A Prospective, Double-lbinded, Controlled Pilot Study to Evaluate the Effects of Cyclosporine (Atopica) on Skin Barrier Function in Canine Atopic Dermatitis by Measurement of Trans-Epidermal Water Loss

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ABSTRACT

Skin barrier dysfunction has been reported to play an important role in both human and canine atopic dermatitis (AD). Inflammation can worsen skin barrier, thus it is reasonable to believe that therapy aimed at reducing inflammation should have a beneficial effect on skin barrier function. The present study aimed to investigate the effect of glucocorticoids and cyclosporine on skin barrier function in dogs with naturally occurring AD. Twenty-seven dogs with AD were randomly allocated to either prednisolone (0.5mg/kg daily for the first week, then every other day for 3 weeks) or cyclosporine (5mg/kg once daily for 4 weeks). Skin barrier was assessed by measuring transepidermal water loss (TEWL) on pinnae, axillae, and groin on days 0 and 28. Clinical signs were scored on days 0 and 28.

For clinical signs, analysis of variance showed a significant effect of time (P=0.03; end<beginning), but no effect of group or group x time interaction. For TEWL, no significant effects of time nor group were found. The only significance for TEWL was found for region (P<.0001, axilla>inguinal>pinna). The reason for lack of significant improvement of TEWL despite improvement of clinical signs is unclear at this time. Larger studies are needed to con-

clusively address the role played by inflammation on skin barrier dysfunction in dogs.

INTRODUCTION

Preliminary evidence exists on the presence of skin barrier dysfunction in canine atopic dermatitis (AD).[1,2,3] It is currently unknown whether this impairment is primary or secondary to inflammation, or both. Canine AD shares many similarities with the human counterpart.[4] In humans, extensive data exist on the impairment of skin barrier in AD, both primary and secondary.[5,6] It is proposed that increased permeability allows enhanced penetration of environmental allergens, thus amplifying the risk for sensitization in genetically predisposed individuals.[7,8]

Skin barrier function is affected by cytokines,[10] thus, once inflammation is triggered, further impairment of the skin barrier ensues,[11] leading to self-perpetuating cycles of sensitizations and progressive worsening of the disease. It is, therefore, reasonable to speculate that treatments aiming to decrease inflammation would have a beneficial effect on skin barrier. Great effort is currently devoted to investigate strategies to improve skin barrier function,[12] as this could halt the progression of AD,[13] but our knowledge of the effects of commonly used anti-inflammatory agents on skin barrier is incomplete.

Skin barrier can be assessed by non-invasive methods such as the measurement of transepidermal water loss (TEWL). This parameter is increased in atopic patients when compared to healthy controls in both humans[14,15,16] and dogs.[17,18]

Glucocorticoids are frequently used to control flare ups of AD in both humans and dogs. They have a beneficial effect in decreasing the inflammatory process, and have been shown to temporarily improve TEWL in human patients with AD.[19] Glucocorticoids, however, induce skin atrophy and compromise lipid synthesis in humans, and may not be an ideal option for long term management.[20] Topical application of calcineurin inhibitors have been reported to have less of a negative effect on skin barrier function,[19,21] although a recent study demonstrated some compromise of stratum corneum integrity in people.[22] No information exists in human medicine on the effects of cyclosporine and TEWL.

Limited information exists on the effects of anti-inflammatory agents on skin barrier in dogs. One open study evaluated the short-term effect of topical glucocorticoids on skin barrier, and showed a decreased of TEWL in dogs with AD.[23] No information exists on the effects of calcineurin inhibitors on skin barrier function in dogs with AD. Thus, the purpose of the present study was to investigate the effects of oral glucocorticoids and cyclosporine on skin barrier function in dogs with AD as measured by TEWL. The hypothesis tested was that both glucocorticoid and cyclosporine administration would improve TEWL at the end of the study.

Experimental Design and Methods

This study was designed as a prospective, double-blinded, controlled, 4-week long study.

Animal Use

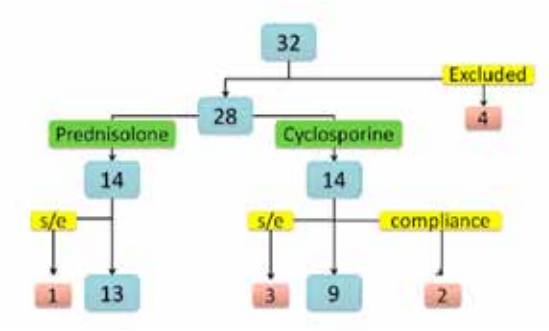
All animal procedures were approved by Institutional Animal Care and Use Committee. A consent form was signed by all owners at the time of enrollment in the study.

Animals and Allocation to Groups

Twenty privately owned dogs diagnosed with AD were recruited. All dogs were judged healthy on physical examination aside from skin disease, and were clear of any secondary skin infections prior to enrolment. Diagnosis of AD was based on suggestive history, compatible clinical signs according to Prelaud criteria, and exclusion of other pruritic skin diseases that may mimic AD.

Once enrolled, dogs were randomly allocated to receive either prednisone (0.5mg/kg once daily for first week then every other day for 3 weeks) or cyclosporine (5mg/kg once daily for 4 weeks). The randomization was done by an assignment of numbers to each dog and blind hat draw. Investigators were blinded in terms of allocation of the dogs to the two groups. Skin cytology was

Table 1. *Flow chart explaining the details of patients allocated to the prednisolone and cyclosporine group.*



done from representative areas at each visit to monitor for secondary infections. No anti-inflammatory therapies were allowed during the trial, nor were changes in the diet or environment.

