Effects of cyclosporine A on clinical and histologic abnormalities in dogs with sebaceous adenitis

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Objective—To evaluate the effects of cyclosporine A in the treatment of sebaceous adenitis in dogs.

Design—Open-label clinical trial.

Animals—12 dogs with sebaceous adenitis.

Procedure—Dogs were treated with cyclosporine A at a dosage of 5 mg/kg/d (2.3 mg/lb/d), PO, for 12 months and reevaluated every 4 months. A clinical score was calculated by grading the extent of alopecia and severity of follicular casts as absent, mild, moderate, or severe in each of 17 body regions. Biopsy specimens were obtained and examined histologically and by means of immunohistochemical staining.

Results—Mean clinical score was significantly decreased, compared with baseline score, after 4 months of treatment and remained low after 8 and 12 months. Histologically, the severity of inflammation was significantly decreased, as were numbers of macrophages, CD3+ T cells, and major histocompatibility complex class II-expressing cells. The percentage of hair follicles with sebaceous glands increased, suggesting regeneration of sebaceous glands. Clinical signs recurred when cyclosporine administration was discontinued.

Conclusions and Clinical Relevance—Results suggest that administration of cyclosporine A at a dosage of 5 mg/kg/d may reduce the inflammation associated with sebaceous adenitis in dogs. Long-term treatment appears to be necessary to control the disease. (J Am Vet Med Assoc 2005;226:59–64)

Sebaceous adenitis (SA) is an uncommon skin disease in dogs. It is most commonly identified in Standard Poodles but is also seen in Akitas, Samoyeds, Vizslas, and German Shepherd Dogs and has been diagnosed in dogs of various other breeds as well as in dogs of mixed breeding.14 Sebaceous adenitis has on rare occasions been identified in other animal species such as cats and rabbits,15,16 but to our knowledge, there are only 2 published reports9,10 of SA in humans.

Clinical signs of SA are usually first identified when dogs are young to middle-aged; there is no apparent sex predilection.13 The clinical features of SA vary among breeds but always include the appearance of silvery white dandruff and scales that are adherent to the hair shafts called follicular casts. It has been suggested that the clinical picture of SA can be divided into 2 groups. The first group is represented by long-coated dogs, with Standard Poodles, Akitas, and Samoyeds being the most extensively studied examples.12,13 In these dogs, the first sign of disease is symmetric multifocal alopecia characterized by broken hair shafts and associated with scaling and a general appearance of a dull and brittle hair coat. Lesions start on the head, pinnae, dorsal aspect of the neck, and tail and extend to the dorsal midline. At this stage, there is little pruritus. As the disease progresses, it may generalize, and secondary bacterial folliculitis with pruritus and malodor is common at this stage.13 The clinical course may wax and wane with no apparent seasonality. The second group of dogs with SA is represented by short-coated dogs such as Vizslas. Lesions tend to be more arciform with coalescing areas of alopecia and fine nonadherent scaling.14,16

Histologic abnormalities in dogs with SA are variable, and their character changes in association with chronicity of the disease.11,13,17 In the early phase, discrete perifollicular inflammatory cells are present at the isthmus level of hair follicles.21,18 Later on, the most common finding is a nodular, granulomatous to pyogranulomatous inflammatory reaction around the sebaceous glands. Sebocytes are destroyed and may no longer be detectable in histologic sections. However, hair follicles themselves and the apocrine sweat glands are not affected by the inflammation. The inflammatory infiltrate consists of histiocytes, lymphocytes, and neutrophils.19,20 In long-coated breeds, a marked orthokeratotic hyperkeratosis is detectable within the follicular infundibulum, whereas in short-coated breeds, the hyperkeratotic changes are reportedly milder.21 In advanced stages of the disease, the sebaceous glands are completely destroyed and the inflammatory reaction becomes sparse. Telogenization of hair follicles or follicular atrophy may occur. If a secondary staphylococcal infection is present, supplicative folliculitis or furunculosis will be seen.

The pathogenesis of SA is still unknown, although it has been reported that SA is inherited as an autosomal recessive trait in Standard Poodles and Akitas.2,12,13,20,21 It has been postulated that SA results from a primary structural defect in the sebaceous glands or their ducts that is responsible for leakage of sebum and a subsequent foreign body reaction.2 Alternatively, SA may represent an autoimmune reac-
tion against the sebaceous glands because an immunohistochemical study has shown that the major cell populations in SA are dendritic, antigen-presenting cells and CD4+CD8+ T-cells as well as CD4+CD8- T-cells. In contrast, B cells are rarely found and circulating or tissue-bound autoantibodies against sebaceous glands have not been detected. Finally, it has been suggested that SA may be a result of abnormalities in lipid metabolism that affect the production of sebum.

