

Evaluation of the Effect of Two Dose Rates of Cyclosporine on the Severity of Perianal Fistulae Lesions and Associated Clinical Signs in Dogs

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Objective—To investigate the effect of cyclosporine (2 or 5 mg/kg every 24 hours) on perianal fistulae (PAF) lesions.

Study Design—Blinded randomized, prospective trial.

Animals—Dogs (n = 20) with perianal fistulae.

Methods—Dogs were randomly assigned to administration of either 2 mg/kg (n = 10) or 5 mg/kg (n = 10) of cyclosporine orally every 24 hours for 8 weeks. Lesion surface area was measured, lesion severity was graded using a visual analog scale, and the presence and severity of clinical signs recorded every 2 weeks.

Results—Lesion variables were significantly reduced in both groups after 8 weeks and owners also reported a reduction in clinical sign severity. The 5 mg/kg dose rate significantly accelerated lesion resolution compared with 2 mg/kg. In the 2 mg/kg group, 20% of dogs had complete resolution of clinical signs and 10% had resolution of lesions. In the 5 mg/kg group, 40% of dogs had complete resolution of clinical signs and 60% had resolution of lesions.

Conclusions—A dose rate of 5 mg/kg every 24 hours was more effective at reducing the surface area and severity of PAF lesions than 2 mg/kg every 24 hours but less effective at resolving PAF lesions than previous studies using dose rates \geq 5 mg/kg every 12 hours.

Clinical Relevance—Cyclosporine at 5 mg/kg every 24 hours may be useful for the palliation of PAF lesions.

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INTRODUCTION

CANINE PERIANAL FISTULAE (PAF) are a chronic, progressive disease characterized by the development of cutaneous and rectocutaneous fistulae, and ulceration within the perianal tissues. The disease occurs most commonly in German shepherd dogs (GSD) although other breeds can be affected.¹ Clinical signs include tenesmus, dyschezia, constipation, and mucopurulent discharge from the perineum.^{2–5}

Treatment of PAF remains challenging because of lack of understanding of its etiopathogenesis. Similarities between the clinical appearance of PAF in dogs and perianal fistulae that develop in humans with Crohn's disease have resulted in speculation that these diseases may share a similar etiopathogenesis.⁶ Preliminary histologic and immunohistochemical examination of perianal tissue from dogs with PAF has demonstrated a possible immune-mediated cause and more recent analysis of cytokine mRNA expression from perianal tissue samples

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found a bias of cytokines associated with a TH-1 T-cell response, further suggesting that PAF may be associated with a failure of immunomodulation.^{7,8}

Cyclosporine has been used successfully for the treatment of PAF in humans and based on these results, Mathews and others reported use of cyclosporine for treatment of PAF in dogs.^{9,10} Cyclosporine is a potent immunosuppressive agent that inhibits T-cell activation and suppresses cell-mediated immune responses.¹¹⁻¹³ The absorption and metabolism of cyclosporine can vary markedly in dogs although the newer microemulsion formulation of cyclosporine has improved bioavailability and pharmacokinetic properties.¹¹⁻¹⁴ Originally reported dose rates of cyclosporine for treatment of PAF were derived from the therapeutic range used for immunosuppression of dogs with renal allografts.^{10,15} Doses varying from 5 to 10 mg/kg every 12 hours resulted in excellent improvement in clinical signs (96–100%) and good lesion resolution rates (72–100%); however, costs associated with these dose rates was relatively high.^{10,16,17}

Unfortunately, the relatively high cost of cyclosporine remains a limitation for its use in the management of PAF and highlights the need to determine the lowest effective dosing protocol. More recent reports suggest that the original dosing recommendations may be higher than necessary and lower dose rates may provide similar efficacy and at the same time reduce treatment costs.^{18,19} Previous studies evaluating lower dose rates (<7.5 mg/kg every 24 hours) of cyclosporine have produced conflicting results about efficacy of these lower dose rates for the management of PAF lesions.^{18,19} Use of ketoconazole as an adjuvant agent to slow the metabolism of cyclosporine is a widely accepted strategy to reduce cost; however, use of ketoconazole introduces the potential for additional side effects.^{20,21} There are very few studies comparing the efficacy of different dose rates of cyclosporine for the management of PAF, and an “ideal” dose rate has yet to be determined.

