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RESEARCH ARTICLE

Effect of Cyclosporine in Canine Atopic Dermatitis: Safety, Clinical Evaluation, and Mechanism Studies

Ha-Jung Kim and Hee-Myung Park*

Department of Veterinary Internal Medicine, College of Veterinary Medicine, Konkuk University, Seoul 143-701, Korea
*Corresponding author: parkhee@konkuk.ac.kr

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A B S T R A C T

Atopic dermatitis (AD) is very common and sometimes the treatment would be challenged in dogs. Cyclosporine A (CsA) has been used in canine AD for its tolerance and efficacy compared to steroids. This study aimed to investigate safety, efficacy, and mechanism of CsA in canine AD.

Blood works and urinalysis for safety verification every 4 weeks and Canine Atopic Dermatitis Extent and Severity Index (CADESI)-03, pruritus, overall coat condition, and scaling scores for clinical evaluation of the atopic dogs (n=7) were performed by every 2 weeks for 8 weeks. In addition, systemic total IgE, Th1/Th2 cytokines, mast cell, CD3-positive T cells in the skin biopsy samples were evaluated in atopic dogs by ELISA, semi-quantative PCR.

Blood works and urinalysis every 4 weeks showed no remarkable. CADESI score reduced significantly (P=0.0034, paired t-test) after 8 weeks, pruritus and scaling scores were improved significantly over times compared to base line. But overall coat condition showed no significant changes. Total serum IgE showed reduction pattern after 8 weeks, but there was not significant compared to the base line.

In mRNA level, IL-2 and TNF-α related to Th1 and IL-4 and IL-10 related to Th2 cytokines showed suppressive tendencies, but no significant difference were remarked after 8 weeks in the skin tissue. In histopathology, the inflammatory changes and mast cell counts showed significantly improved after 8 weeks.

In conclusion, CsA has efficacy and safety in atopic dogs, suppression of Th1 and Th2 cytokines were suspected mechanism in this study.

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INTRODUCTION

Atopic dermatitis (AD) is a complicated skin disease which could be triggered by various environmental factors. The developing mechanisms are very confusing and management often faces challenges. Most atopic dogs need long-term anti-inflammatory drugs (e.g., steroids, cyclosporine) (Olivry and Sousa, 2001; Forsythe and Paterson, 2014).

Cyclosporine A (CsA) has been approved by potent immunosuppressive actions, especially for T cells (Matsuda and Koyasu, 2000). Oral CsA showed a significant improvement to be similar to that of prednisolone. Moreover, side effects in blood parameters are rarely noticed in dogs (Olivry *et al*., 2002). The drug could block the transcription of cytokine genes in activated T cells (Forsythe and Paterson, 2014). It targets interleukin-2 (IL-2) by inhibiting calcineurin, prevents mast cell degranulation, prostaglandin production and cytokine releasing (Wershil *et al*., 1995; Furuta *et al*., 1997; Warbrick *et al*., 1997). However, the correct changes of clinical outcomes and mechanism of the CsA were required more investigation basis in dogs. Especially, there have not been studies with correct clinical improvement over times by CsA. The present study aimed to evaluate safety, clinical efficacy, and the mechanism of CsA in canine AD by time.

MATERIALS AND METHODS

Dogs: Dogs enrolled were client-owned (n=7) and the diagnosis of AD was made by history and clinical (Willemse, 1986). Other skin diseases with food allergy were ruled out. Three dogs were male and 4 were female. The average of age was 7.86±4.26 years and breeds were various.

Dogs were given only microemulsified CsA (NEORAL capsule 25mg, Novartis Pharma, Basel, Switzerland) at the dosage of 5 mg kg^{-1} once a day orally.

Assessment of clinical signs: Canine Atopic Dermatitis Extent and Severity Index was evaluated (CADESI)-03 every 2 weeks (Olivry *et al*., 2007). Pruritus score was also assessed every 2 weeks by the owners (Table 1). Overall coat condition and scaling were evaluated by investigator. Overall coat condition was also scored (Table 2). Scaling was scored from 0 to 4, corresponding to almost normal (0-1), mild, moderate, severe, respectively.

Assessment of safety: A complete blood count, serum chemistry panel and urinalysis were repeated at 0, 4, and 8 weeks after treatment. Any adverse events were monitored by owner interview at each visit.