Currently, there is no gold standard for the treatment of SA in dogs, and a variety of treatment protocols have been published. Administration of corticosteroids at immunosuppressive dosages has been reported to be effective in some short-coated dogs but had no effect in others. Synthetic retinoids have been used for their anti-inflammatory properties, their effects on keratinocyte differentiation, and their inhibitory effect on sebaceous glands. They have been reported to be effective in Vizslas but in other breeds, no or almost no effects have been seen.

Both corticosteroids and retinoids are associated with severe adverse effects when administered long term and therefore are not the treatment of choice, particularly if they are only partially effective. Oral administration of essential fatty acids at high dosages seems to ameliorate clinical signs and topical treatment with anti-inflammatory shampoos, emollient rinses, and benzoyl peroxide, alone or in combination with systemic therapy, has been shown to provide good results in some dogs. For dogs with severe disease and no response to other treatments, a variety of immunosuppressive agents, including glucocorticoids, azathioprine, and cyclosporine A have been used in an attempt to diminish the plugging of the follicular infundibulum with keratinaceous material in affected areas.

Cyclosporine A (CyA) can induce hypertrichosis and as a potent growth factor for T cell proliferation. Cyclosporine A selectively suppresses the induction and proliferation of cytotoxic T cells by blocking gene transcription of T cell cytokines, especially interleukin-2, which is a potent growth factor for T cell proliferation. Cyclosporine A can induce hypertrichosis and as such, might be able to diminish the plugging of the follicular infundibulum with keratinaceous material in affected dogs. In mice, CyA is a potent inducer of the hair growth phase (anagen) and thus could inhibit the telogenization or atrophy of hair follicles that is often observed in dogs with chronic SA. In isolated reports, CyA has been shown to provide good to excellent results when used alone or in combination with ketoconazole for the treatment of SA. However, there is as yet no systematic study of the effects of CyA in dogs with SA. Therefore, the purpose of the study reported here was to determine the effects of CyA in dogs with SA. In addition to changes in clinical signs, changes in histologic, immunohistochemical, and histomorphometric findings were used to assess the effects of CyA.

Materials and Methods

Dogs—Twelve dogs with idiopathic SA were included in the study. Dogs were recruited from 2 veterinary practices in Germany over a period of 9 months. The 12 dogs consisted of 4 Hovawarts, 3 Akitas, 2 Bernese Mountain Dogs, a mixed-breed dog with Bernese Mountain Dog phenotype, a Muensterlander, and a Schnauzer. Mean age was 5.5 years (range, 3 to 7 years). There were 5 sexually intact males, 3 castrated males, 3 sexual-ly intact females, and 1 spayed female. Mean time since the onset of disease was 11.5 months (range, 0.5 to 3 years).
isthmus level was counted and also expressed as a percentage of all hair follicles visible in the tissue section. For each hair follicle with perifollicular inflammation, the severity of the inflammatory reaction was graded as absent, mild, moderate, or severe. Inflammation was considered mild if only a few (<10/hpf) inflammatory cells were seen and inflammatory cells did not obscure the architecture of the sebaceous glands or follicular infundibulum. Inflammation was considered moderate if >10 inflammatory cells were seen per high-power field, but inflammatory cells were restricted to the isthmus region of the hair follicles. Inflammation was considered severe if abundant inflammatory cells (>30/hpf) were seen, and inflammatory cells obscured the isthmus area, spreading into the perifollicular region of the infundibulum and the lower portion. The percentage of hair follicles with mild inflammation was multiplied by 1, the percentage with moderate inflammation was multiplied by 2, and the percentage with severe inflammation was multiplied by 3. Numbers were added, resulting in an inflammation score that represented severity and extent of perifollicular inflammation. Finally, in each biopsy section, infundibular hyperkeratosis was graded as absent, mild, moderate, or severe.

**Treatment**—After the diagnosis of SA was confirmed, dogs were treated with CyA (5 mg/kg/d [2.3 mg/lb/d], PO). Cyclosporine A was administered 2 hours before a meal, as recommended by the manufacturer. Treatment with CyA was continued for 12 months. No other treatment was allowed, except that administration of a single 3- to 4-week course of antimicrobials was allowed in dogs that developed secondary pyoderma.

**Follow-up examinations**—Dogs were reevaluated every 4 months. A clinical score was assigned on the basis of extent of alopecia and severity of follicular casts. Serum urea nitrogen and creatinine concentrations and serum alanine aminotransferase and aspartate aminotransferase activities were measured, and owners were asked whether they had noticed any adverse effects. Two biopsy specimens were again taken from the right shoulder region close to the neck and from the lumbar region and were submitted for histologic examination, immunohistochemical staining, and histomorphometry.

