Efficacy of Cyclosporine for Chronic, Refractory Stomatitis in Cats: A Randomized, Placebo-Controlled, Double-Blinded Clinical Study

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Summary:

Sixteen cats with chronic stomatitis, that had previously undergone premolar-molar or full-mouth extractions, were randomly assigned a group to receive 2.5 mg/kg cyclosporine or placebo orally twice daily. Neither the clinician nor the clients were aware of the group assignments. Cats were evaluated prior to treatment and every 2 weeks for 6 weeks using a 30 point Stomatitis Disease Activity Index (SDAI) score. Mean improvement in SDAI scores among cats in the treatment group after 6 weeks was 52.7 %. This was significantly different from the mean improvement (12.2 %) of cats in the placebo group. During the 6 week study period, 7 of the 9 cats in the treatment group (77.8 %) showed a > 40 % improvement in SDAI score, while 1 of 7 cats in placebo group (14.3 %) showed a > 40 % improvement in SDAI score. This difference was statistically significant. Individual variability in the absorption of orally-administered cyclosporine was high. Trough whole-blood cyclosporine levels ranged from 32.1 ng/ml to 1,576.2 ng/ml. At the end of the 6 week observation period, there was a statistically significant difference among cats with trough whole-blood cyclosporine levels > 300 ng/ml (72.3 % improvement) compared with cats with cyclosporine levels < 300 ng/ml (28.2 % improvement). Whole-blood cyclosporine levels > 300 ng/ml were associated with significant improvement in oral inflammation in cats with chronic stomatitis that had previously undergone premolar-molar or full-mouth extraction. ***J Vet Dent 30 (1); 8-17, 2013***

Introduction

Feline chronic stomatitis is characterized by inflammation of the gingiva, buccal mucosa, and caudal oral mucosa (the tissue lateral to the palatoglossal folds, often incorrectly referred to as the "fauces").[1-5] It may be differentiated from periodontal disease by the presence of inflammatory lesions affecting the caudal oral mucosa as well as the alveolar and buccal mucosa adjacent to the premolar and molar teeth (Fig. 1). Clinical signs may include halitosis, poor grooming, difficulty eating, pain when eating or yawning, irritability, and weight loss. Microscopic examination of affected tissues typically reveals infiltrates of lymphocytes and plasma cells, with varying numbers of neutrophils.[1,2] While it is believed that feline stomatitis results from an inappropriate immune response to oral antigenic stimulation, the initiating cause is usually not identified, may differ from case to case, and is likely multifactorial. A variety of potential contributing factors have been explored, including infectious agents (feline calicivirus, feline herpesvirus, feline leukemia virus, feline immunodeficiency virus, and *Bartonella henselae*), tooth resorption (feline odontoclastic resorption lesions/FORL), periodontal disease, food allergies, and hypersensitivity to plaque bacteria.[1-14] Two recent comparisons of stomatitis cats with age-matched, healthy control cats suggest that, of the infectious agents, only feline calicivirus is commonly associated with chronic stomatitis in cats.[13,14] Although a distinct underlying immunological abnormality has not been identified, increased expression of mRNA for specific inflammatory mediators (IL-2, IL-4, IL-6, IL-10, IL-12 and IFN-γ) has been identified in cats with chronic stomatitis.[15] In addition, immunohistochemical analysis has revealed that the predominant cells infiltrating the caudal oral mucosa in affected cats are primarily CD79a+ IgG isotype plasma cells, along with CD8+ (cytotoxic) T cells, supporting the hypothesis that viral infection may be one initiating factor in the development of this disease.[16] Stimulation of the immune system by plaque bacteria appears to contribute to ongoing inflammation, and successful treatment of chronic stomatitis requires minimizing oral bacteria. Because daily plaque removal by mechanical means (e.g., toothbrushing) is difficult in these painful cats, reduction of plaque-retentive surfaces by extracting teeth has proven to be most effective in eliminating plaque and reducing oral inflammation.[17,18] It has been demonstrated that 60-80 % of cats with chronic stomatitis will significantly improve following extraction of all premolar and molar teeth.[17,18] However, for the remaining 20-40 %, oral inflammation persists, and these refractory cases present a therapeutic challenge to veterinarians. The mainstay of medical management of refractory stomatitis has been administration of corticosteroids, with or without antibiotics. Since these medications may result in undesirable side effects and decreasing efficacy over time, alternative therapies for this condition are sought.

Cyclosporine is a lipophilic, cyclic peptide derived from fungi which has long been used in both human and veterinary medicine to prevent organ rejection in transplant recipients. Cyclosporine inhibits T cell activation by blocking the transcription of genes coding for specific pro-inflammatory cytokines, including IL-2 and IL-4.[19] Its mechanism of action is partially through inhibition of calcineurin, a calcium-binding cytoplasmic protein which dephosphorylates nuclear factor of activated T cells (NFAT), allowing NFAT to enter the cell nucleus and initiate cytokine gene expression.[20] Activated T cell secretion of IL-2 drives proliferation and further expansion of T cells, resulting in a positive feedback loop of increasing T cell numbers.[20] By reducing IL-2 expression, T cell proliferation will also be reduced. This process is thought to be the primary mechanism of immunosuppression by cyclosporine.[21] While less is known about the direct effects of cyclosporine on B cells, it has been demonstrated that calcineurin is required for B-cell-receptor-induced proliferation and differentiation, and cyclosporine has been shown to inhibit B cell reproduction *in vitro*.[22] Successful treatment of plasma cell

Figure 1

Photograph showing a 3-year-old, neutered/male, domestic long-haired cat at study entry (A). Note the marked inflammation of the caudal oral mucosa, in the areas lateral to the palatoglosssal folds. Photograph of the same cat taken 10 weeks (B) after increasing cyclosporine dose to 5.0 mg/kg PO BID. The SDAI score had decreased from 24 at entry to 12.5 at this post-treatment evaluation, representing a 47.9 % improvement. The proliferative-appearing tissue in the caudal oral cavity is greatly diminished, although some of this effect may be due to positioning of the oral cavity, as the mouth was opened more fully for this post-treatment evaluation and photograph.