Serum total immunoglobulin E concentration in atopic dogs: Serum total IgE concentrations were measured at week 0 and 8 weeks by using a total dog IgE test kit (Immunology Consultants Laboratory). Measurement was instructed by supplied protocol.

Semi-quantitative Reverse transcription polymerase chain reaction (RT-PCR): Skin samples of the dogs (n= 5) taken and RNA was extracted using a commercial kit (easy-BLUE™ Total RNA Extraction kit, iNtRON Biotechnology Inc., Seongnam, Korea) according to manufacturer's instruction. Primer sets for PCR are described in the Table 3. β-actin was used as the housekeeping gene.

Toluidine blue staining for mast cell counting: The processed sections were stained with toluidine blue working solution (toluidine blue O, Sigma, 1% sodium chloride) according to company's instruction.

Mast cell counts were conducted by modified method of the previous study (Auxilia and Hill, 2000). The counts were performed by another three investigators.

Withdrawal: Criteria for withdrawal from the study is listed: lack of compliance, adverse events, owner desire to withdraw, development of concurrent disease, loss of dog to follow-up.

Statistical analyses: To assess the changes in mean CADESI, pruritus, overall coat condition, and scaling score throughout the study, repeated measures ANOVA (Wilks' Lambda, RM ANOVA) was used. To compare the clinical scores between base line and each week, paired t-tests were used. The percentage of changes from base line in mean CADESI, pruritus, overall coat condition, and scaling score at each visit were compared using ANOVA. Quantities of the cytokines in skin biopsy sample were compared by paired t-test. Differences of sum of mast cell counts between base line and 8 week were analyzed by paired t-test. The statistical significance was considered at $P<0.05$.

RESULTS

Safety profile: In blood works and urinalysis, there were no significant changes in all dogs (data not shown).

Table 1: Scoring system for the owners' assessment of pruritus

Score	Definition
0-1	Dog not pruritic at all, or scratches occasionally like a normal dog
2	Dog scratches/bites occasionally, and is generally comfortable
3	Dog scratches and bites frequently, but not excessively
4	Dog scratches and bites frequently, often seems uncomfortable
5	Dog scratches and bites almost constantly, is a lot of discomfort

Modified from Nagale *et al.* (2001).

Table 2: Scoring system for overall coat condition

Score	Definition
0	Normal coat
1	Mildly dull but likely normal coat
2	Dull coat with mild alopecia
3	Hair loss over <10% of body surface area
4	Hair loss over 10-25% of body surface area
5	Hair loss over >25% of body surface area

Modified from Nagale *et al.* (2001).

Table 3: Cytokine primers and optimum conditions for RT-PCR

		Forward		Reverse
IL-2	K9IL2-1	caactcctgccacaatgtac	K9IL2-2	ctgtaatggttgctgtctcg
	K9IL2-3	cttgcatcgcactgacgc	K9IL2-4	tccttggtgtctgtcaagtg
IL-4	K9IL4-1	tgagcctctcctagtaaac	K9IL4-2	ccatgctgctgaggttcc
	K9IL4-1	gggtctcacctcccaac	K9IL4-4	cctgtagagtcctctgag
IL-10	K9IL10-1	atactgctgaccgggtc	K9IL10-2	tcggctctcctacatctc
	K9IL10-3	ttggaggaggtgatgccc	K9IL10-4	agagttgccatcctgggtg
TNF-a	K9TNFa-1	cctcttgcccagacagtc	K9TNFa-2	gtcctacccccttacagg
	K9TNFa-3	ccaacggcgtggagctg	K9TNFa-4	tacacctgcccggactc
β-actin	CAb1	Caggcgacgacgcccc	CAb2	Cgctcggcggtggtgg
	CAb3	Gccgtcttcccctccatc	CAb4	agctgtagccacgctccg

The nucleotide sequences of the primers were based on the GenBank nucleotide sequences for the canine genes as follows: β-actin (Accession no. AB038240), IL-2 (U28141), IL-4 (AF239917), IL-10 (U33843) and TNF-α (DQ923808).