**Statistical analyses**—The following parameters were recorded every fourth month during the 12-month treatment period: clinical score, inflammation score, percentage of hair follicles with sebaceous glands, number of MAC387-reactive macrophages, number of MHC class II-expressing cells, and number of CD3+ T cells. Mean values were calculated for each evaluation time point (0, 4, 8, and 12 months of treatment) and were compared over time by use of the general linear model procedure and the Dunnett 1-tailed t test. Standard software was used for all analyses. Values of $P \leq 0.05$ were considered significant.

**Results**

Serum urea nitrogen and creatinine concentrations and serum alanine aminotransferase and aspartate aminotransferase activities were within reference limits in all dogs throughout the study. All 12 dogs completed the study; however, in 1 dog, biopsy specimens could not be obtained at the completion of the study for unrelated reasons.

Mean clinical score after 4 months of treatment with CyA was significantly decreased, compared with baseline mean clinical score (Figure 1). There was a slight, although insignificant, further decrease in mean clinical score between the 4th and 12th month of treatment. One dog had a substantial increase in clinical
score between months 4 and 8, but clinical score had decreased again by month 12. Two dogs had substantial increases in clinical score between months 8 and 12.

Mean inflammation score after 4 months of treatment with CyA was also significantly decreased, compared with mean baseline score, and remained decreased at months 8 and 12 (Figure 2).

Results of immunohistochemical staining indicated that prior to treatment with CyA, there were high numbers of MAC387-reactive macrophages, MHC class II-expressing cells, and CD3+ T cells in the perifollicular area in biopsy specimens (Figures 3–5). In 2 dogs, low numbers of MAC387-reactive macrophages and MHC class II-expressing cells in biopsy specimens after 12 months of treatment. One dog had an increase in the number of MHC class II-expressing cells coincident with the development of lymphoplasmacytic lichenoid dermatitis, which was interpreted as a drug eruption. Administration of CyA was discontinued, and the lichenoid dermatitis resolved within 4 weeks.

The percentage of hair follicles with sebaceous glands increased significantly during treatment with CyA (Figure 6). At the beginning of the study, only 2% of hair follicles had sebaceous glands, but after 4 months of treatment with CyA, 32% did, and after 12 months of treatment, 40% did. When results for individual dogs were examined, it was found that in 4 dogs, ≥70% of hair follicles had sebaceous glands after 12 months of CyA treatment and in 3 dogs, 20% to 50% of hair follicles had sebaceous glands. In the remaining 5 dogs, <10% of hair follicles had sebaceous glands after 12 months of CyA treatment.

The severity of infundibular hyperkeratosis did not change significantly during the course of the study.

### Discussion

The 12 dogs included in the present study were quite heterogenous in regard to age, sex, breed, duration of disease, and previous treatment. Age at the onset of clinical signs and duration of signs prior to diagnosis in these dogs were similar to what has been described in the literature. Three of the dogs were Akitas, a breed that is reported to have a predisposition for SA. Four were Hovawarts, a breed that is relatively common in Germany and in which, in our experience, SA occurs relatively commonly. The fact that no Standard Poodles were included in the study may reflect the relative rarity of this breed in Germany or may suggest that Standard Poodles in Germany are from a different genetic pool, compared with Standard Poodles in North America, where most studies of SA involving Standard Poodles have been conducted.

In the present study, mean clinical score was significantly decreased, compared with baseline score, after 4 months of treatment with CyA. Subjectively, both the extent of alopecia and the severity of scaling improved in all dogs, resulting in an overall much better quality of the hair coat. Clinical improvement was most evident within the first 4 months after the initiation of treatment, with little additional improvement seen at months 8 and 12.

This improvement in clinical score was accompanied by an improvement in the severity and extent of
perifollicular inflammation at the isthmus level of the hair follicles, as reflected by the inflammation score. The inflammatory reaction had almost completely resolved after 12 months of CyA administration. Specifically, the numbers of MAC387-reactive macrophages and CD3+ T cells in the sebaceous gland region decreased so that few were seen at the 12-month follow-up examination. Macrophages and T cells are known to be effector cells in cell-mediated autoimmune diseases. The decrease in their numbers during treatment with CyA in the present study therefore supports the theory that SA in dogs is a cell-mediated autoimmune disorder. Additionally, the number of cells expressing MHC class II, which is involved in antigen presentation and maintenance of autoimmunity, was reduced with CyA administration.