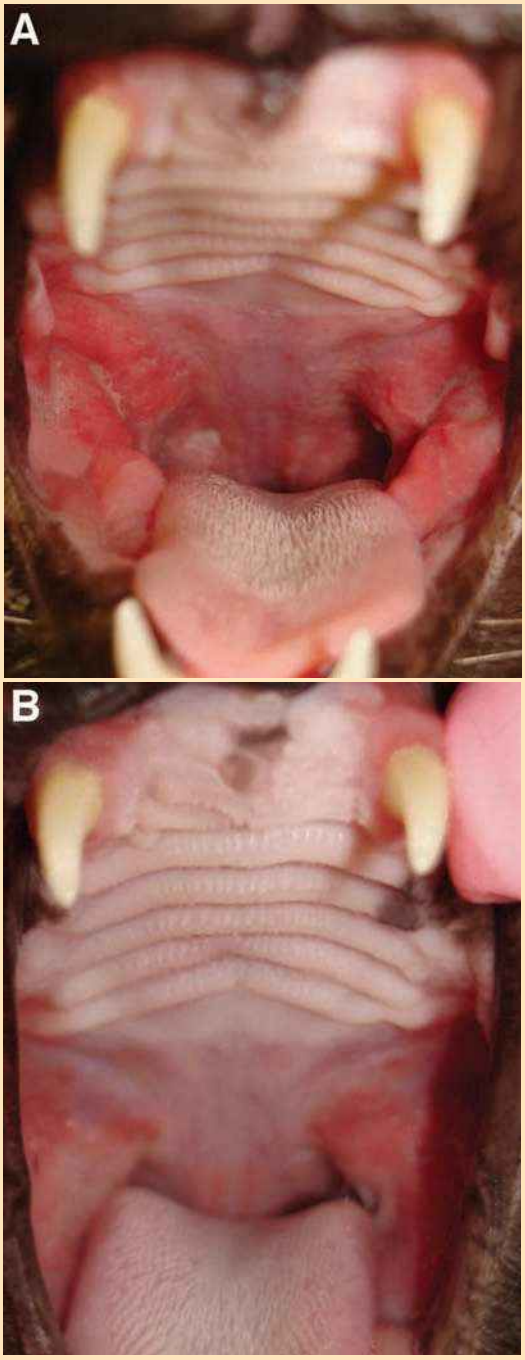


Table 1

Inclusion Criteria.

- Inflammatory lesions on the gingiva, alveolar/buccal mucosa, and caudal mucosa persisting for at least 1 month following extraction of all premolar and molar teeth
- Indoor-only
- FIV/FeLV negative
- CBC, serum biochemistry profile, and urinalysis within 6 weeks of starting the study
- Absence of immunosuppressive disease (e.g. diabetes) or liver disease
- Fed a commercial feline diet that remains consistent throughout the study
- Has not received injectable corticosteroids within 6 weeks of starting the study
- Has not received oral corticosteroids within 1 week of starting the study

cheilitis in humans using topical calcineurin inhibitors further supports the concept of a partially-calcineurin dependent B cell activation and differentiation cascade.[23-25]

The purpose of this study was to evaluate the efficacy of orally-administered microemulsified cyclosporine for treatment of cats with chronic, refractory stomatitis following premolar-molar or full-mouth extraction.

Materials and Methods

Study population: Client-owned cats with chronic, refractory stomatitis (defined as gingivitis, buccal mucositis, and caudal stomatitis persisting for at least 4 weeks following extraction of all premolar and molar teeth) were recruited for the study. If not already performed by the referring veterinarian within 6 weeks prior to presentation, CBC, chemistry profile, and urinalysis were completed at the time of initial evaluation. Cats with evidence of diabetes mellitus, liver disease, and FeLV or FIV infection were excluded from the study. Cats who were allowed outdoors, were fed a raw diet, or those that had received injectable corticosteroids within 6 weeks or oral corticosteroids within 1 week of presentation were also excluded (Table 1). All clients signed an informed-consent form prior to enrollment. Eighteen cats were initially enrolled, however 2 were eliminated from the study within the first 2 weeks due to failure to adhere to the medication administration protocol (1 cat) or failure to maintain indoor-only status (1 cat). Characteristics of the study population at entry are summarized in Table 2.

Patient preparation: At the initiation of the study, cats that had not been previously treated by the author were anesthetized (including premedication with an opioid +/- anticholinergic, induction with propofol/diazepam, ketamine/diazepam or etomidate/diazepam, intubation, and maintenance with isoflurane/O2). Photographs were taken and full-mouth radiographs were obtained using bisecting angle, parallel, and near-parallel extra-oral techniques as described previously.[26] Periodontal treat-

Table 2

Characteristics of the Study Population at T0*.