Our objective was to investigate the effect of 2 and 5 mg/kg of cyclosporine every 24 hours for 8 weeks on the severity of PAF lesions and associated clinical signs. Our hypothesis was that 5 mg/kg of cyclosporine every 24 hours would be more effective than 2 mg/kg every 24 hours at resolving PAF lesions and associated clinical signs in dogs with PAF.

MATERIALS AND METHODS

Study Population

Twenty consecutive dogs admitted for treatment of PAF were included. Each dog had a physical examination, complete blood count, and serum biochemistry profile before entry into the study. PAF diagnosis was based on history, clinical

signs, and exclusion of other causes of PAF by histopathologic evaluation of lesion biopsies. Persistent disease was observed in all dogs. None of the dogs had been administered cyclosporine within 3 months of entry into the study. Dogs were not administered any other medication during the study and diet was not standardized. Dogs were randomly assigned to administration of either 2 mg/kg (n=10) or 5 mg/kg (n=10) of cyclosporine (Neoral, Novartis, Basel, Switzerland) orally every 24 hours for 8 weeks. Owners were unaware of the dose rate administered to their dog and signed an informed consent form, and were advised of their right to withdraw their dog from the study at any time.

Clinical Assessment

Before treatment, each owner was asked to grade the severity of their dog's clinical signs on a scale from 0 to 10 (with 0 being not present to 10 being most severe) using a standardized questionnaire. The clinical signs evaluated included: licking and chewing of the perianal lesions, discharge from the perianal region, straining to defecate, and bleeding from the perianal region. A combined clinical score was calculated for each dog by summation of the severity grades assigned by the owner for each individual clinical sign.

A single investigator (A.K.H.) performed all PAF lesion measurements, and was the primary clinician responsible for patient care and was not blinded to the treatment each dog was administered. For the initial examination the dogs were anesthetized. A digital rectal examination was performed and PAF lesions photographed. Presence of rectal or anal strictures and anal sac involvement was recorded. A stricture was defined as the inability of the investigator to easily insert the 3 middle fingers of the right hand (approximately 4.1 cm diameter) into the anus because of abnormal tissue in the anal or rectal structures. Anal sac involvement was defined as the presence of thickening within the region of the anal sac on palpation or the identification of a fistula communicating with the anal sac.

Photographs were taken of the lesions and the surface area (mm²) calculated from the photographs using computer-imaging software (Scion Image software, Scion Corporation, NY). To standardize lesion photography, dogs were positioned in sternal recumbency, the perianal region was clipped to remove hair and the tail was held in a vertical position. The camera was positioned level with, and parallel to, the anus. All photographs included a scale with 1 mm graduations placed on the skin surface immediately ventral to the anus. The surface area of each lesion was calculated by importing digital images of the photographs into computer-imaging software and tracing the interface between the healthy skin and the lesion. The software calculated the area of the region traced with reference to the scale on the image.

Digital images of the photographs were assessed by a second investigator (S.P.G.), who was blinded to the treatment received by each dog. PAF lesion severity was graded using a visual analog scale (VAS) that was 100 mm in length. One end of the scale was marked as 0, which represented no lesion present and the other end was marked as 100, which

represented a case of PAF in which the lesions surrounded the entire circumference of the anus and complete destruction of perianal tissues had occurred. The investigator marked on the scale the position between these two points that best represented the severity of the lesion. The score was determined as the distance (millimeters) on the scale from 0 (no lesion present).