In all dogs, sebaceous glands were largely absent from skin biopsy specimens obtained at the beginning of the study, a finding that is common in dogs with sub-acute to chronic SA. Surprisingly, the reduction in the inflammatory reaction during treatment with CyA was accompanied by an increase in the percentage of hair follicles with sebaceous glands, suggesting that sebaceous glands regenerated. Indeed, small sebaceous glands were found in some dogs after 4 and 8 months of treatment. Regeneration of sebaceous glands was not further investigated morphologically (eg, by use of proliferation markers), but it is likely that regeneration was a result of proliferation of reserve or stem cells located in the hair follicle isthmus area. That said, the percentage of hair follicles with sebaceous glands after 12 months of CyA treatment, and in 2 of these dogs, ≥ 82% had sebaceous glands, a value that, in our experience, is comparable to the value for healthy dogs. In 3 dogs, regeneration was limited, with only 20% to 50% of hair follicles having sebaceous glands at the end of the study, and in the remaining 5 dogs, there was hardly any regeneration of the sebaceous glands. Currently, why some dogs apparently had sebaceous gland regeneration and some did not is not known. No correlation with duration of disease, age, or breed was found.

Although most dogs had tremendous clinical improvement after 12 months of treatment, in 2 dogs, clinical scores at month 12 were higher than baseline scores despite continuous treatment with CyA. One of these dogs had marked clinical worsening, with hyperkeratosis of the pinnae, dorsum, and tail, during the last third of the study that was associated with a slight increase in inflammation score and a marked decrease in the percentage of hair follicles with sebaceous glands. However, the clinical worsening was likely unrelated to SA since this dog developed concurrent eosinophilic (possibly allergic) dermatitis. The other dog responded well to CyA treatment for the first 4 months. However, because the disease was so long standing in this dog (> 3 years), there were no sebaceous glands at the beginning of the study and no perifollicular inflammation that could be suppressed by CyA. Clinical improvement in this dog was likely related to an anagen-initiating effect of CyA since this dog had been treated with glucocorticoids and hair follicles were therefore in the telogen phase. We conclude that CyA did not have an affect on severity of SA in this dog.

In 1 dog, a temporary worsening of the clinical condition was observed between months 4 and 8. Because this increase in clinical score was not associated with an increase in the inflammation score, number of T cells, or number of MAC387-reactive macrophages or a decrease in the percentage of hair follicles with sebaceous glands, it was not considered to be related to SA. It is possible that climatic conditions played an important role in that clinical worsening was observed during high temperatures in the summer and the dog was known to respond to high temperatures with a worsening of coat quality.

Ten dogs in the present study had marked improvement after 12 months of treatment with CyA. In 2 of these dogs, administration of CyA was continued after the end of the study and clinical status remained constant. In the other 8 dogs, CyA administration was discontinued. Two of the 8 had a recurrence of SA verified histologically. In both, treatment with CyA was reinstated and clinical improvement was again seen. In the remaining 6 dogs, treatment with topical emollients resulted in some clinical benefit after CyA administration was discontinued. In 1 dog, SA did not recur despite discontinuation of treatment. Therefore, we conclude that although complete remission of disease might be possible in rare cases, most dogs will require life-long treatment with CyA to control SA.

Treatment with CyA was considered safe since few adverse effects were noticed during this study. One dog developed a drug eruption after 8 months of treatment, a rare adverse event in dogs. Administration of CyA was discontinued, and the lichenoid dermatitis resolved. Other adverse events that were observed included vomiting, diarrhea, and gingival hyperplasia, which are also well-known adverse effects of CyA.

In summary, treatment with CyA resulted in clinical improvement in dogs with SA, with the greatest improvement evident within 4 months after the initiation of treatment. There was some evidence that CyA was of limited benefit in dogs with chronic disease in which the perifollicular inflammatory reaction had already resolved. Therefore, treatment with CyA should be initiated as early as possible during the course of the disease. Clinical improvement was associated with a marked reduction in perifollicular inflammation, reduced expression of MHC class II, and reduced numbers of T cells and macrophages. Simultaneously, sebaceous glands appeared to regenerate since more hair follicles with well-developed sebaceous glands could be found after initiation of treatment with CyA. Life-long treatment appears to be necessary since cessation of treatment was associated with relapses. The treatment was well tolerated.

Additional studies are needed to evaluate the benefit of CyA in larger numbers of dogs with SA. Since SA is generally progressive without treatment and spontaneous remission has only been reported anecdotally, inclusion of a group of dogs treated with a placebo in the present study was not considered reasonable. Instead, treatment with CyA should be compared
against topical treatment with emollients. A further group should evaluate the potential effects of combined CyA administration and topical treatment.


b. Clone TAL.185, DAKO Cytomation, Hamburg, Germany.

c. Clone CD3-12, Serotec GmbH, Duesseldorf, Germany.

d. Dianova GmbH, Hamburg, Germany.

e. Vector, Burlingame, Calif.

f. Neoral soft gelatin capsules, distributed in the United States by Novartis Pharmaceuticals Corp. East Hanover, NJ.

g. SAS version 6.12, SAS Institute Inc, Cary, NC.

References