Characteristic		Treatment	Control	Significance
Age (years)	Range	2-15	2-11	
	Mean (SD)	8.0 (4.0)	5.8 (2.9)	p = 0.25
Weight (kg)	Range	2.90-11.50	3.15-5.46	
	Mean (SD)	5.42 (3.17)	4.27 (0.85)	p = 1.0
	Median	4.60	4.23	
SDAI	Range	10-26	9.5-21	
	Mean (SD)	15.9 (5.5)	16.6 (4.5)	p = 0.79
Male/female		5/4	6/1	p = 0.31
Previous steroid administration		6	7	p = 0.48
Edentulous vs. canine +/- incisor teeth present		4/5	1/6	p = 0.31

T0 = beginning of study. P values < 0.05 considered significant.
SDAI = Stomatitis Disease Activity Index

Table 3

Owner Evaluation of Cat at Study Entry*.

Appetite	3 = eats only pureed food, or only when hand fed
	2 = eats wet food; cannot eat dry food
	1 = eating wet and dry food, but less than normal amount
	0 = eating normally
Activity Level	3 = no interest in people or other pets, spends most of time sleeping
	2 = low activity level, but will play occasionally when engaged by people or other pets
	1 = plays spontaneously, but not frequently
	0 = normal activity level (playful and active)
Grooming Behavior	3 = will not groom
	2 = grooms occasionally but not at 'pre-illness' level
	1 = grooming excessively
	0 = grooming normally
Perceived Comfort	
	On a scale of 0-3, with 0 being most comfortable and 3 being most painful, rank
	your cat's present comfort level: _____

* The average of the scores above (total score divided by 4) was entered in the "owner evaluation" box on the initial SDAI (Stomatitis Disease Activity Index) tabulation sheet.

ment was performed on any remaining canine and incisor teeth using an ultrasonic scaler[a] and air-polisher[b]. Presence and location of any retained root remnants beneath the gingiva was recorded. If dental radiographs revealed significant periodontitis or tooth resorption of any remaining canine or incisor teeth, or root remnants of premolar and molar teeth protruding from the gingiva, affected teeth were extracted and the cat was temporarily disqualified from the study. If subsequent examinations at least 4 weeks postoperatively revealed persistent oral inflammation, the cats were enrolled in the study at that time. If root fragments were identified completely surrounded by bone, with no evidence of periodontal or endodontic disease, this was recorded and the cat was allowed to enter the study. For cats whose extractions had been performed within 3 months of presentation and postoperative radiographs had confirmed absence of retained roots (n = 8), anesthetization for dental radiographs and repeat periodontal treatment was not required.

Initial evaluation and group assignment: Clients completed a brief questionnaire and the numbers were averaged to provide the initial client evaluation score (Table 3). Using the scoring guidelines for weight and oral inflammation (Table 4), clinical findings were recorded by placing checkmarks in the appropriate boxes on the initial Stomatitis Disease Activity Index (SDAI) form (Table 5) and the total SDAI

Table 4

Scoring Criteria for Stomatitis Disease Activity Index (SDAI) Tabulation.

Owner Evaluation* (See Table 3):
0 = significant improvement
1 = mild improvement
2 = no change
3= worse

Weight (compared with most recent visit):
0 = gain > 0.5kg
1 = gain > 0.25kg but < 0.5kg
2 = < 0.25 kg gain
3 = weight loss

Inflammation (specified sites as graded by clinician)*:
0 = none
1 = mild
2 = moderate
3 = severe

* Intermediate value scores were accepted in these categories; e.g., a cat that had shown moderate improvement could receive a score of 0.5 in the "owner evaluation" category. As a result, the final SDAI scores may not be whole numbers.

Table 5

Stomatitis Disease Activity Index (SDAI) Score Tabulation.

STOMATITIS DISEASE ACTIVITY INDEX	0	1	2	3
Owner evaluation				
Weight (compared to most recent visit)				
Maxillary buccal mucosal inflammation				
Mandibular buccal mucosal inflammation				
Maxillary attached gingival inflammation				
Mandibular attached gingival inflammation				
Inflammation lateral to palatoglossal folds				
Molar salivary gland inflammation				
Oropharyngeal inflammation				
Lingual and/or sublingual inflammation				
TOTAL SCORE (max = 30)				

score was calculated. A request form was faxed to Golden Gate Veterinary Pharmacy, where each cat was randomly assigned to the treatment or control group based on a coin toss by the participating pharmacist. Assignment was recorded by pharmacy personnel and kept confidential: neither the investigator nor the cats' caretakers were aware of each animal's assigned group. For the treatment group, the dispensed medication (60 ml) was custom-compounded to include a dose of cyclosporine equivalent to 2.5 mg/kg per 1.0 ml, in a cod liver oil base with tuna flavoring added. Cats assigned to the control group received 60 ml of cod liver oil with tuna flavoring added. The medication was mailed directly from the pharmacy to the clients, with instructions to administer 1.0 ml, by mouth, twice daily. In summary, cats assigned to the treatment group received 2.5 mg/kg microemulsified cyclosporine[c] PO BID. Cats assigned to the control group received 1.0 ml of the same cod liver oil base with tuna flavoring added PO BID.

Follow-up evaluation: Examinations were performed every 2 weeks for 6 weeks. At each of the 3 follow-up examinations, cats were graded according to the SDAI reevaluation form (Table 5) using the aforementioned criteria (Table 4). At the second post-treatment examination (4 weeks), clients were instructed not to give the medication that morning, in order for blood to be collected from the cats for evaluation of trough (12 hours post-dose) whole-blood cyclosporine levels. At the 6 week post-treatment examination, final scores were recorded and the treatment group was revealed (Group A = treatment, Group B = placebo). If the patient was revealed to be on placebo, the option was given to administer cyclosporine at 2.5 mg/kg at no charge. Evaluations continued for an additional 6 weeks at no charge, with post-treatment examinations every 2

Table 6

Mean Stomatitis Disease Activity Index (SDAI) Scores at Each Time Point.

