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Original article

Cyclosporin A treatment in intrinsic canine atopic dermatitis (atopic-like dermatitis): open trial study

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Abstract

In this study, dogs were separated into two groups and treated with immunosuppressant (Cyclosporin A: CsA). The first group was the canine atopic dermatitis (CAD) group, which is similar to extrinsic atopic dermatitis (AD) in humans (treated with a CsA dose of 2.5-5.5 mg/kg, n=8), and the second group was the canine atopic-like dermatitis (ALD) group, which is similar to intrinsic AD in humans (treated with a CsA dose of 2.5-6.5 mg/kg, n=14). The canine atopic dermatitis extent and severity index (CADESI)-4 was evaluated before treatment (PRE) and after treatment (POST) to assess the effectiveness of CsA for the two groups. In the CAD group, CADESI-4 showed no change (PRE:79±29, POST:77±28) and out of the eight dogs, no dogs showed complete remission, three dogs showed partial remission, and five dogs showed no effect. Whereas in the ALD group, CADESI-4 showed a significant reduction (PRE: 61±42, POST: 32±25, $p<0.01$) and out of the 14 dogs, 11 dogs showed complete remission, two dogs showed partial remission, and one dog showed no effect. The results indicate that the immunosuppressant showed effectiveness for the dogs diagnosed with ALD. One dog had to be treated for a year and eight months, which was the longest period in the study, this dog presented with hyperplasia of the lymphoidgland and mammary tumor.

Key words: cyclosporine A, canine atopic dermatitis, canine atopic-like dermatitis, intrinsic

Introduction

In humans, atopic dermatitis (AD) has been divided into the extrinsic and intrinsic type (Brenninkmeijer et al. 2008, Kabashima et al. 2013). Extrinsic AD has high concentrations of total IgE and positive allergen-specific IgE levels whereas intrinsic AD is characterized by the absence of allergen-specific IgE (Brenninkmeijer et al. 2008). In the immune system, extrinsic AD is Th2-dominant which shows immediate (type 1) hypersensitivity and constitutes about 80% of AD, and intrinsic AD is Th1-dominant and constitutes about 20% of AD (Kabashima et al. 2013). In the veterinary field, there are some reports of dogs diagnosed with AD with the absence of allergen-specific IgE. The task force on canine atopic dermatitis (ACVD) classified CAD as follows: 1) associated with sensitization to environmental allergens, classified as

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canine atopic dermatitis (CAD); 2) clinical signs of CAD but no demonstrable allergen-specific IgE and skin tests, classified as canine atopic-like dermatitis (ALD) (Halliwell et al. 2006). Cyclosporin A (CsA) inhibits the phosphatase activity of calcineurin when complexed with specific binding proteins, and exerts its immunosuppressive effects through the down-regulation of nuclear factor of activated T cells (NFAT) transcription factor, thus preventing the transcription of T cell effector cytokines such as IL-2 (Prince et al. 1986, McCaffrey et al. 1993, Salowe et al. 1998, Hemenway and Heitman 1999).

Oral immunosuppressants such as CsA are used for organ transplantation in humans. In contrast, in the veterinary field, it is widely used for treatment of CAD (Olivry et al. 2002, Forsythe et al. 2014, Nuttall et al. 2014). In a previous study, CsA (10 mg/kg/BID for 8 days) was administrated to healthy dogs orally and IL-2 was significantly suppressed, whereas IL-4 was not suppressed (Archer et al. 2011). IL-2 is a Th1 related cytokine and intrinsic AD in humans is Th1-dominant (Kabashima et al. 2013), so CsA might be more effective against Th1-dominant conditions. Since ALD and intrinsic AD are similar in pathology for the absence of allergen-specific IgE we hypothesized that CsA may be effective for ALD. Our study, set out to assess effectiveness of CsA for the treatment of ALD.

