Topical 0.1% Tacrolimus for the Treatment of Discoid Lupus Erythematosus and Pemphigus Erythematosus in Dogs

Topical 0.1% tacrolimus was used for treatment of localized lesions associated with 10 cases of discoid lupus erythematosus (DLE) and two cases of pemphigus erythematosus (PE) either as a sole therapy (n=2) or as an adjunctive treatment (n=10). Eight of 10 dogs with DLE and both dogs with PE were improved following 8 weeks of topical application. In six of the eight dogs that improved, other medications were discontinued. No adverse effects in clinical or laboratory parameters were noted throughout the study. **J Am Anim Hosp Assoc 2004;40:29-41.**

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Introduction

Discoid lupus erythematosus (DLE) and pemphigus erythematosus (PE) are immune-mediated diseases with lesions often localized to the nasal planum and dorsal muzzle and, less commonly, the pinnae, periocular skin, and lips.[1] Discoid lupus erythematosus is a relatively benign cutaneous disease with no systemic involvement, and it is the second most common immune-mediated dermatitis of the dog.[1,2] Pemphigus erythematosus is thought to represent a more benign form of pemphigus foliaceus or possibly a crossover syndrome between pemphigus and lupus erythematosus.[1,3]

The initial presentation of both DLE and PE may be characterized by erythema, depigmentation, and scale with loss of normal appearance and texture of the nasal planum that frequently progresses to erosion or ulceration and crust, and may extend onto the dorsal muzzle. Lesions may be exacerbated by sun exposure and may have a waxing and waning course. In the early phases, sun avoidance, topical sunscreens, topical glucocorticoids, and systemic vitamin E and omega-3/omega-6 fatty acids may be helpful in treating these diseases.[1,4] Commonly used therapy for progressive cases includes the addition of tetracycline and niacinamide.[5] For more severe or refractory cases, oral glucocorticoids may be needed in addition to other systemic immunosuppressive drugs such as azathioprine or chlorambucil.[1] While vitamin E, tetracycline, and niacinamide have minimal long-term adverse effects, their success may be more limited or may require frequent oral dosing (e.g., *tid* in the case of tetracycline/niacinamide) for optimum results.[5] Glucocorticoids, azathioprine, and chlorambucil are generally more successful in managing these cases, but they may be associated with systemic toxicity, potential long-term adverse effects, and must be monitored closely; therefore, they should be used with caution.[1,6]

Topical therapies for localized lesions of DLE and PE have, to date, consisted primarily of medium- to high-potency corticosteroids and may be plagued by limited success, epidermal atrophy, and potential adverse