Time point	Mean SDAI Group A	Mean SDAI Group B	Significance
T0	15.9	16.6	(0.7949)
T1	10.2	15.8	(0.0801)
T2	8.7	14.1	(0.3681)
T3	7.2	14.7	(0.0340)

T0 = initial; T1 = 2 weeks post-treatment; T2 = 4 weeks post-treatment; T3= 6 weeks post-treatment
Group A = treatment; Group B = placebo

Table 7

Percent (mean) Stomatitis Disease Activity Index (SDAI) Improvement at Each Time Point.

Time point	Group A	Group B	Significance
T1	0.324 (32.4%)	0.057 (5.7%)	No (0.0500)
T2	0.421 (42.1%)	0.153 (15.3%)	No (0.1118)
T3	0.527 (52.7%)	0.122 (12.2%)	Yes (0.0444)

Group A = treatment; Group B = placebo

weeks and trough whole-blood cyclosporine levels evaluated at 4 weeks after starting treatment (Group B-2). For cats from Group A with cyclosporine levels < 300 ng/ml at the 2.5 mg/kg BID dose and who showed minimal improvement, the dosage was increased to 5.0 mg/kg BID and the cats were examined every 2 weeks for an additional 6 weeks, with trough whole-blood cyclosporine levels evaluated at week 4 (Group A-2). It should be noted that the scoring of cats in Groups A-2 and B-2 was not performed in a blinded fashion. Observations made beyond the initial 6 week study period are provided for additional information but were not subjected to statistical analysis.

Data analysis: The Mann-Whitney test was used to establish that the mean age, weight, and initial SDAI scores were not significantly different between treatment and control groups at the start of the study. Percentage improvement was calculated for each cat at each post-treatment examination based on initial SDAI score and post-treatment SDAI score (% improvement = initial SDAI – post-treatment SDAI/initial SDAI). Student's *t*-test for independent samples and the Mann-Whitney test were performed to compare the mean SDAI scores and mean improvement of the treatment group with the control group at each time point. (As the Mann-Whitney test is the more robust of the two, stated p-values comparing mean SDAI scores and mean improvement between treatment and control groups are from the Mann-Whitney calculations.) A two-tailed p-value of less than 0.05 was considered significant. The Mann-Whitney test was also used to compare the change in weight from T0 to T3 for each group. Student's *t*-test was used to evaluate effects of previous steroid administration and to compare edentulous cats to cats with canine and incisor teeth in Group A. Fisher's exact probability test was performed to determine statistical significance of differences between Groups A and B in regards to ratio of males to females; prior steroid administration; presence of canine and/or incisor teeth; incidence of side effects; requirement for rescue medications; and percentage of cats showing > 40 % improvement in SDAI scores at week 6. Again, a two-tailed p-value of less than 0.05 was considered significant. For the latter three parameters, 95 % confidence intervals (CI) were also calculated.

Results

Efficacy assessment: Mean SDAI scores were calculated for each group at each time point (Table 6). At T0, there was no significant difference for the mean SDAI score between Groups A (15.9) and B (16.6) [p = 0.7949]. At T1, there was no significant difference for the mean SDAI score between Groups A (10.2) and B (15.8) [p = 0.0801]. At T2, there was no significant difference for the mean SDAI score between Groups A (8.7) and B (14.1) [p = 0.0719]. At T3, there was a significant difference for the mean SDAI score between Groups A (7.2) and B (14.7) [p = 0.0340] (Fig. 2).

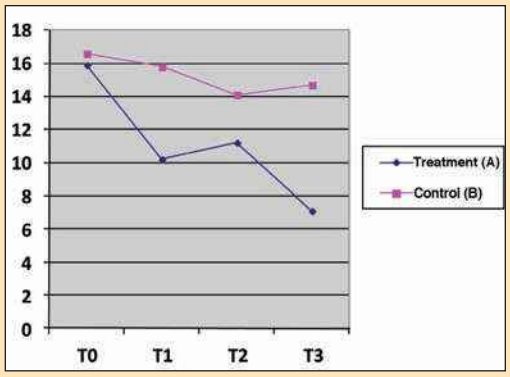
SDAI scores recorded at each post-treatment examination (T1, T2 and T3) were compared with scores at initial evaluation (T0), and the percent improvement was calculated (e.g., T1-T0/T0) for each cat at each time point (Table 7). Mean improvement in SDAI scores did not differ significantly between groups at T1 (32.4 % in Group A, 5.7 % in Group B) or T2 (42.1% in Group A, 15.3 % in Group B). At T3, mean improvement in SDAI scores among cats in Group A (52.7 %) was significantly different (p = 0.0444) compared with 12.2 % in Group B cats (Fig. 3).

Response rate: In Group A, 7/9 cats (77.8 %) demonstrated > 40 % improvement in SDAI, compared with 1/7 of cats in Group B (14.3 %), at the end of the 6 week evaluation period. This difference was statistically significant (p = 0.040; 95 % CI = 0.1421-0.8324).