Dogs were re-examined every 2 weeks and the lesions assessed and photographed. Photographs of the lesions were obtained using the protocol described for the initial examination with the modification that the dogs were not anesthetized and were standing. Lesion surface area and lesion severity were assessed as described for the initial examination.

Owners were asked to grade the severity of clinical signs using the same standardized questionnaire. Owners were also questioned as to whether any side effects were observed while their dog was administered cyclosporine. After 8 weeks of cyclosporine treatment, owners with dogs that had persistent clinical signs were asked if they perceived these persistent clinical signs to be negatively affecting the dog's quality of life.

Trough blood levels of cyclosporine (measured by monoclonal radio-immunoassay using a whole blood sample taken 24 hours after the previous dose of cyclosporine¹²) were measured 2 and 4 weeks after initiation of therapy.

Data Analysis

Differences in the lesion variables (surface area and visual analog score for lesion severity) were assessed within treatment groups at admission and after 8 weeks of treatment using a Wilcoxon Signed Ranks test. A *P*-value of $<.05$ was considered significant. Differences in the cyclosporine trough levels between groups were analyzed using a Mann-Whitney U-test of association, with a significance set at $P<.05$.

To assess the differences in surface area of the lesions and the VAS of lesion severity between groups during the 8 weeks, fitted models to each response using generalized estimating equations (GEE) were used. The Gaussian distribution with auto regressive correlation structure was used to model the responses. Time was included as a continuous variable after

assessing the linearity of its effect. The interaction between dose rate and time was included in each initial model and retained if significant ($P<.05$).²² Regression models were created by use of commercial software (PROC GENMOD, SAS Institute Inc., Cary, NC).

Efficacy of randomization was assessed by comparing the lesion variables at the beginning of the study between groups. All analysis was performed using commercial software (PROC GENMOD, SAS Institute Inc. and SPSS 11.5, SPSS Inc., Chicago, IL).

RESULTS

Study Population

2 mg/kg Group. There were 8 GSD, 1 Staffordshire bull terrier and 1 mixed-breed dog; mean age was 5 years 10 months (range, 3 years 6 months to 9 years 1 month). Six dogs were male (3 neutered) and 4 dogs were female (3 neutered).

5 mg/kg Group. There were 9 GSD and 1 Border collie; mean age was 6 years 5 months (range, 3 years 8 months to 8 years 11 months). Five dogs were male (1 neutered) and 5 dogs were female (4 neutered).

Before admission all dogs had been administered antibiotics, 7 had been administered corticosteroids, and 3 surgical treatment of PAF. No significant difference in lesion surface area and VAS score for lesion severity between groups at study start was identified.

Clinical Signs

The presence of each clinical sign in order of greatest prevalence was: licking and chewing of the perianal lesions, straining to defecate, discharge from the perianal region, and bleeding from the perianal region (Table 1). At the end of 8 weeks, 2 of 10 (20%) dogs in the 2 mg/kg

Table 1. Prevalence of Clinical Signs and the Grade of Severity Assigned by the Owner of Each Dog for Each Clinical Sign

Time (week)	Dose (mg/kg)	N	Licking and Chewing	Straining to Defecate	Discharge from Perianal Region	Bleeding from Perianal Region
0	2	10	8 , 0-8 (3)	5 , 0-9 (1.5)	7 , 0-6 (2)	3 , 0-5 (0)
	5	10	8 , 0-7 (2.5)	8 , 0-10 (2)	5 , 0-8 (2)	6 , 0-8 (1)
2	2	10	10 , 1-6 (1.5)	5 , 0-6 (0)	2 , 0-5 (0)	2 , 0-4 (0)
	5	10	8 , 0-7 (1.5)	7 , 0-10 (2)	4 , 0-5 (0)	0
4	2	10	8 , 0-6 (2)	3 , 0-4 (0)	2 , 0-1 (0)	2 , 0-3 (0)
	5	10	5 , 0-4 (0.5)	4 , 0-10 (0)	3 , 0-2 (0)	1 , 0-2 (0)
6	2	8	8 , 1-5 (1)	2 , 0-4 (0)	0	2 , 0-3 (0)
	5	10	3 , 0-2 (0)	4 , 0-9 (0)	1 , 0-1 (0)	0
8	2	10	7 , 0-3 (1)	3 , 0-4 (0)	0	2 , 0-1 (0)
	5	10	4 , 0-2 (0)	3 , 0-7 (0)	1 , 0-3 (0)	0