Clinical Evaluation (CADESI)

On days 0 and 28 severity of clinical signs was scored at the beginning and at the end of the clinical trial using a validated scoring system (Canine Atopic Dermatitis and Extent Severity Index 03).[25] Briefly, the dog's body was divided into small sections, each of which will receive a score based on the clinical signs evaluated. The total score was calculated by adding the scores of all clinical signs and body sites. Total score was used in the statistical analysis.

Skin Barrier Evaluation (TEWL)

On days 0 and 28 skin barrier function was assessed by the measurement of TEWL using a closed chamber device (VapoMeter, Delfin Technologies Ltd, Kuopio, Finland). The dogs were allowed to acclimatize to the room for at least 30 minutes before measurements were taken. The measurements of each dog were taken in the same temperature regulated exam room. The device was re-calibrated before each dog. Transepidermal Water Loss was measured in three representative areas, which have been shown to be significantly increased in atopic dogs compared to normal dogs. These areas include the pinnae, the axillae, and inguinal area, which had been shown to be significantly increased in atopic dogs compared to normal dogs.[17] Measurements from each area were taken in triplicates, at 10 seconds per measurement, and the mean and standard deviation were used for analysis. Values were expressed in g/m^2hr.

Statistics

Pre- and post-therapy CADESI and TEWL measurements were compared using a mixed model ANOVAs. A p value less than 0.05 was considered significant.

RESULTS

Animals

Of the 32 recruits, 28 were selected to participate in the study based on clinical signs. Initially, four were excluded. Exclusion criteria included: dogs that received corticosteroids or cyclosporine within 2 months prior to study day 0, presence or history of malignancy, presence of uncontrolled ectoparasite

Table 2. *Details on the age, gender and coat length for the prednisolone and cyclosporine group.*

	Prednisolone	**Cyclosporine**
Age	Range: 1 - 10 YO Mean: 5.4 YO	Range: 2 - 10 YO Mean: 5.2 YO
Gender	Males: 6 Females: 7	Males: 3 Females: 6
Hair Coat Length	Short: 9 Long: 4	Short: 7 Long: 2
Seasonality	All Year: 8 Spring/Summer: 5	All Year: 4 Spring/Summer: 5

Table 3. *Clinical scores expressed as CADESI on days 0 and 28 of the study for both groups of dogs.*

	Mean Day 0	Standard deviation	Mean Day 28	Standard deviation
Prednisolone	35.19	40.24	12.38	9.99
Cyclosporine	25.22	9.85	17.56	11.99

or microbial infestation, dogs unavailable for the entire duration of the study, and dogs that would require vaccination or allergen specific immunotherapy during the study. Of the remaining 28 dogs, 14 were randomly allocated into each group.

- In the Prednisolone group, one dog withdrew due to adverse effects of polyuria and polydipsia, leaving 13 for analysis.

In the cyclosporine group, three withdrew due to adverse effects of vomiting and diarrhea, and two withdrew due to lack of owner compliance, leaving nine for analysis at the end of the 4 weeks. See Table 1 for details on the cases enrolled. Each group was represented by: a wide range of ages, both genders, dogs with varying lengths of hair (Table 2).

CADESI

For CADESI, ANOVA only showed a significant effect of time, where the scores at day 28 were less than those at day 0 ($p<0.03$). No significant difference between the prednisolone and cyclosporine groups was found (Table 3).

TEWL (Table 4)

No significant effects of time nor group were found for TEWL. The only significance for TEWL was found for region, where the measurements of the axilla were greater than those of the inguinal area, which were greater than those of the pinnae on day 0 and day 28 for both the prednisolone and cyclosporine groups.

DISCUSSION

In our study, we found no effect of either glucocorticoids or cyclosporine on skin barrier as measured by TEWL. We decided to use a non-invasive method to assess skin barrier function. Although the close chamber device is the best device to measure TEWL, this methodology has been demonstrated to show large variability. Lau-Gillard et al warned that the significant site to site, day-to-day, and dog to dog variations would make changes induced by medications very difficult to reliably detect and this may be indeed the case of our study. TEWL also requires for the patient to be perfectly still during measurements. The individual personality of each dog and the resulting challenge in keeping them sufficiently still during the TEWL measurements might have also played an important factor in the resulting values.

So, it is possible that the only way to reliably evaluate the effects of skin barrier

Table 4. *Mean TEWL measurements on days 0 and 28 of the study. Values are expressed in g/m²hr.*

	Mean day 0	St dev	Mean day 28	St dev
Prednisone				
Pinna	14.427	6.073	13.882	3.886
Axilla	22.608	8.997	20.846	6.969
Inguinal	14.804	6.325	15.511	7.757
Cyclosporine				
Pinna	11.917	2.954	11.298	3.116
Axilla	20.511	8.340	19.549	7.161
Inguinal	14.022	7.893	14.931	7.046

would be by electron microscopy as done by Jensen et al in humans to evaluate the effect of these therapies on lipid synthesis and ultrastructure of the upper layers of the epidermis.

Due to the large variability of the results, a larger sample size may be beneficial in future studies. The length of the treatment could also be prolonged in order to hopefully see more of the beneficial effects of cyclosporine, especially since this is a life-long treatment option. In our study, there was a wide range of breeds of dogs. Future studies may have less variability if dogs of similar hair coat (such as only short-haired dogs) were used, especially since this length of hair coat can very easily affect the readings on the Vapometer.

Age could also be a complicating factor. Dogs as young as 1year old and as old as 10 years old were used in the study. Age and nutrition can influence TEWL[17], thus it may be beneficial to restrict age groups and standardize diets in future studies to minimize variability.

CONCLUSION

In our pilot study, no effect on skin barrier function was found after 4 weeks of either oral prednisolone or cyclosporine. Additional studies with more invasive methods or different methodology to measure TEWL should be done to investigate the effects of anti-inflammatory therapies in dogs with AD.

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