Effect of previous steroid administration: In Group A, cats never having received steroids prior to entering the study (n = 3) demonstrated a mean improvement of 68.8 % over 6 weeks, compared

Figure 2

Progression of Stomatitis Disease Activity Index (SDAI) Scores. Mean SDAI scores decreased more dramatically for Group A than for Group B cats between study entry (T0) and the 6 week post-treatment (T3) examination. At T3, the mean SDAI for Group A was 7.2, and for Group (B) it was 14.7. This difference was statistically significant (p = 0.034).



with mean improvement of 44.7 % among the 6 cats who had previously been administered steroids. This finding was not statistically significant (p = 0.2957).

Difference between edentulous cats and those with canine and/or incisor teeth present: Of cats in Group A, 5 had canine and/or incisor teeth and 4 were edentulous. Mean percent improvement of cats with teeth was 56.3%, while that of edentulous cats was 48.3%. This difference was not statistically significant (p = 0.7223).

Client perception of improvement: At any time during the 6 week observation period, more Group A clients reported "significant improvement" than did clients with cats in Group B, and more clients with cats in Group B reported "no change" than did clients with cats in Group A (Fig. 4). For Group A clients at T1, 4 considered their cats to have shown mild improvement, 3 reported moderate improvement, and 1 each reported significant improvement or no change. For clients of Group B at T1, 1 reported significant improvement, and 2 each reported mild improvement, no change, or worse. At T2, 5 clients of Group A considered their cats to have improved significantly (compared with only 1 client in Group B), 2 noted mild improvement (as did 2 owners of cats in Group B), 1 noted moderate improvement (none in Group B), and 1 reported no change (compared with 4 clients of Group B cats who reported no change). At the final post-treatment examination (T3), 7 owners of Group A cats reported significant improvement (while 2 owners of Group B cats felt their cats had improved significantly), 1 reported mild improvement (none in Group B) and 1 reported no change (while 5 owners of Group B cats reported no change). These differences were not statistically significant at any of the three post-treatment time points. Correlation between client perception of improvement and overall improvement in SDAI scores was high. In Group A, the cat whose owner reported mild improvement at T3 had an improvement of 26.3 %, while the cat whose owner reported no change had a slight increase in SDAI score from T0 to T3 resulting in an "improvement" of -7.1 % (Table 8). In Group B, the two cats whose owners reported significant improvement had decreases in SDAI scores corresponding to 22 % and 68 % improvement overall, while the remaining 5 cats (whose owners all reported no change at T3) had improvement in SDAI scores of 5 % or less.

Figure 3

Percentage improvement. At each recheck, the mean improvement in SDAI scores increased for Group A, with a mean improvement of 52.1% (compared with 12.2% for Group B) after 6 weeks of treatment. This difference was statistically significant (Mann-Whitney test p-value = (0.0444).

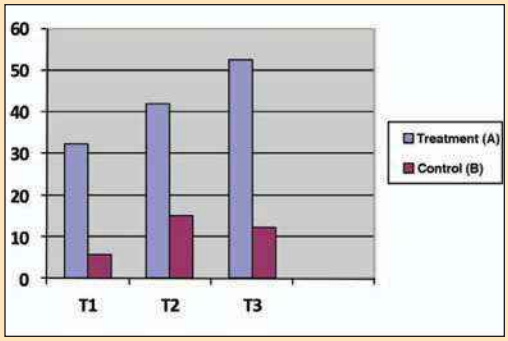


Figure 4

Client perception of improvement over time. At each recheck, the number of clients reporting "significant improvement" increased for patients in the treatment group (A), while the number of clients reporting "no change" increased for patients in the control group (B). These differences were not statistically significant.

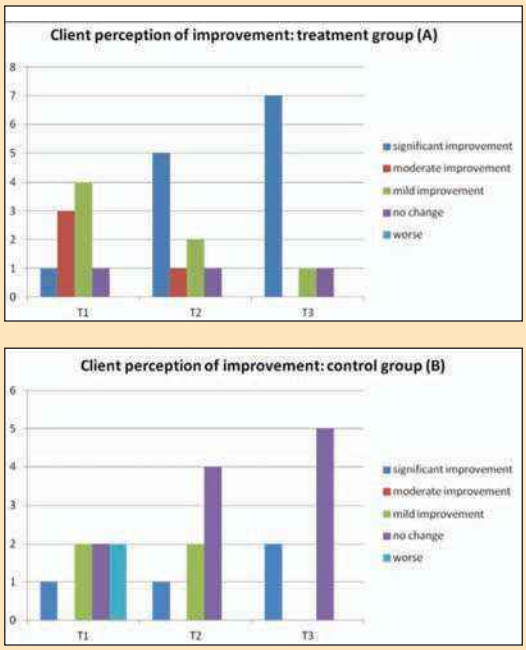
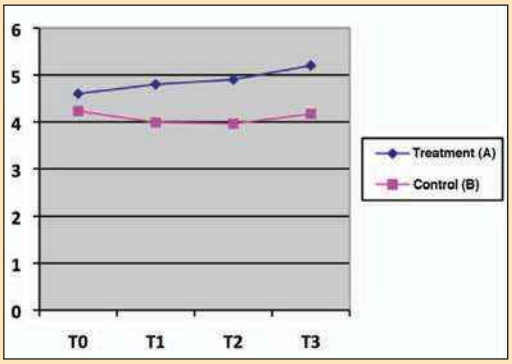