The number of dogs affected by each clinical sign in each group is in bold, followed by the range and median (within parentheses) of the severity grades assigned by owners in each treatment group at each 2 week examination.

group and 4 of 10 (40%) dogs in the 5 mg/kg group had complete resolution of all clinical signs.

A reduction in the calculated combined clinical score was observed for all dogs during the 8 week treatment period. "Licking and chewing of the perianal lesions" and "straining to defecate" were the most persistent clinical signs at the end of 8 weeks (Table 1). Of the 8 dogs in the 2 mg/kg group with persistent clinical signs, only 2 were perceived by their owners to have signs affecting the dog's quality of life. Similarly of the 6 dogs in the 5 mg/kg group with persistent clinical signs, only 1 was perceived by the owner to have signs affecting the dog's quality of life.

Rectal Examination

At admission, 3 dogs in both groups had evidence of an anal stricture and 7 dogs in both groups had evidence of anal sac involvement. Detailed rectal examination was not consistently possible on follow-up examinations because dogs were not anesthetized, thus precise changes in anal sac involvement or anal strictures could not be determined.

Lesion Surface Area and VAS

A significant reduction in the lesion surface area and VAS was observed within each treatment group after 8 weeks of treatment ($P \leq .04$; Figs 1 and 2). The 5 mg/kg every 24 hours dose rate resulted in significantly faster resolution of PAF lesion surface area and VAS compared with the 2 mg/kg every 24 hours dose rate, the effect being significant from week 2 for surface area ($P = .008$), and at week 6 for VAS ($P = .025$). After 8 weeks of treatment, 1 of 10 (10%) dogs in the 2 mg/kg group and 6 of 10 (60%) dogs in the 5 mg/kg group experienced resolution of external PAF lesions.

The 1 dog in the 2 mg/kg group that had resolution of external lesions had an anal stricture on admission and continued to have tenesmus after resolution of the external lesion. Two of 6 dogs in the 5 mg/kg group that had closure of external lesions had persistent clinical signs. One of these dogs had evidence of anal sac involvement on admission and had mild persistent tenesmus. The other dog had no evidence of strictures or anal sac involvement and continued to lick the perianal region. The converse was observed in 2 dogs in the 2 mg/kg group that had resolution of clinical signs as these dogs had persistent lesions.

Cyclosporine Trough Levels

Data from 2 dogs were excluded from the 2 mg/kg group at both 2 and 4 weeks because of owners admin-

