

Investigations on the Effects of a Topical Ceramides-Containing Emulsion (Allerderm Spot on) on Clinical Signs and Skin Barrier Function in Dogs with Topic Dermatitis: a Double-Blinded, Randomized, Controlled Study

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ABSTRACT

Skin barrier is impaired in canine atopic dermatitis (AD) and topical application of ceramides has been suggested to be beneficial. This study evaluated the effects of a ceramides-containing emulsion on clinical signs and skin barrier in a double-blinded, placebo-controlled trial. Dogs diagnosed with AD were enrolled and divided into

two equal groups. Half received ceramide emulsion (Allerderm spot-on) 3x/week for 4 weeks, and the rest received control. Both investigators and owners were blinded to group allocation. Treated areas included pinna, antebrachial, axilla, and groin.

Dogs were evaluated on day 0, 14, and 28 for clinical signs and skin barrier function. Clinical signs were scored using the Canine Atopic Dermatitis Extent and Severity Index (CADESI) score and skin barrier was assessed by measurement of Transepidermal Water Loss (TEWL). Owners scored pruritus and overall improvement. One investigator did all clinical evaluations

while TEWL measurements were done by two different investigators.

For CADESI, analysis of variance showed significant effect of time (week 4<0) and group (Allerderm<control). For owners' assessment no statistically significant difference in pruritus scores was found between groups and over-time. Analysis of TEWL measurements was done separately by operator due to possibility of operator effect and found no significant differences for one operator and a significant effect of group (Allerderm<control) in antebrachial and axillary area and time (week 4<0) for the other operator. It is concluded that topical ceramides-containing emulsion has beneficial effects in canine AD and that TEWL results must be carefully interpreted as affected by operator's skills.

INTRODUCTION

Numerous studies have demonstrated that dogs with atopic dermatitis (AD) have some skin barrier impairment (Marsella, 2011). Although it is not clear whether this dysfunction is primary or secondary to inflammation or both, it has been documented that this impairment is, at least in part, linked to ceramides deficiency (Reiter et al, 2009; Shimada et al, 2009; Yoon et al, 2011). Skin barrier impairment has important consequences on the disease process as it increases the risk for sensitization (Olivry et al 2011) leading to vicious cycles of sensitization and additional inflammation, trauma, and skin damage. For this reason much effort has been devoted to the identification of therapies to improve skin barrier in dogs with AD (Marsella, 2013).

Ceramides are sphingo-lipids of great importance for skin barrier function (Elias, 2012) and have been used successfully for the treatment of AD in humans (Hon et al, 2012; Sajić et al, 2012). In veterinary medicine, several studies have demonstrated that topical application of a sphingo-lipid emulsion has the ability to correct the ultrastructure abnormalities in the stratum corneum (Piekutowska et al, 2008) in dogs with AD and ameliorate the lipid deficien-

cies of affected dogs (Popa et al, 2012). After this initial proof of concept, a reasonable question was whether this chemical and ultrastructural improvement would translate into a decrease of severity of clinical signs. A recent open study showed a positive clinical effect with this emulsion in patients that had refractory AD and had already tried more conventional therapies (Fujimura et al, 2011). Although these results were encouraging about the potential of this treatment, the enthusiasm about the findings was limited by the fact that the trial was not controlled and it could be speculated that some of the perceived improvement of the clinical signs might have been partially biased by the lack of a control group. For this reason, the present study aimed to investigate the clinical effect of this sphingo-lipid emulsion in a placebo controlled trial where both owners and the investigator are blinded as far as treatment allocation.

MATERIALS AND METHODS

Experimental Design

This study was designed as a prospective, double-blinded, placebo-controlled clinical trial. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Florida.

Animals

Dogs with naturally occurring AD were recruited through the clinics of the Small Animal Hospital at the University of Florida, College of Veterinary Medicine. Informed consent was signed by all owners prior to the enrollment in the study.

Study Subjects

Inclusion Criteria

In order to be enrolled, dogs had to be judged healthy upon physical examination aside from skin disease, and had to be clear of any secondary skin infections. Diagnosis of AD was based on suggestive history, compatible clinical signs according to Prèlaud criteria (Prèlaud et al, 1998), and exclusion of other pruritic skin diseases that may mimic AD.