Figure 5

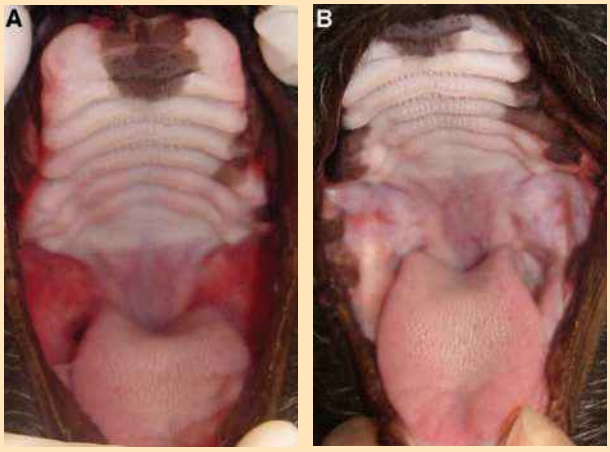
Progression of Median Weight* (kg) Over Time. The cats in Group A tended to gain weight (generally a desired effect for cats with stomatitis), while those in Group B maintained or lost weight.



* Median weight is shown because it is less likely to be influenced by outliers (e.g. the 2 obese cats in the treatment group) than is mean weight.

Figure 6

Photograph showing a 6-year-old spayed/female Maine coon cat with a Stomatitis Disease Activity Index (SDAI) score of 10 (A). Photograph of the same cat 15 months later (B) with a SDAI score of 4 while receiving cyclosporine (5.0 mg/kg BID).



Body weight: Mean body weight did not differ between the two groups at entry or at any of the post-treatment evaluations. Mean body weight of cats in Group A increased by 0.18 kg (from 5.42 kg to 5.60 kg) during the 6 week observation period (T0 to T3). Mean body weight of the cats in Group B increased by 0.16 kg (from 4.27 kg to 4.42 kg) from T0 to T3. This difference was not statistically significant (p = 0.8729). However, due to the presence of 2 obese cats in Group A, changes in median body weight more accurately reflected weight gain by the non-obese cats than changes in mean body weight. Median body weight for Group A cats increased by 0.6 kg (from 4.60 kg to 5.20 kg) over the 6 week observation period. Median body weight for Group B cats decreased by 0.06 kg (from 4.23 kg to 4.17 kg) from T0 to T3 (Fig. 5).

Need for rescue medications: Rescue medications were dispensed by the investigator when a client reported his or her cat demonstrating persistent drooling, pawing at the mouth, vocalizing after eating, or difficulty eating. None of the cats in Group A required rescue medications, while 4 of the 7 cats (57.1 %) in Group B received analgesics during the study period. This difference was statistically significant (p = 0.0192; 95 % CI = 0.1327-0.8418).

Side effects: Side effects were reported at T1, and included vomiting (2 cats in Group A and 1 in Group B), lethargy/depression (3 cats in Group A), and hair pulling (1 cat in Group B). There was no statistically significant difference in report of side effects between groups (p = 0.3575; 95% CI = -0.1881-0.5966).

Cyclosporine blood levels: Individual variability in the absorption of orally-administered cyclosporine was high. Among cats in Group A, trough whole-blood cyclosporine levels ranged from 32.1 ng/ml to 1,576.2 ng/ml (Table 8). The two cats in Group A that showed less than 40 % improvement in SDAI scores at the end of the 6 week study period each had trough whole-blood cyclosporine levels below 300 ng/ml. The mean improvement of cats in Group A with blood levels > 300 ng/ml (n = 5) was 72.3 %, while mean improvement of cats with blood levels < 300 ng/ml (n = 4) was 28.2 %. This difference was statistically significant (p = 0.0188).

Increased cyclosporine dosage: At the end of the 6 week evaluation period, for the 2 cats from Group A that had shown less than 40 % improvement in SDAI scores (as well as a third cat with trough whole-blood cyclosporine levels below 100 ng/ml who had shown 41.2 % improvement in SDAI scores), the dosage of cyclosporine was increased to 5.0 mg/kg BID, and monitoring (non-blinded) continued for an additional 6 weeks. Each of the 3 cats then demonstrated trough cyclosporine levels >300 ng/ml, and the mean improvement in SDAI scores at the end of the second 6 week period was 53.8 % for this subgroup.

Cross-over: Following the initial 6 week blinded evaluation period, 6 of the 7 cats in Group B were placed on cyclosporine at 2.5 mg/kg BID and monitoring (non-blinded) continued for an additional 6 weeks. Of the 6 cats, 5 achieved therapeutic blood levels of cyclosporine at 2.5 mg/kg BID. These cats showed an average improvement of 46.5 % over the second 6 week time period. One cat failed to achieve therapeutic levels even after increasing the dose of cyclosporine to 5.0 mg/kg BID and failed to respond, showing only a 5.6 % improvement.

Long-term findings: Although no longer blinded, observations of 11 of the study cats were continued at regular intervals for a longer term (currently ranging from 6 to 42 months) (Fig. 6). Five of the 11 cats (45.5 %) were clinically cured (defined as having no residual oral inflammation) after having received cyclosporine for 3 months or more. All 5 have been weaned off of cyclosporine and continue to have normal-appearing oral cavities, with follow-up ranging from 4 to 39 months since discontinuing cyclosporine. Six cats remained on cyclosporine indefinitely; 3 of those cats have since died of unre-

Table 8

Correlation of Client Perception of Improvement and Stomatitis Disease Activity Index (SDAI) Scores, and Effect of Cyclosporine Blood Levels Among Group A Cats.*