Clinical scores: CADESI scores showed improved patterns during the study (Fig.1). The scores between base line and 8 week showed significantly differences ($P=0.0034$, paired t-test), however, there were no significant improvement for each week throughout the study. Five dogs (71.4%) of in C group showed more than 50% improvement from base line on 8 week.

In pruritus, dogs showed improvement until 8 weeks significantly compared to base line in each time (Fig. 1, paired t-test). Overall skin condition was reduced over time, but there were no significant differences in any weeks from base line and previous tests (Fig. 1, paired t-test). Significant differences from base line were noted from at 4 week to the end of the study in scaling scores (Fig. 1, paired t-test). However, no parameters showed significant changes over times (Fig. 1, ANOVA).

Serum total IgE concentrations of the atopic dogs: Dogs showed reduced concentrations of serum total IgE after 8 weeks, but statistical significance was not noticed (Fig. 2).

Quantification of mRNA by RT-PCR: IL-4, TNF-α, IL-10, and IL-2 were shown reduced relative quantities at 8 week compared to the base line, but statistical significances were not shown (Fig. 2).

Histopathology and mast cell population: Most of the atopic dogs showed epidermal hyperkeratosis and hyperplasia with perivasculitis on dermal area (Fig. 3).

After 8 weeks of treatment, the epidermal hyperplasia and inflammation in dermis of the skin were improved (Fig. 3). The number of mast cells in the dermis was also reduced significantly. Mast cell populations are confirmed by toluidine blue stain (Fig. 3).

DISCUSSION

In this study, the efficacy and mechanism of CsA were evaluated based on the objective clinical parameters and molecular works.

Many studies have been studied that have demonstrated the safety and efficacy of CsA in the management of canine AD. The reports show that around one- to two-thirds of dogs will show a 50 per cent or more reduction in pruritus and lesion scores within four to eight weeks (Forsythe and Paterson, 2014). Some studies showed oral CsA provides 'good-to excellent' reduction of clinical scores compared to oral prednisolone and methylprednisolone in the same dosages (5 mg kg^{-1}, once a day) (Olivry *et al*., 2002; Steffan *et al*., 2003). In this study, CsA has also shown the clinical improvement significantly after 8 weeks. There were no significant defferences between 6 and 8 week of the changes in the dogs. Therefore, it have shown fully works within 6 week. In deed, some previous studies which investigate anti-allergic efficacy of treatments compared to CsA were degined for 6 weeks (Olivry *et al*., 2002; Steffan *et al*., 2003).

In pruritus score, it was improved significantly at all time points compare to base line by CsA, therefore, CsA would be an efficient treatment option if pruritus were serious in AD dogs. In overall coat condition, it was not significant different after treatment. The score of improvement in this study was much broader, including not only pruritus but also coat quality, alopecia and self-trauma. Self-induced alopecia included in CADESI-03 was only reflected a 'pruritus'. Our study has investigated alopecia with two methods, CADESI and overall coat condition to get reasonable data of it. The scaling score was reduced significantly from 4 weeks in the dogs. Therefore, CsA could also improve scaling in the skin after 4 weeks in atopic dogs.

Many studies have suggested that balancing the Th1/Th2 types of reactions may be a fundamental approach to AD treatment (Hussain *et al*., 2014). IL-2 and TNF-α related to Th1 and IL-4 and IL-10 related to Th2 cytokines selected to confirmed the previous study. Our results demonstrated that CsA showed suppression of Th1/Th2 cytokine expression tendency in the skin although it was not statistically different.

In addition to the effect on T cells, action of CsA has been recently reported to influence both innate and adaptive immune responses and there is an increasing list of other cells involved in inflammatory and immune responses that may be affected by ciclosporin including B cells, antigen presenting cells, keratinocytes, endothelial cells, mast cells, basophils and eosinophils (Fric *et al*., 2012).

This study enrolled only small population that is why it could not show the significances in the Th1/Th1 cytokine expressions. Further mechanism studies are needed on canine AD.