Materials and Methods

Diagnosis of dogs

The diagnosis of CAD was made by ruling out other causes of the itch. All dogs received flea control and appropriate treatment for scabies mites. If bacterial pyoderma and yeast (*Malassezia dermatitis*) was diagnosed by cytology, it was treated mainly by shampoo therapy. All dogs underwent an elimination diet using hypoallergenic foods (Hill's prescription diet canine z/d Ultra: Hill's Pet Nutrition, KS, USA; or Royal Canin Veterinary Diet Sensitivity Control: Royal Canin, France; or Iams Veterinary Formulas FP: OH, USA) for at least 6 weeks. Diagnosis of CAD was based on compatible history and clinical signs of Favrot's criteria (Favrot et al. 2010). For all dogs diagnosed with CAD, on intradermal skin test (IDST) and/or allergen-specific IgE test (Animal Allergy Clinical Laboratories, Inc., Kanagawa, Japan) was performed and dogs which showed negative result for all environmental allergens were diagnosed with ALD or otherwise diagnosed with CAD. 22 dogs were enrolled in this study; eight in the CAD group and 14 in the ALD group.

Intradermal skin test and allergen-specific IgE test against environmental allergens

To determine the identity of the sensitized allergen, on intradermal allergy test was performed for 24 selected antigens. These antigens were subdivided into six environmental antigen groups (house dust mite (HDM) mix; *Dermatophagoides farina* and *Dermatophagoides pteronyssinus*, dust, epithelia, tree, weed, grass, mold) and flea antigen. The majority of commercial allergen preparations were purchased from Greer Laboratories (Lenoir, NC, USA). The remainder (Japanese cedar) was obtained from Torii Medicine (Tokyo, Japan). The mixed HDM extract was used at a concentration of 1,000 PNU/ml and 200 PNU/ml. House dust extract was used at a concentration of 100 PNU/ml and other antigens were at a concentration of 1,000 PNU/ml. All extracts were prepared and diluted as sterile diluents. During the intradermal allergy test, dogs were premedicated with atropine sulfate (0.04 mg/kg, subcutaneously) and sedated with xylazine (0.15 mg/kg, intravenously).

Allergen-specific IgE was measured with commercially available quantitative fluorometric enzyme-linked immunosorbent assay (Animal Allergy Clinical Laboratories, Inc., Kanagawa, Japan) (Okayama et al. 2011). The specific IgE measured in this assay included 22 environmental allergens (*Dermatophagoides farinae, Dermatophagoides pteronyssinus*, flea, mosquito, cockroach, mugwort, ragweed, goldenrod, dandelion, daisy, orchardgrass, sweet vernal, timothy, rye, bermuda, Japanese cedar, birch, alder, *Aspergillus fumigatus, Alternaria alternata, Cladosporium herbarum*, and *Penicillium notatum*).

Evaluation criteria

The criterion for remission was as follows: 1) dogs requiring only shampoo therapy to maintain the remission period and neither steroid treatment nor other anti-inflammatory treatments being needed were classified as complete remission (CR); 2) dogs requiring some percutaneous steroid treatment to maintain the remission period but no oral steroid treatment were classified as partial remission (PR); 3) dogs requiring oral steroid treatment to control the atopic dermatitis were classified as no effect.

CADESI-4 (Canine Atopic Dermatitis Extent and Severity Index)

CADESI-4 was used to assess lesion severity. The severity of erythema, lichenification and alopecia/ex-

Table 1. CADESI-4 score before and after CsA treatment of CAD (Extrinsic AD) group.

No.	Breed	CsA dose (mg/kg)	Sensitized allergen	CADESI-4		Treatment period and effectiveness
				PRE	POST	
1	Shih Tzu	3.8	HDM	45	51	No effect(2.5 m)
2	French Bulldog	2.5	HDM	117	98	PR(4 m)
3	Toy Poodle	5.2	HDM	98	103	No effect (1 m)
4	Shih Tzu	3.8	HDM	80	87	Noeffect (1.5 m)
5	Shiba Inu	4.7	HDM	84	75	PR (6 m)
6	Miniature pinscher	5.5	HDM	51	39	PR (3 m)
7	Shih Tzu	5.2	penicillium	45	48	No effect (3 m)
8	mongrel	4.5	HDM	111	114	No effect (1 m)
			Mean ± SD	79 ± 29	77 ± 28	

CR: Complete remission. PR: Partial remission, y: year, m: month

Table 2. CADESI-4 score before and after CsA treatment of ALD (Intrinsic AD) group.