Patient	SDAI T0	SDAI T3	Percent improvement	Client perception of improvement at T3	Blood cyclosporine levels (ng/ml)
8-year-old MN Burmese	10.5	5	52.4 %	significant	175.8
11-year-old FS DSH	19	14	26.3 %	mild	276.0
15-year-old FS DSH	14	15	-7.1 %	no change	32.1
6-year-old FS Maine coon	17	10	41.2 %	significant	95.0
10-year-old MN DMH	11	6	45.5 %	significant	1,576.2
3.5-year-old MN DSH	22	2	90.9 %	significant	410.8
7-year-old MN DMH	14	5	64.3 %	significant	1,349.1
10-year-old MN DMH	26	5	80.8 %	significant	1,201.8
2-year-old FS DSH	10	2	80.0 %	significant	900.0

* The mean improvement of cats in the treatment group (Group A) with blood levels > 300 ng/ml (n = 5) was 72.3 %, while the mean improvement of cats with blood levels < 300 ng/ml (n = 4) was 28.2 %.This difference was statistically significant (p = 0.0188).

lated causes, and 3 cats are still receiving treatment. Five cats were lost to long-term assessment.

Discussion

Although extraction of all premolar and molar teeth (with or without extraction of the canine and incisor teeth) results in significant improvement in 60-80 % of cats with chronic stomatitis,[17,18] the remaining 20-40 % of cats with persistent inflammation typically require medical intervention to preserve their quality of life. Various immunomodulatory therapies including gold salts,[2] bovine lactoferrin,[27,28] and even thalidomide[28] have been attempted. Recently, a European study reported that recombinant feline interferon omega (rFeIFN-ω) delivered transmucosally was as effective as prednisolone in decreasing clinical lesions and pain scores.[29] Although these are promising data, this product is not yet commercially available in the United States. Glucocorticoids remain the most commonly prescribed medication for management of refractory stomatitis, however administration is not consistently helpful and may be accompanied by deleterious effects such as behavior changes, thinning of the skin, polyuria, polydypsia, and potential for development of diabetes mellitus.[30-32] Thirteen of the 16 cats who completed this study (6 from Group A and 7 from Group B) had previously received steroid therapy, which was discontinued due to inefficacy (10 cats) or significant side effects (3 cats), which included diabetes mellitus (2 cats) and subcutaneous fat necrosis (1 cat).

While glucocorticoids exert wide-reaching effects on the immune system, which include decreasing neutrophil diapedesis, redistributing lymphocytes to extravascular compartments, and down-regulating maturation of antigen-presenting cells,[30] cyclosporine's effects are more targeted to T cells and, to a lesser extent, B cells[20-22]. Since lymphocytes and plasma cells are the predominant cell type found in stomatitis,[1,2] it makes sense that cyclosporine would be explored as a treatment option.

One of the challenges of using orally administered cyclosporine is the variable bioavailability of different formulations, compounded by individual variation in intestinal absorption and hepatic metabolism.[21,33,34] It has been demonstrated that a microemulsified formulation[c] has a more predictable, linear dose response than the originally-available, oil-based product[d], that requires emulsification by bile salts and digestion by pancreatic enzymes in the gastrointestinal tract.[34] As a result, much higher doses of the oil-based product must be administered to achieve adequate blood levels. When cyclosporine was first used for immunosuppression of renal transplant recipients, the initially administered dosage was 7.5 mg/kg BID to achieve trough whole-blood levels of 400-600 ng/mL.[35] Although a microemulsified, encapsulated cyclosporine product is commercially available and FDA-approved for use in dogs[e], a custom-compounded microemulsified liquid formulation[c] was selected for this study due to: 1) the ability to give each individual a precise 2.5 mg/kg dose; 2) relative ease of administering a liquid rather than a pill to a cat with a painful mouth; and, 3) ease of preparing a placebo with similar characteristics. The custom-compounded formula in a fish oil base used in this study was accepted well by cats and clients. Only 1 of the 16 cats hid from the client to avoid receiving the medication, and this behavior ceased within the first 2 weeks.

Individual variation in absorption was evident, and affected treatment outcome even with the use of the microemulsified formulation. The 2 cats in Group A that showed less than 40 % improvement in SDAI scores at the end of the 6 week study period each had trough whole-blood cyclosporine levels below 300 ng/ml. In these 2 cats (as well as a third cat with trough whole-blood cyclosporine levels below 100 ng/ml that had shown 41.2 % improvement in SDAI score), the dosage of cyclosporine was increased to 5.0 mg/kg BID, and monitoring continued for another 6 weeks. Each of these 3 cats then demonstrated trough cyclosporine levels > 300 ng/ml, and the mean improvement in SDAI scores at the end of the second 6 week period significantly increased. This is consistent with published recommendations for immunosuppression of transplant recipients by achieving trough levels of 300-500 ng/mL.[36,37]

Although 3 cats of Group A had blood levels > 1000 ng/mL, side effects reported were mild (lethargy in 2 of the 3 cats). Other side effects reported among the 16 cats included vomiting (2 cats in Group A), soft stool (1 cat in each group) and hair pulling behavior (1 cat in Group B). In addition, most clients reported a fishy odor and deposition of the fish oil on fur during grooming behavior. The incidence of side effects was not statistically significant between groups. Diarrhea is reportedly the most common side effect of cyclosporine administration in cats.[38] More serious side effects including severe upper respiratory tract infections and disseminated toxoplasmosis have been rarely reported.[39-42] Although seizures, diabetes mellitus, hypertension, and a higher-than-normal risk of malignant neoplasia have been reported in feline renal transplant recipients receiving prednisolone and cyclosporine,[36,39,43-47] further investigation is required to determine what role cyclosporine administration may play in the development of these conditions. In order to minimize the risk of life-threatening infections, cats receiving cyclosporine should be kept indoors and should not be fed raw meat.