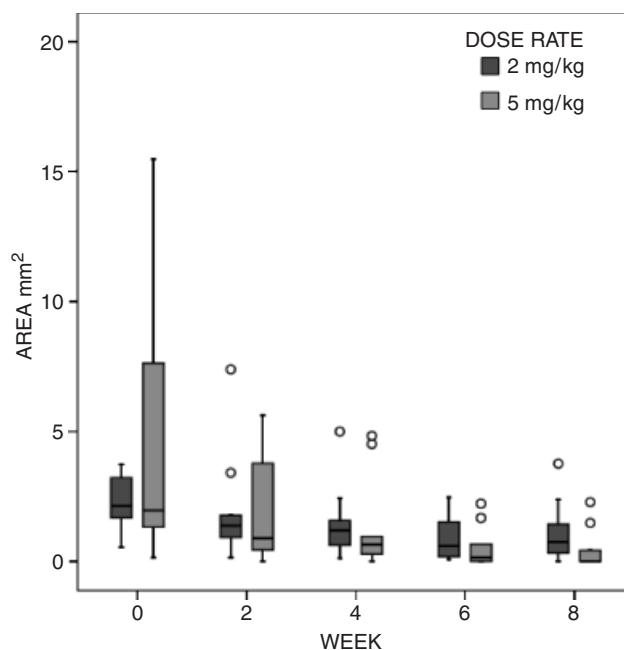


Fig 1. Box-and-whisker plots of external perianal fistulae lesion surface area in mm². The box represents the interquartile (25–75th percentiles) range. The horizontal line in the box represents the median. The whiskers represent 1.5 times the interquartile range. Open circles represent outliers.

istering cyclosporine on the morning of blood sampling. Similarly, 5 and 2 measurements were excluded from the 5 mg/kg group at week 2 and 4, respectively, because owners administered cyclosporine on the morning of blood sampling. Mean trough blood concentrations of cyclosporine measured after 2 and 4 weeks were 30 ng/mL (range, 25–54.7 ng/mL) and 39 ng/mL (range, 25–76.8 ng/mL) for the 2 mg/kg group and 102 ng/mL (range, 69.4–133.9 ng/mL) and 122 ng/mL (range, 47.4–236.6 ng/mL) for the 5 mg/kg group. At both 2 and 4 weeks 5 mg/kg every 24 hours resulted in significantly higher cyclosporine trough levels ($P = .003$).

Side Effects

The only consistent side effect observed during the study was excessive hair shedding followed by hirsutism in 6 dogs (4 dogs from the 2 mg/kg group; 2 dogs from the 5 mg/kg group).

DISCUSSION

Our results indicate that cyclosporine at a dose rate of 5 mg/kg every 24 hours was more effective than 2 mg/kg every 24 hours at reducing lesion surface area severity in dogs with PAF. Although both dose rates reduced PAF

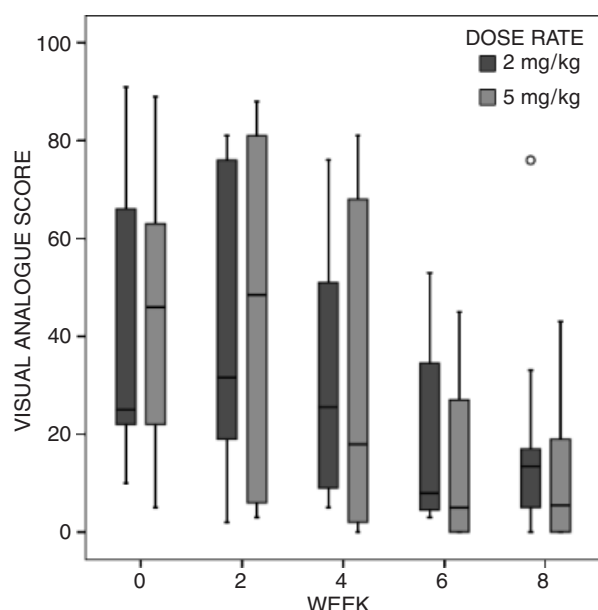


Fig 2. Box-and-whisker plots of visual analog score for lesion severity. The box represents the interquartile (25–75th percentiles) range. The horizontal line in the box represents the median. The whiskers represent 1.5 times the interquartile range. Open circles represent outliers.

lesion severity and associated clinical signs compared with initial presentation, neither dose rate was as effective as higher dose rates reported in previous studies.