The following inclusion criteria were ad-

opted:

- Patients with a history of 1–6 years duration and mild to moderate severity AD based on a subjective clinical evaluation. The severity of atopic dermatitis was evaluated by the clinicians according to these cut-off Canine Atopic Dermatitis Extent and Severity Index (CADESI-03) values : remission: 0-15; mild AD: 16-59; moderate AD: 60-119; and severe AD: > 120 recommended in the International Task Force on Canine Atopic Dermatitis (Olivry et al, 2008).
- Dogs with food as flaring factor for AD were not eliminated as long as that component of the diet was under control and no dietary changes were allowed once dogs had been included in the study.
- Dogs had to be on a flea control program starting at least 1 month prior to inclusion in the study, to minimize the potential for clinical improvements due to flea control. This program included a variety of flea products to be used no less than once a month. No change in flea control was allowed during the trial.
- Dogs were off of oral cyclosporine for a minimum of 1 month, oral fatty acid supplements, long-acting injectable glucocorticoids for a minimum of 2 months, and oral and topical glucocorticoids for 2 weeks.
- All dogs were off a concurrent allergen-specific immunotherapy, unless treatment had been ongoing for at least 1 year. The dose and frequency was not changed during the study.

Exclusion Criteria

The following exclusion criteria were also adopted:

- Dogs with exceptional severity of AD that did not allow the discontinuation of previous treatments were excluded. If the owner determined that the dog was not doing well enough

to be off other medications, that was considered severe AD regardless of the CADESI scores.

- Outdoor dogs were excluded due to the potential for environmental factors (eg, rain) to decrease the residual effect of topical therapy.
- All dogs with concurrent bacterial or Malassezia infections as determined by the presence of clinical signs (eg, papules, pustules, epidermal collarettes, scaling) and supportive cytology were excluded. If infection developed in the course of the trial, the dog was removed from the study and the last value was carried forward for the purpose of statistical analysis.

Randomization

The randomization was done by an assignment of numbers to each dog and blind hat draw.

Treatment Allocation

Half of the dogs were allocated to receive topical application of sphingo-lipid emulsion (Allerderm Spot on, Virbac, Fort Worth, Texas) 3 times weekly on pinnae, antebrachial, axilla, and groin and the other half was to receive de-ionized water for a total of 4 weeks. Owners and investigators were blinded to the allocation to the groups. Dogs were rechecked every 2 weeks (day 0, 14, 28). The volume applied to each site at each application was 250 microliters for dogs weighing less than 15 kg (ie, a pipette of 2ml per dog) and 500 microliters for dogs weighing more than 15 kg (ie, a pipette of 4ml per dog).

Clinical Evaluation by the Investigator

At each visit clinical signs were evaluated using a modified version of the Canine Atopic Dermatitis and Extent Severity Index 03 (CADESI). Clinical signs were evaluated using a modified version (Marsella et al, 2010) of the validated Canine Atopic Dermatitis and Extent Severity Index-03 (Olivry et al, 2007). The two modifications in relation to CADESI-03 were that papules were added as a clinical sign and that the scores for each

sign ranged from 0 to 3 rather than from 0 to 5. The dog's body was divided into sections, each of which received a score based on the clinical signs evaluated. The total score was the sum of all body sites. Clinical signs evaluated included diffuse erythema, erythematous macules, papules, excoriations and alopecia. The total score was used in the statistical analysis.

Owner Assessment

Owners were asked to score pruritus every 2 weeks using a visual analogue scales (PVAS) ranging from 0 (no pruritus) to 10 (worst possible pruritus with interruption of daily activities and waking up at night to scratch). At the end of the study the owner was also asked to provide feedback about the experience with the product and whether there was an overall improvement in pruritus and lesions. The feedback about improvement overall was simply a yes or no answer and did not involve assigning a score.

Measurement of TEWL

TEWL was measured using a closed-chamber evaporimeter (VapoMeter, Delfin Technologies Ltd, Kuopio, Finland) in an ambient temperature of 20–26° C. Dogs were allowed 30 minutes to acclimatize to the examination room prior to TEWL measurements. The assessment was done three

times: at baseline (day 0, before any topical application), 2 weeks (day 14 of treatment), and at the end of the study (day 28 of treatment). All TEWL readings were done in triplicate, and the mean of the reading were used for statistical analysis. Unclipped skin sites were evaluated and included the concave surface of pinnae, axillary, antebrachial, and inguinal areas. These sites have been selected since they are commonly affected sites, but also sites for which a significant difference in TEWL has been reported between increased in atopic dogs compared to normal breed and age matched controls (Hightower et al, 2010). TEWL were done by two different operators. Ten dogs were enrolled and measured by one researcher and 22 dogs by another investigator.