One of the most interesting findings of this study was the cat receiving placebo who demonstrated 68 % improvement during the 6 week observation period. Fish oil is high in long-chain omega-3 (ω3) polyunsaturated fatty acids (PUFA). Diets high in fish oil lead to replacement of ω6 PUFA in lymphocyte membrane phospholipids with ω3 PUFA[48] and have been reported to inhibit lymphoproliferative responses and reduce production of proinflammatory cytokines in humans and a variety of animals.[49,50] It was hypothesized that the fish oil base in both groups may have contributed an anti-inflammatory effect, supported by the previous demonstration of an immune-modulating effect of a diet high in ω3 PUFA in cats.[51] A recent study investigated the effects of 2 different dietary ratios of ω6:ω3 PUFA on wound healing and oral inflammation for 4 weeks following premolar-molar extractions in a population of 14 cats with stomatitis.[50] Although higher dietary levels of ω3 were associated with lower plasma levels of certain prostaglandins and leukotrienes, a clinical effect was not appreciated. The role of PUFA in inflammation is an area anticipated for further investigation.

Another thought-provoking finding was the relatively greater improvement in cats of Group A having never received steroids prior to entering the study compared with cats that had previously been administered steroids. Although this difference was not statistically significant, it may have been influenced by the very small sample size reducing statistical power, and precluding robust statistical analysis with a Mann-Whitney test. Since most of the cats enrolled in this study were presented to the investigator as a secondary and sometimes tertiary care provider, detailed information about the type, dosage, frequency, and duration of corticosteroid therapy was often lacking. A larger scale investigation comparing response rates of these two subsets of cats would be very interesting, the results of which may potentially influence how newly-presented cases are managed in the future. In the author's practice, stomatitis cats are treated surgically rather than medically (i.e., dental extractions are performed soon after diagnosis) and refractory cases are usually prescribed cyclosporine rather than prednisolone. If glucocorticoid administration has a negative effect on response to alternative therapies such as cyclosporine, it would be prudent to recommend against the use of steroids in these cats. Again, this remains to be demonstrated, and further studies are needed.

This study has several limitations, the most obvious being the small number of cats and the resulting lack of statistical power when evaluating observations such as effect of previous steroid administration, changes in body weight, and client perception of improvement. Recruitment was difficult given the strict inclusion criteria, notably the requirement for an indoor-only lifestyle. The presentation of the medication in fish oil with tuna flavoring eliminated several prospective cats also, as some clients reported that their cats do not like fish. Although it is possible to formulate cyclosporine in vegetable or peanut oil and to add flavoring such as poultry or beef, it was important for the oil base and flavoring agents to be consistent for all cats in the study, and fish oil with tuna flavoring was selected based on the belief that it would be well accepted by most cats. Another reason for failure to enroll prospective cats was client resistance due to the chance of receiving a placebo. Placebo was selected instead of an alternative treatment such as prednisolone in part due to the hydrophobic, oil-based nature of cyclosporine. A compounded prednisolone suspension would not have had the same consistency, potentially resulting in non-blinded observations. Giving prednisolone or placebo tablets was not considered, as client compliance with administering both a liquid and a tablet by mouth twice daily to a painful group of cats seemed unlikely. As a result of choosing a placebo-controlled protocol, we elected to limit the observation period to 6 weeks. With the allowed use of opioid analgesics and non-steroidal anti-inflammatory medications, the 6 week time frame was short enough to expect compliance with the study protocol even for those cats assigned to the placebo group. The six week observation period in this study was obviously insufficient to see the full beneficial effects of cyclosporine administration, but was long enough to demonstrate that a majority of cats receiving the drug had substantial reductions in oral inflammation.

Another challenge was the inclusion of 2 obese cats in Group A. With the scoring system used, weight loss adds 3 points to the SDAI score, increasing the overall score even if the weight loss was desired. This may have resulted in underestimation of the beneficial effects of cyclosporine in these 2 cats. This was an unforeseen limitation of the scoring system, as most cats with chronic stomatitis have difficulty eating and maintaining body weight. Modifying the scoring index to reflect body condition score (BCS) rather than body weight (with a lower BCS corresponding to a higher point total) may be a better approach.

Following the initial 6 week observation period, cats revealed to be in Group B were then allowed to "cross over" and receive active medication at no charge for an additional 6 week observation period. Observations of this group were not blinded at this stage, and therefore the data were not subjected to statistical analysis. However, the information obtained is of value, as is the information obtained from longer-term observation of cats from Group A that continued to receive cyclosporine after the study period.

In conclusion, microemulsified cyclosporine administered orally was found to be well tolerated and effective in reducing oral inflammation in the majority (8/9) of cats with refractory stomatitis. Cats with trough cyclosporine blood levels above 300 ng/mL demonstrated a greater than 70 % reduction in their disease activity index scores over the 6 week observation period. In addition,

progressive improvement was noted at later time points for those cats that continued receiving the medication after the study was completed.

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[a] 42-12, IM3 Incorporated, Vancouver, WA

[b] Cavitron ProphyJet, Densply Professional, York, PA

[c] Neoral, Novartis Pharmaceuticals Corporation, East Hanover, NJ

[d] Sandimmune, Novartis Pharmaceuticals Corporation, East Hanover, NJ

[e] Atopica, Novartis Pharmaceuticals Corporation, East Hanover, NJ

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