Dose rates of 5–10 mg/kg every 12 hours have achieved lesion resolution rates of $\sim 95\%$ which is substantially better than the resolution rates we achieved, refuting the previous suggestion that lower dose rates may be equally effective for treating PAF in dogs.^{10,16,17} The effect of the longer duration (10–20 weeks) of treatment used previously compared with the 8 weeks we studied is unknown, but should be considered when making direct comparisons between results.^{10,16–18}

When examining the effect of dose rate on clinical signs, 5 mg/kg every 24 hours was more effective at resolving clinical signs than 2 mg/kg every 24 hours; however, the percentage of dogs that had complete resolution was low in both groups. Compared with other studies, the percentage of dogs that had resolution of clinical signs in both groups was less than that reported in studies using higher dose rates of cyclosporine (5–10 mg/kg every 12 hours for 10–20 weeks, 7.5 mg/kg every 24 hours for 13 weeks) or cyclosporine 1–3.5 mg/kg every 24 hours combined with ketoconazole 8 mg/kg every 24 hours.^{10,16–18} The percentage of dogs that had resolution of clinical signs treated with 5 mg/kg every 24 hours was comparable with studies using lower dose rates of cyclosporine (1.5 or 3 mg/kg every 24 hours for 13 weeks), azathioprine 50 mg every 24 hours combined with

metronidazole 400 mg every 24 hours for 6 weeks or prednisolone (2 mg/kg every 24 hours for 2 weeks followed by 1 mg/kg every 24 hours for 4 weeks).^{22–24}

Similarly, when examining the effect of dose rate on lesion variables, both dose rates we used resulted in a significant reduction in lesion surface area and severity after 8 weeks of treatment. The 5 mg/kg every 24 hours dose rate resulted in a significantly faster reduction in lesion surface area and severity and a higher percentage of dogs had complete resolution of lesions compared with the 2 mg/kg every 24 hours dose rate. Despite the greater efficacy of the 5 mg/kg every 24 hours dose rate, the percentage of dogs that had complete resolution of lesions was lower than that reported for dogs treated with 5–10 mg/kg every 12 hours or cyclosporine 1–3.5 mg/kg every 24 hours combined with ketoconazole 8 mg/kg every 24 hours.^{10,16,17,20,21} However, the percentage resolution was comparable with that reported for dogs treated with cyclosporine at 7.5 mg/kg every 24 hours, and higher than other studies where dogs were treated with cyclosporine at 1.5, 3 or 5 mg/kg every 24 hours, azathioprine 50 mg every 24 hours combined with metronidazole 400 mg every 24 hours for 6 weeks or prednisolone (2 mg/kg every 24 hours for 2 weeks followed by 1 mg/kg every 24 hours for 4 weeks).^{18,23,24} In contrast, the percentage of dogs from the 2 mg/kg group that had complete resolution of lesions was less than all other studies except for those using cyclosporine 1.5 mg/kg every 24 hours or azathioprine and metronidazole combination.^{10,16–18,23,24}

At present, no uniform or accurate classification system exists for grading PAF lesion severity.^{10,16–19,23,25} To improve our ability to evaluate response to treatment, both objective and subjective data evaluating lesion severity and extent, plus clinical signs associated with the disease were collected and evaluated. The clinical signs selected for evaluation included; licking and chewing of the perianal lesions, straining to defecate, discharge from the perianal region, and bleeding from the perianal region, as these are all signs commonly reported by owners of dogs with PAF.^{1–5} Of the clinical signs evaluated, straining to defecate was the least specific to PAF lesions and may be associated with other diseases such as colitis however, an association between PAF and colitis has been reported.⁵ Use of the owner's assessment of clinical signs was an attempt to document both the clinical response of each dog to treatment and the owner's perception of the response to treatment. The combined clinical score decreased for all dogs at the conclusion of the 8-week study and it appeared that both dose rates provided a degree of palliation of clinical signs that was acceptable to most owners despite the presence of persistent lesions and clinical signs. Unfortunately, a clinical definition was not provided for each score on the grading scale used by

the owners on the standardized questionnaire, therefore the scoring system used lacked repeatability and comparison between the two dose rate groups was not able to be performed.

