed by treatment-related changes in secondary parameters, i.e. objectives evaluated by histopathology. Differences in these secondary objectives between groups might reflect the differences in the mechanism of action between topical therapy and CsA. In all three treatment groups, sebaceous glands were nearly absent at the beginning of the study. This is a feature which is commonly observed in subacute to chronic ISA.[14,15,20] In all groups, mean sebaceous gland scores after 4 months of therapy were higher compared to baseline scores. Interestingly, there was a statistically significant difference in sebaceous gland scores between the *CsA* and the *Placebo/Top* group and between the *CsA/Top* group and the *Placebo/Top* group. The higher mean sebaceous gland score in both CsA groups suggests that CsA allows for better regeneration of sebaceous glands than topical treatment, as described by Reichler.[9]

Perifollicular inflammation (i.e. inflammation involving sebaceous glands) was reduced in all treatment groups after 4 months of therapy. In the *CsA* and the *CsA/Top* group, this decline in inflammation most likely reflects the activity of *CsA*, which is a known immunomodulator. The fact that inflammation resolves most in the *CsA/Top* group and the fact that there is a decline of inflammation in those patients that received placebo and topical treatment only, suggest some anti-inflammatory properties of the topical treatment, although its precise mode of action is yet unclear.

Topical treatment was not associated with any adverse events. Treatment with CsA was also considered safe, since few adverse effects were noted during this study. Adverse effects included vomiting and/or diarrhoea, gingival hyperplasia and hypertrichosis. These adverse effects have been reported previously[31-34] and rarely require withdrawal of the medication. Hyperadrenocorticism is not a reported side effect of CsA treatment. Thus, the development of hyperadrenocorticism in one patient from the *CsA* group was not considered to be related to the therapy.
Despite the clear evidence of efficacy of either CsA or topical therapy, some cases are refractory to treatment. The reasons for this are unknown. Unresponsiveness could be related to the chronicity of disease, particularly in cases where all sebaceous glands have been

destroyed prior to the commencement of therapy and where perifollicular inflammation has already resolved.[20] It is also possible that clinical worsening may be caused by other concurrent diseases, such as atopic dermatitis, or by diseases that reduce the compliance to treatment (as was seen in one patient from the *Placebo/Top* group). Some dogs might need higher doses of CsA, as is known from other autoimmune diseases.[30,31] Additionally, as no trough levels were measured, we cannot exclude the possibility that in some dogs variability in absorption did not render therapeutic levels.

Conclusion

Both CsA and topical therapy have clearly demonstrated efficacy in this study. Differences between the treatment protocols are marginal. Topical treatment, both alone and in combination with CsA, appears to reduce scaling more effectively than CsA alone. Both therapies reduce alopecia. There is evidence of a synergistic benefit on both scaling and alopecia, if both treatment options are combined. Inflammation of the sebaceous glands is also best reduced by a combination of both CsA and topical therapy. There is evidence that regeneration of sebaceous glands is best achieved by CsA, either given alone or in combination with topical treatment.

Both treatment options have specific advantages and disadvantages that need to be taken into account when a patient is subjected to therapy. Ciclosporin A is frequently considered too expensive by owners, while topical treatment is considered too labour intensive by others. When owners participating in this study were retrospectively asked about their experience, six of 20 that used topical treatment considered this therapy impractical. Two owners even discontinued the topical treatment. Therefore, it is merely a question of technical feasibility and costs that should determine the first choice for one or the other therapy, with some evidence that a combination of both therapies provides further benefit for the patient.

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