STATISTICS

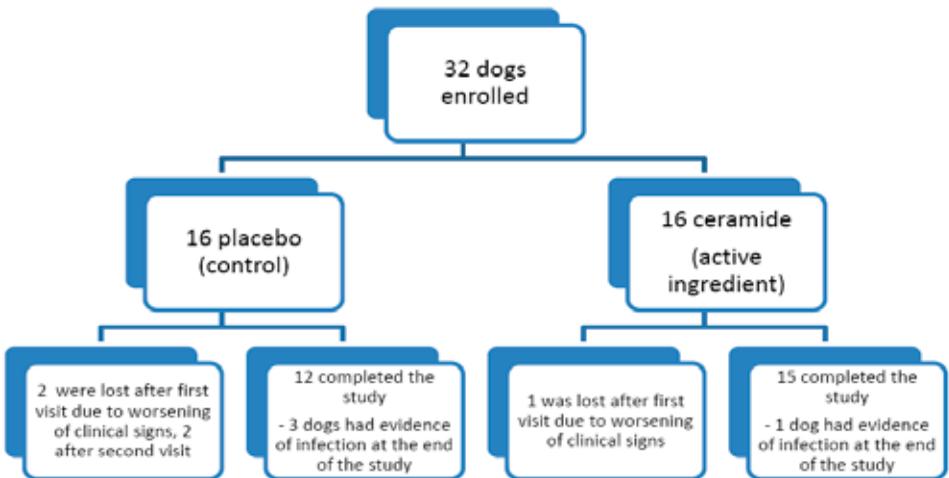
Analysis of Variance (ANOVA) was used to compare the total CADESI scores, pruritus scores and TEWL between groups and over-time. JMP 9.0.3 through the SAS Institute, Inc (Cary, NC, USA) was used for data analysis. P <0.05 was considered significant.

RESULTS

Animals

Thirty-two dogs total were enrolled in the study, with age ranging from 2 to 8 years, half of them were allocated to the placebo

Figure 1. Summary of follow-up of enrolled dogs.

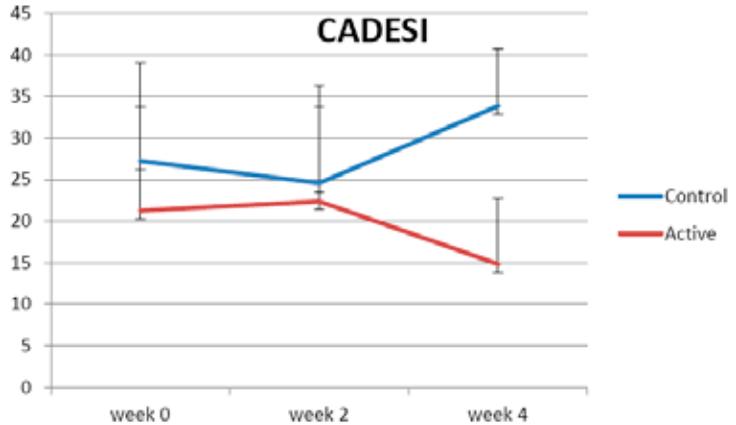


group (de-ionized water) and half of them to the active group (sphingo-lipid emulsion). The mean age in the control group was 3.4 years and 3.8 years in the active ingredient group. The mean reported age of onset of the disease was 1.4 years of age.

All the animals in this study were neutered; 14 were males and 18 were females. Forty-eight percent of the dogs were mixed breed dogs. Of the purebreds, 32% were Pit pulls. Other breeds that were represented included Yorkshire Terrier, German Shepherd, Beagle, Standard Poodle, English Setter, French Bulldog, Vizla, and Rhodesian Ridgeback. Eighty-seven percent of the dogs were non seasonal at the time of the study. All of the dogs had had seasonal signs when allergies were first noticed.

Twenty-seven dogs completed the study (Figure 1). A higher number of dogs were

Figure 2. Shows the means and standard deviation of the total CADESI for the two groups over time.



lost in the placebo group (n=4) compared to the treatment group (n=1).

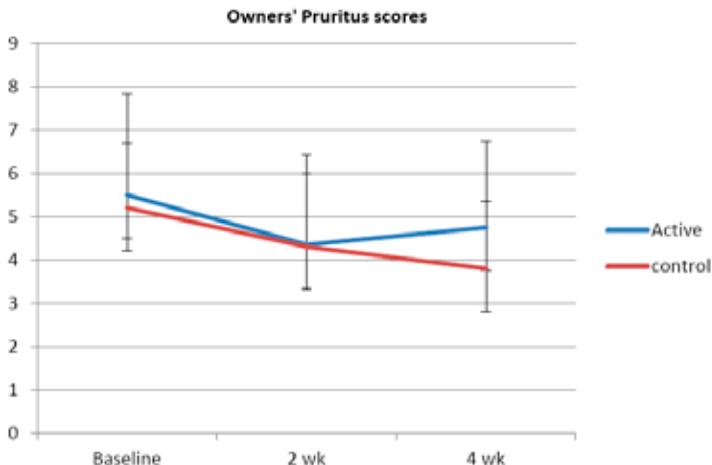
Clinical scores by investigator

For total CADESI ANOVA found a significant effect of time (week 4<0, p=.028) and group (active<control, p=.018), and group x time interaction (p=.0106). By the end of the study, the mean of the active ingredient group improved by 69% (baseline mean total CADESI of 21.25, week 4 mean total CADESI 14.84), while the control group mean worsened by 24% (baseline mean total CADESI 27.25, week 4 mean total CADESI 33.83).