PAF lesions typically involve the perianal skin within the region of the mucocutaneous junction but can also involve perianal structures such as the anal sacs, anal sinuses, and rectal mucosa.²⁶ The presence of persistent clinical signs despite resolution of external PAF lesions in some dogs in our study could have been the result of concurrent disease of the colon or rectum making it difficult to accurately assess response to treatment. However, in view of the high incidence of anal sac involvement and anal stricture formation observed at the onset of the study (as well as the lack of other clinical signs such as diarrhea or hematochezia), the persistent clinical signs seen in some dogs were most likely because of ongoing anal sac involvement or anal stricture despite resolution of the external lesions. The importance of PAF lesions extending into perianal, anal and rectal structures on treatment outcomes is unknown and warrants further investigation.

After 2 and 4 weeks, the 5 mg/kg every 24 hours dose rate resulted in significantly higher blood trough concentrations than the 2 mg/kg every 24 hours dose rate. Based on the cyclosporine trough concentrations achieved after 4 weeks of therapy in the 5 mg/kg group, it can be inferred that mean trough blood concentrations of ~ 122 ng/mL can be expected to result in improvement in clinical signs, lesion extent, and severity with complete resolution of PAF lesions occurring in some dogs within an 8 week treatment period. In contrast, mean trough blood concentrations of ~ 39 ng/mL (achieved in the 2 mg/kg group) can lead to a degree of improvement in both clinical signs and the extent and severity of PAF lesions, but are unlikely to result in complete resolution of PAF lesions or associated clinical signs within an 8-week period. Whole blood cyclosporine measurements can be performed using either high-performance liquid chromatography (HPLC) or monoclonal radioimmunoassay methods. Measured trough concentrations vary depending on the method used however there is a significant correlation between the results from each assay. The monoclonal radioimmunoassay technique provides clinically reliable information and is readily available.²⁷

The relatively high cost of cyclosporine remains a major limitation for its use in dogs and highlights the need to determine the lowest effective dosing protocol and more effective treatment strategies. Potential factors contributing to the difficulty in determining an ideal dosage for this disease include the wide variation in severity and extent of lesions, individual differences in bioavailability, absorption and metabolism of cyclosporine, and variations in local and systemic factors affecting inflammation with-

in the lesions and wound healing.^{7,10,14,28} Eventually, as additional studies are performed, it may be possible to establish a more accurate correlation between clinical response and cyclosporine dosage, trough blood concentrations, and lesion severity. Based on our study, a dose rate of 5 mg/kg every 24 hours is recommended over 2 mg/kg every 24 hours for the management of PAF; however, most dogs in both groups were still troubled by persistent lesions.

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REFERENCES

1. Ellison GW: Treatment of perianal fistulas in dogs. *J Am Vet Med Assoc* 206:1680-1682, 1995
2. Robins GM, Lane JG: The management of anal furunculosis. *J Small Anim Pract* 14:333-342, 1973
3. Lane JG, Burch DGS: The cryosurgical treatment of canine anal furunculosis. *J Small Anim Pract* 16:387-392, 1975
4. Houlton JEF: Anal furunculosis: a review of seventy cases. *J Small Anim Pract* 21:575-584, 1980
5. Jamieson PM, Simpson JW, Kirby BM, et al: Association between anal furunculosis and colitis in the dog: preliminary observations. *J Small Anim Pract* 43:109-114, 2002
6. Day MJ, Weaver BMQ: Pathology of surgically resected tissue from 305 cases of anal furunculosis in the dog. *J Small Anim Pract* 33:583-589, 1992
7. Day MJ: Immunopathology of anal furunculosis in the dog. *J Small Anim Pract* 34:381-389, 1993
8. House A, Gregory SP, Catchpole B: Expression of cytokine mRNA in canine anal furunculosis lesions. *Vet Rec* 153:347-358, 2003
9. Present DH, Lichtiger MD: Efficacy of ciclosporine in treatment of fistula of crohn's disease. *Dig Dis Sci* 39:374-380, 1994
10. Mathews KA, Ayers SA, Tano CA, et al: Cyclosporine treatment of perianal fistulas in dogs. *Can Vet J* 8:39-41, 1997
11. Thomson AW: The effects of cyclosporin A on non-T cell components of the immune system. *J Autoimmun A(Suppl)*: 167-176, 1992
12. Vaden SL: Cyclosporine, in Kirk RW, Bonagura JD (eds): *Current Veterinary Therapy XII*. Philadelphia, PA, Saunders, 1995, pp 73-77
13. Bennett WM, Norman DJ: Action and toxicity of cyclosporine. *Annu Rev Med* 37:215-224, 1986
14. Kovarik JM, Mueller EA, van Bree JB, et al: Reduced inter- and intra-individual variability in cyclosporine pharmacokinetics from microemulsion formulation. *J Pharm Sci* 83:444-446, 1994
15. Mathews KA, Holmberg DL, Johnston K: Renal allograft survival in outbred mongrel dogs utilizing a combination immunosuppressive drug therapy and donor bone marrow. *Vet Surg* 3:347-357, 1994