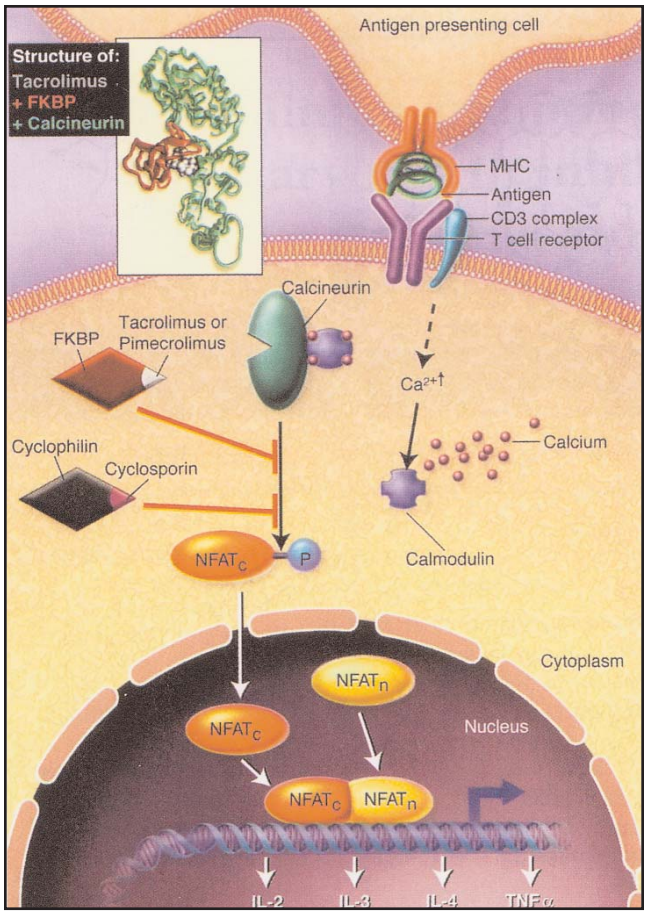


Figure 1—Molecular mechanism of inhibition of the immune response by tacrolimus, pimecrolimus, and cyclosporine. T-lymphocyte activation is initiated by interaction of antigenic peptide presented in the major histocompatibility complex (MHC) to the appropriate T-cell receptor. Activation signals from the CD3 complex cause an increase in intracellular calcium and induce the synthesis of the nuclear subunit of the nuclear factor of activated T cells (*NFATn*). Elevated free calcium in the cell binds to calmodulin, which binds and activates calcineurin, a critical calcium-activated protein phosphatase. Calcineurin causes the dephosphorylation of the cytoplasmic subunit of NFAT (NFATc), allowing it to translocate to the nucleus. The newly synthesized nuclear subunit (NFATn) then binds to NFATc and this essential complex facilitates transcription of numerous cytokines including tumor necrosis factor α (TNFα) and interleukins 2, 3, and 4 (IL-2, IL-3, IL-4). Tacrolimus, pimecrolimus, and cyclosporine block this normal activation pathway by inhibiting calcineurin function. First, drug binds its intracellular ligand: tacrolimus or pimecrolimus bind FKBP and cyclosporine binds cyclophilin. In each case, these complexes gain the ability to bind calcineurin and block its ability to dephosphorylate NFATc. In other cell types, such as mast cells, degranulation is a calcium-dependent event and is also blocked by tacrolimus or cyclosporine. *Inset:* The crystal structure of the complex of FKBP (in red), tacrolimus (in white), and calcineurin (in green) is modified from the x-ray crystal structure solved in 1995. The groove bound by FKBP-tacrolimus is adjacent to the active site on calcineurin and blocks the ability of substrate to interact with calcineurin effectively. (Reprinted with permission from Nghiem P, Pearson G, Langley RG. Tacrolimus and pimecrolimus: From clever prokaryotes to inhibiting calcineurin and treating atopic dermatitis. J Am Acad Dermatol 2002;46:228-241.)

effects from systemic absorption.[7] Topical cyclosporine has been used as an adjunctive therapy for localized lesions of DLE and PE with anecdotal reports of benefit.[8]

The objective of this study was to explore the use of 0.1% tacrolimus in an attempt to identify a therapy that is both successful and safer than many of the current treatment modalities for DLE and PE, either as a sole therapy or as an adjunctive therapy.

Tacrolimus (formerly known as FK506)[9] is a 23-member macrolide produced by *Streptomyces tsukabaensis*, a fungus found in the soil of Mount Tsukuba, Japan. Since its discovery in 1984, the intravenous and oral formulations have been used worldwide in the prevention of organ rejection following allogenic liver, kidney, pancreatic, heart, and lung transplantation in humans.[10-13] Tacrolimus, like cyclosporine, is an immunomodulator whose primary mechanism of action is the inhibition of calcineurin, an important factor in the intracellular signal transduction pathway [Figure 1]. Potency of tacrolimus, however, has been estimated at 10 to 100 times greater than cyclosporine. Inhibition of the phosphatase activity of calcineurin results in suppression of antigen-presenting T cells, inhibition of the production and release of inflammatory cytokines including many interleukins (i.e., IL-2, IL-3, IL-4), granulocyte-macrophage colony-stimulating factor (GM CSF), tumor necrosis factor α (TNFα), and interferon-χ (IFNχ).[14] Tacrolimus has also been shown to down-regulate cytokine expression in other cells, including mast cells, basophils, eosinophils, keratinocytes, and Langerhans' cells.[15,16] In topical formulations, tacrolimus has been extensively studied in humans and recently approved for the treatment of atopic dermatitis in children and adults. It is associated with minimal systemic absorption, has a wide margin of safety, and is not atrophogenic.[17-24]

Materials and Methods

Twelve dogs with a diagnosis of DLE or PE were selected for inclusion by compatible history, clinical signs, and histopathological findings. Prior to beginning topical tacrolimus, blood was obtained from each dog for complete blood count (CBC) and serum biochemical analysis. Each dog's lesions were assessed for degree of erythema, crust, ulceration or erosion, depigmentation, and scarring. Each of these parameters was graded on a scale of 0 to 3 (0=absent; 1=mild; 2=moderate; 3=severe). The distribution and nature of lesions observed were recorded for each of the six surface areas of the nasal planum and the dorsal muzzle [Figure 2]. Current medications were recorded, and clinical photographs were taken. The owner was instructed to apply a thin film of 0.1% tacrolimus ointment topically to the affected areas of the nasal planum and the dorsal muzzle every 12 hours. No special handling precautions were recommended due to the wide margin of safety in numerous human studies. In an attempt to minimize immediate licking of accessible areas after application, the owner was advised to apply the topical ointment prior to feeding or other distracting activities when possible. Dogs were evaluated 2, 4, and 8 weeks after starting therapy. At each evaluation, clinical

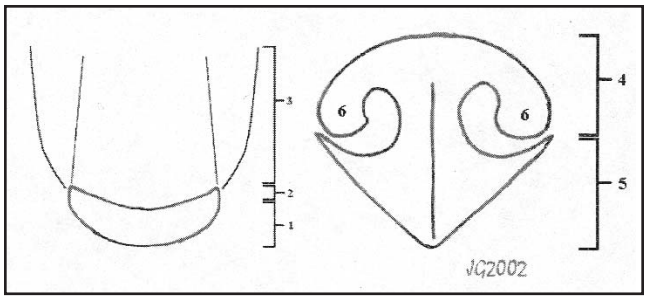


Figure 2—Six surface areas of muzzle and nasal planum evaluated in 12 dogs with either discoid lupus erythematosus (n=10) or pemphigus erythematosus (n=2).