No.	Breed	CsA dose (mg/kg)	Sensitized allergen	CADESI-4		Treatment period and effectiveness
				PRE	POST	
1	Chihuahua	4.3	–	18	8	CR (4 m)
2	Miniature Dachshund	4.5	–	92	34	CR (9 m)
3	West Highland White Terrier	5.5	–	114	105	CR (1y8m)
4	Shih Tzu	4.4	–	50	41	PR (1y5m), relapse
5	Toy Poodle	5.1	–	20	24	PR(8 m), relapse
6	Labrador Retreiver	2.5	–	116	31	CR (4 m), relapse
7	Miniature Dachshund	4.5	–	157	55	CR (8 m)
8	Miniature pinscher	6.5	–	39	41	No effect (1 m)
9	Pomeranian	6.2	–	39	13	CR (6 m)
10	Chihuahua	4.2	–	53	19	CR (6 m)
11	Toy Poodle	6.2	–	45	30	CR (4 m)
12	Toy Poodle	5	–	23	15	CR (3 m)
13	Bichon Frise	5.1	–	30	3	CR (4 m)
14	Miniature Schnauzer	3.7	–	53	29	CR (4 m), ADR
			Mean ± SD	61 ± 42	32 ± 25	

CR: Complete remission, PR: Partial remission, y: year, m: month, ADR: Adverse reaction

Table 3. Evaluation of CsA effectiveness.

CAD (Extrinsic AD) group			ALD (Intrinsic AD) group		
Evaluation	Number of dogs (%)		Evaluation	Number of dogs (%)	
No effect	5	(62.5)	No effect	1	(7.1)
PR	3	(37.5)	PR	2	(14.3)
CR	0	(0.00)	CR	11	(78.6)
Total	8		Total	14	

coriation was assessed at 20 body sites using a scale from 0-3 (0=none, 1=mild, 2=moderate, and 3=severe) (Olivry et al. 2014)

Cyclosporin A

In the present study, two different formulations of CsA called Atopica® (Novartis Animal Health Inc. Basel, Switzerland) or Ciclocap® (Generic preparation.Veterinarian medical development Co Ltd. Saitama, Japan) were used for treatment. The Administeed dose of CsA was 2.5 to 5.5 mg/kg (median: 4.6 mg/kg) for the CAD group and 2.5 to 6.5 mg/kg (median: 4.8 mg/kg) for the ALD group, respectively. CsA was administeed orally between meals by the dogs' owners. Blood serum chemistry was examined before administration and after administration (once a month) for all dogs. The frequency of administration was as follows: daily for the first 2 months, every

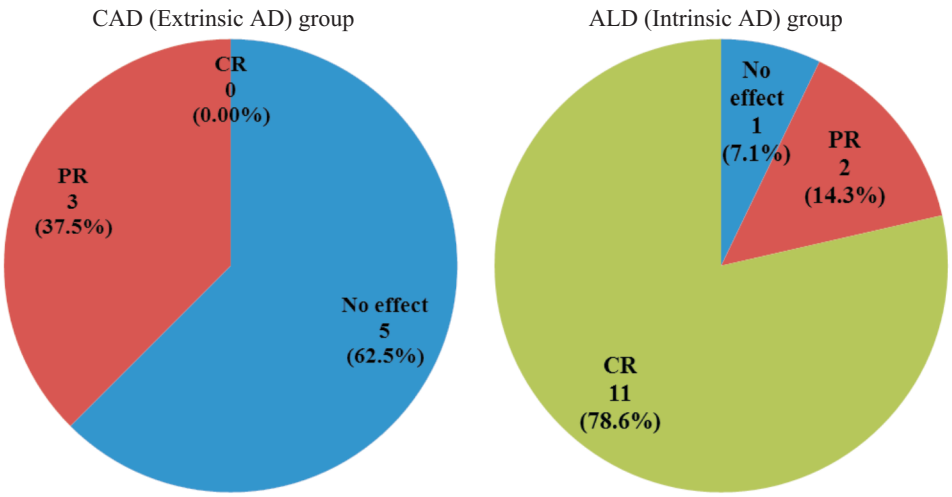

Fig. 1. Evaluation of CsA effectiveness. Shown in green is CR, red is PR, and blue is no effect, respectively.