16. Mathews KA, Sukhiani HR: Randomized controlled trial of cyclosporine for treatment of perianal fistulas in dogs. *J Am Vet Med Assoc* 211:1249–1253, 1997
17. Griffiths LG, Sullivan M, Borland WW: Cyclosporine as the sole treatment for anal furunculosis: preliminary results. *J Small Anim Pract* 40:569–572, 1999
18. Doust R, Griffiths LG, Sullivan M: Evaluation of once daily treatment with cyclosporine for anal furunculosis in dogs. *Vet Rec* 152:255–229, 2003
19. Wooldridge JD, Gregory CR, Mathews KG: Clinical evaluation of the efficacy of leflunamide alone, leflunamide and cyclosporine and cyclosporine at varying dosages in the treatment of perianal fistulas in dogs. *Proc 28th Annual American College of Veterinary Surgeons Forum*, Chicago, IL, 1998
20. Patricelli AJ, Hardie RJ, McAnulty JF: Cyclosporine and ketoconazole for the treatment of perianal fistulas in dogs. *J Am Vet Med Assoc* 20:1009–1016, 2002
21. O'Neill T, Edwards GA, Holloway S: Efficacy of combined cyclosporine A and ketoconazole treatment of anal furunculosis. *J Small Anim Pract* 45:238–243, 2004
22. Caulkett N, Read M, Fowler D, et al: A comparison of the analgesic effects of butorphanol with those of meloxicam after elective ovariohysterectomy in dogs. *Can Vet J* 44:565–570, 2003
23. Tisdall PL, Hunt GB, Malik R: Management of perianal fistulae in five dogs using azathioprine and metronidazole prior to surgery. *Aust Vet J* 7:374–378, 1999
24. Hardie RJ, Gregory SP, Tomlin J, et al: Cyclosporine treatment of anal furunculosis in 26 dogs. *J Small Anim Pract* 46:3–9, 2005
25. Harkin KR, Walshaw R, Mullaney TP: Association of perianal fistula and colitis in the German shepherd dog: response to high dose prednisolone and dietary therapy. *J Am Anim Hosp Assoc* 2:515–520, 1996
26. Johnston DE: Rectum and anus, surgical diseases, in Slatter DH (ed): *Textbook of Small Animal Surgery* (ed 1). Philadelphia, PA, Saunders, 1985, pp 770–793
27. McAnulty JF, Lensmeyer GL: Comparison of high performance liquid chromatography and immunoassay methods for measurement of cyclosporine A blood concentrations after feline kidney transplantation. *Vet Surg* 27:589–595, 1998
28. Papadakis KA, Targan SR: Role of cytokines in the pathogenesis of inflammatory bowel disease. *Annu Rev Med* 51:289–298, 2000