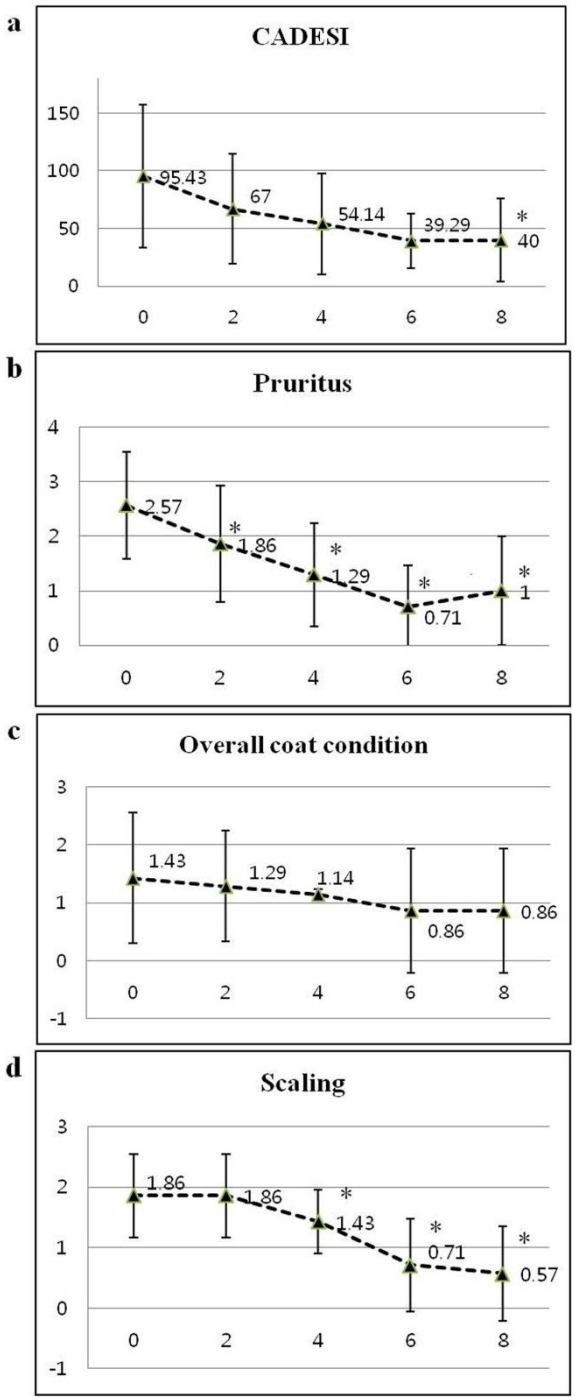

Fig. 1: Changes of clinical signs during the course of the study in atopic dogs (n=7). **a**: Canine Atopic Dermatitis Extent and Severity Index (CADESI) scores.; **b**: pruritus score; **c**: overall coat condition score; **d**: scaling score. *P<0.05, significantly different from base line. X axis means weeks. *P<0.05.

In human medicine, serum total IgE concentrations are used a diagnostic indicator of AD (Wu and Scheerens, 2014; Makris *et al*., 2014). However, no such differences have been found in dogs, which have been shown to have not correct correlation than people (Koebrich *et al*., 2012; Oldenhoff *et al*., 2014). Although the statistical significances were not presented, the reduced patterns after treatments of AD could not neglected in this study. Recoveries from diseases of atopic dogs could be related the patterns in this study. Further study of serum total IgE levels was required.