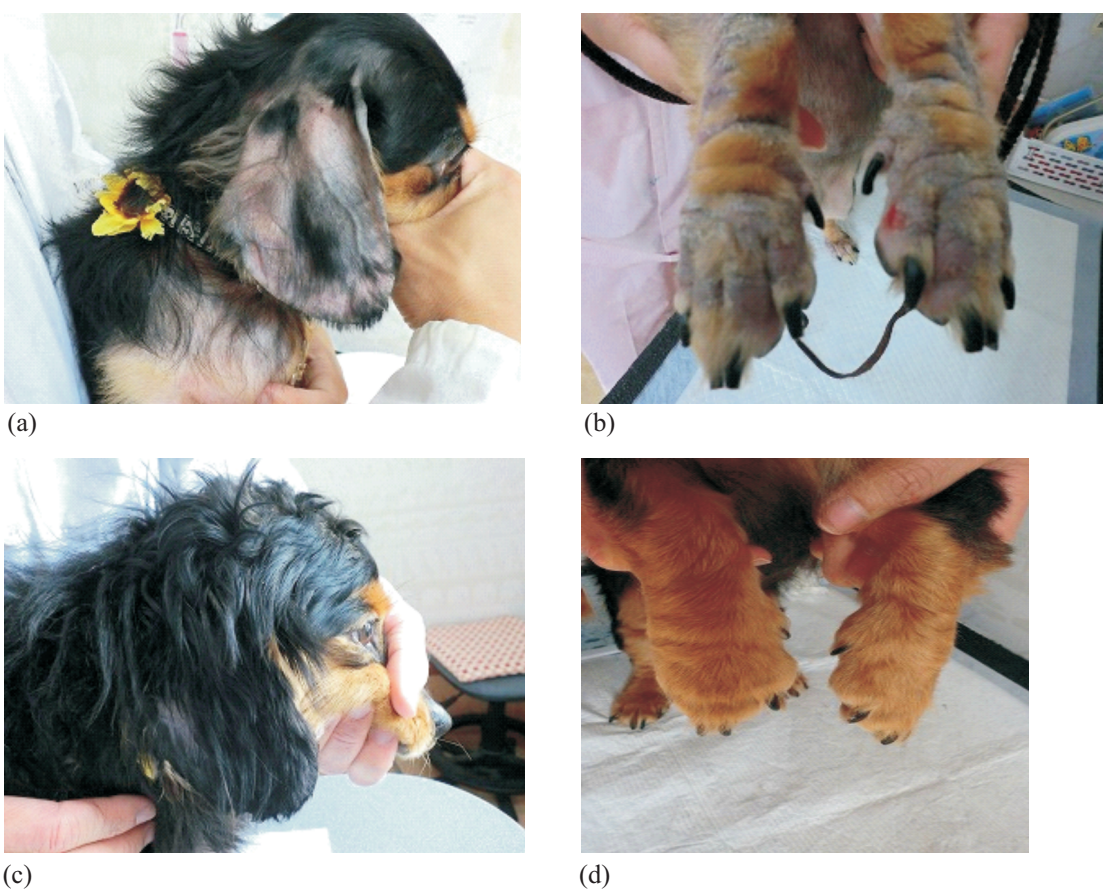

Fig. 2. Clinical characteristics of Dog no. 2 at start of the treatment (Pre) are shown in (a) and (b), and after 9 months of the treatment (Post) are shown in (c) and (d), respectively.

2 days for the third month, and twice a week thereafter. During the trial, the treatment was stopped for any dogs that showed increased CADESI-4 and these were regarded as having no effect.

Statistical Analysis

Statistical analysis was performed using the Wilcoxon signed-rank test, and statistical significance was defined as $p<0.01$.