Owner's Assessment

No significant difference in pruritus scores was found between the two groups and over time (Figure 3). For the overall assessment, in the active ingredient group, an overall improvement in pruritus was reported by 55% of the owners and no worsening of condition was described by the owners of the dogs that completed the trial (n=15). In the active

Figure 3. Shows the pruritus scores over the course of the study. No significant difference between groups and overtime were found.



ingredient group, an improvement in skin lesions was reported by 33% of the owners. None of the owners of the dogs allocated to the sphingo-lipid emulsion complained about the application of the product or reported any adverse effects.

For the overall assessment in the placebo group, of the dogs that completed the trial (n=12), 45% reported an improvement of pruritus and 33% an improvement of clinical lesions. Approximately 67% of owners reported no change or worsening of skin lesions. Approximately 20% of the owners of the dogs allocated to the control group complained about the volume and the fact that the solution did not appear to “stay on the skin”.

TEWL Measurements

For TEWL taken by one operator (n=10) no statistically significant differences were found. For TEWL taken by the second operator (n=22) a significant effect of group (Allerderm<control) in antebrachial and axillary area as well as of time (week 4<0) were found. An effect of laterality was also found (right>left).

DISCUSSION

This study aimed to evaluate the clinical effects of a sphingo-lipid suspension in a blinded fashion and confirmed the positive clinical effect that Fujimura (Fujimura et al, 2011) had reported. Our study was shorter than Fujimura's clinical trial, which lasted 12 weeks and reported a significant improvement of clinical signs after 6 weeks of twice weekly application. Although our study was shorter, a positive effect was evident at 4 weeks using this emulsion three times weekly on areas prone to AD. It is possible that a twice weekly regimen could be used long term for maintenance and could decrease the likelihood of flare ups. More studies are needed to investigate a preventative effect on flare ups. As AD is a clinical syndrome that may involve different skin alterations in different patients, skin barrier dysfunction and repair may have diverse impact from patient to patient. Topical application of ceramides in human medicine is best

used as adjunctive therapy to minimize the frequency and severity of relapses as part of a long term management plan (Sajić et al, 2012, Chamlin et al, 2002). It is reasonable to speculate that similar approach would be most beneficial in dogs with AD too.

In our study we considered also the owner's perception of the dog's condition. This assessment included a scoring of pruritus every 2 weeks and a simple feedback on whether they thought that the dog was any better, the same, or worse at the end of the study. Interestingly, while the pruritus scores were not significantly different between the two groups and over time, the feedback at the end of the study supported a beneficial effect more marked for the active ingredient group since no worsening was reported in the active ingredient group but it was reported in the placebo group. This observation was consistent with the investigator's clinical scores. It important to note that investigator scored the dogs without any knowledge of the feedback provided by the owners, which was collected by another person. The improvement reported by several owners in the placebo group is also interesting and is hard to explain it with anything other than a placebo effect. This emphasizes the importance of double blinded placebo controlled studies before final conclusions can be made regarding the efficacy of a product.

In terms of skin barrier as measured by TEWL, our study showed some improvement with the measurement taken by one operator but not the other. Both individuals had undergone the same training and measurements were taken under the same room conditions. These findings confirm the temperamental nature of this methodology in veterinary medicine as reported by Lau-Gillard (Lau-Gillard et al , 2010), even when a closed chamber device is used, as it was done in this study. This is an important consideration when the efficacy of a skin repair product is only assessed by TEWL. In the case of this ceramides-containing emulsion other studies had already demonstrated

a positive ultrastructural and chemical effect (Piekutowska et al, 2008; Popa et al, 2012) thus it is more likely that the beneficial effect found by the second operator is the most reliable assessment. If, however, in some studies, only TEWL is used to evaluate skin barrier repair, it could be possible to draw incorrect conclusions. Interestingly, an effect of laterality was found (right>left) in our study. Different readings from side to side had already been reported by Lau-Gillard highlighting the difficulty of interpreting TEWL results in the context of a study to assess repair of skin barrier function.

In summary, based on the results of this double blinded placebo controlled trial, it is concluded that topical application of ceramides-containing emulsion has a beneficial effect on clinical signs in dogs diagnosed with AD. The product was well tolerated and has a place as adjunctive treatment in dogs with mild to moderate disease.

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