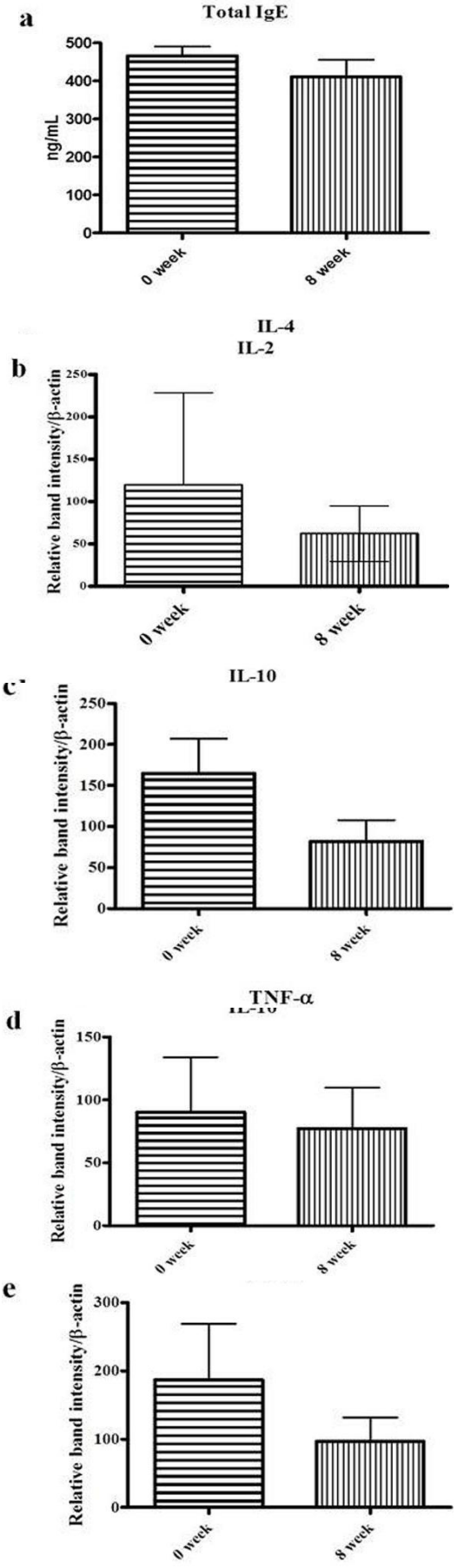

Fig. 2: mRNA quantification of Th1/Th2 cytokines by semi-quatitative PCR in the atopic skin tissues after 8 week CsA treatment. They showed reduced quantities at 8 week from base line. There were no significant differences between 0 and 8 week in all cytokines (P<0.05).

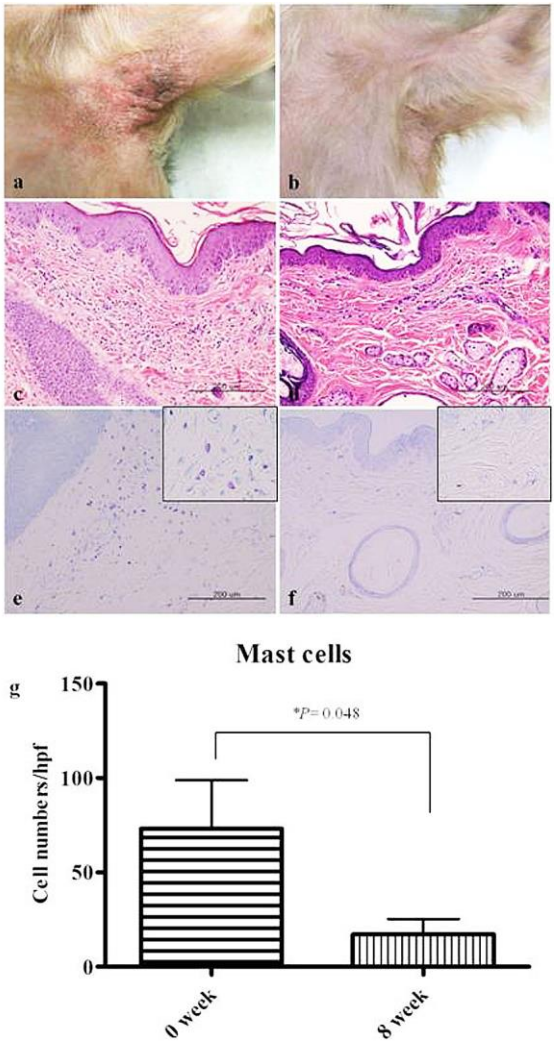

Fig. 3: Histopathology and toluidine blue staining in the atopic skins after 8 week CsA treatment. **a** and **b**: clinical features in the base line and 8 week; **c** and **d**: H&E stain (× 200); **e** and **f**: toluidine blue stain (× 200). The purple colored cells are mast cells. Note the mast cell population is marked reduced after 8 weeks (**f**). **g**: Mast cell counts. The mast cell populations were significantly reduced (P<0.05).

Conclusions: CsA has efficacy and safety in atopic dogs to improve clinical signs and suppression of Th1 and Th2 cytokines were suspected mechanism in this study. Further studies of CsA on canine AD are needed to identify correct mechanism and efficacy for long management.

Author's contribution: HJK performed the study and analyzed the data, and wrote this paper. HMP designed the study and analyzed the data, and revised the paper.

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