Results

In the CAD group, CADESI-4 showed no change (PRE:79±29, POST:77±28), and out of eight dogs, no dogs showed CR, three dogs showed PR, and five dogs showed no effect (Table 1 and 3, Fig. 1). The sensitized allergens were HDM for seven dogs and penicillium for one dog.

In the ALD group, CADESI-4 was significantly reduced (PRE: 61±42, POST: 32±25, $p<0.01$), and out of 14 dogs, 11 dogs showed CR, two dogs showed PR, and one dog showed no effect. The administration period for CR was three to four months for six dogs, six months for two dogs, eight to nine months for two dogs, and one year and eight months for one dog. For PR, the administration period was eight months for one dog, and one year and five months for one dog (Table 2, 3, Figure 1, 2). No severe adverse effects were observed during the study in the CAD group except that one dog (No. 14) in the ALD group showed severe liver damage (GPT>450 IU/L) a week after administration. The dog did complete the study after treatment for the liver condition with a decreased dose of CsA. Dog No.6 in the ALD group showed complete remission but relapsed 6 months after the study. Dog No. 3 in the ALD group had to be treated for a year and eight months which was the longest period in the study. This dog showed hyperplasia of lymphoid and mammary tumor but it is unclear if this was related to CsA treatment. Dog No. 8 showed no effectiveness with 6.5 mg/kg CsA, but 2 mg/kg oral steroid showed moderate effectiveness to pruritus.

Discussion

Previous studies have shown that ALD constitutes 14 to 25% of CAD, which is similar to that of intrinsic AD in humans (20%)(Tokura 2010, Fujimura 2011, Kabashima et al. 2013, Kawano et al. 2013, Suto et al. 2014). From what we know of the mode of action of CsA, it may be more effective against intrinsic AD, because IL-2 is a Th1 related cytokine and intrinsic AD in humans is Th1-dominant. Since ALD and intrinsic AD are similar in pathology for the absence of allergen-specific IgE, CsA might be effective in treating ALD. The results of our present study show that this is the case.

Contact allergy such as metal allergy mainly consists of intrinsic AD in humans (Kabashima et al. 2013), but this remains controversial (Karimkhani et al. 2015) and may be due to autoallergy (Hradetzky et al. 2015). On the other hand, the International Committee on Allergic Diseases of Animals (ICADA) reported that it is still unknown whether there is indeed an absence of allergen-specific IgE in ALD or if there is just a failure to find a specific allergen upon examination (Pucheu-Haston et al. 2015). For these reasons, the ICADA categorize both CAD and ALD collectively as CAD. The cause of the condition might be due to an unknown allergen-specific IgE or complication of CAD and food allergy but these are difficult to diagnose correctly. Interestingly, the result of histopathology and cytokine profile of CAD and food allergy induced CAD are similar, so it is possible that ALD is related to delayed-type food allergy induced CAD (Suto et al. 2014, Pucheu-Haston et al. 2015).

Our study showed CR in 11 of the 14 dogs in the ALD group. From this result CsA might be especially useful in ALD. Low doses of CsA against AD showed effectiveness in a human study (Brandt et al. 2009) and therefore may also be useful for treatment of ALD in the veterinary field. In the present study, dog No. 6 in the ALD group showed CR with low-dose CsA (2.5 mg/kg) but relapsed 6 months later. In a previous study, CsA was administered to 51 dogs (5 mg/kg orally) for six to 24 months and 11 dogs (22%) showed PR and 12 dogs (24%) showed CR (Radowicz and Power 2005). This study did not distinguish ALD from CAD, but since the percentage of CR (24%) was close to that of the ALD percentage (14 to 17%), there is a possibility that the dogs exhibiting CR had ALD.

The present study showed that CsA is effective against ALD, which is more difficult to treat than CAD. These results suggest an answer to ICADA's question, the mechanism of ALD. Our study, which is based on clinical observations, suggests that the next step would be to examine if cytokines are involved.

Conclusion

In conclusion, CsA was more effective against ALD than CAD. These results suggest that there is a difference between CAD and ALD, and that it will be important to correctly diagnose ALD and CAD so that effective treatment for either condition can be prescribed.

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