parameters were again evaluated and recorded, a CBC and serum biochemical profile were performed, and photographs were taken to aid in assessing clinical response. After 8 weeks, clinical evaluation scores were summarized for each case, and differences in each parameter were calculated from day 0 to week 8. Dogs with a reduction of >2 in two or more of the clinical evaluation scores from day 0 to week 8 were considered to have had an excellent response. Those with a reduction of >1 in two or more of the clinical scores were considered to have had a partial response, while those with fewer changes were considered to have had no response.

Whole blood was also obtained 2 to 4 hours after application for evaluation of systemic absorption of the drug. Tacrolimus levels were assayed via Abbot IMx microparticle enzyme immunoassay (MEIA). This test is used routinely for therapeutic drug monitoring in human transplant patients and is preferred over previously used plasma enzyme-linked immunosorbent assays (ELISA) due to its ability to better detect lower drug levels.[25] It is the preferred method for therapeutic drug monitoring in human patients and is available in many human hospitals.[26] This test has not yet been verified for use in veterinary medicine.

At each reevaluation, the owner was questioned about any observable adverse effects, including increased redness, pruritus, or pain response following application. When rapid improvement allowed, concurrent medications such as oral prednisone, azathioprine, or tetracycline and niacinamide were decreased or stopped.

The relatively small number of cases evaluated and the lack of a placebo-controlled group made statistical analysis impossible.

Results

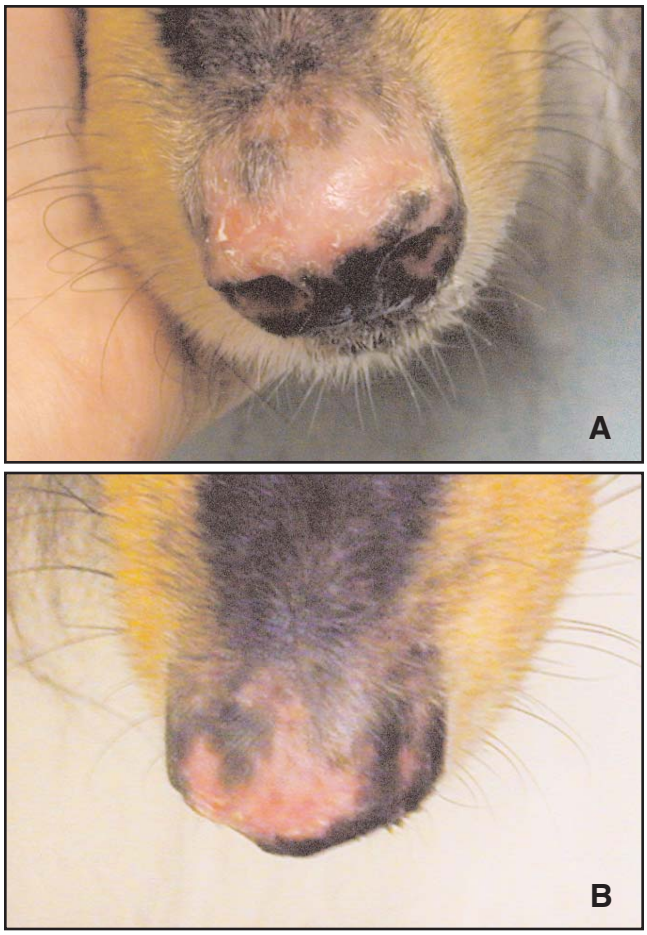
Patient Population

Two dogs were enrolled in the study immediately after the diagnosis of DLE. The remaining cases were diagnosed from 6 months to 7 years before beginning the study and had been treated with a variety of medications, including vitamin E, tetracycline and niacinamide, topical and oral glucocorticoids, and, in two cases, glucocorticoid and azathioprine combination therapy. Of these, seven had an incomplete response to previous therapy, one had adverse effects from niacinamide, one exhibited unsatisfactory glucocorticoid effects (i.e., polyuria and polydipsia [PU/PD]), and one exhibited elevated liver enzyme values, warranting discontinuation of previous medication (i.e., azathioprine). The age range of all study dogs was 3 to 10 years (mean, 5.25 years; median, 5 years). Breeds represented included the Akita (n=3), chow chow (n=1), springer spaniel (n=2), Australian shepherd (n=1), collie (n=1), border collie (n=1), Jindo (n=1), and mixed-breed dogs (n=2) [Table 1].

Clinical Evaluation

Five of the cases evaluated had an excellent response, five had a partial response, and two dogs showed no clinical response to topical tacrolimus therapy [Table 1]. Of the two cases (case nos. 1, 11) treated immediately after diagnosis, both were diagnosed with DLE and had lesions predominantly affecting the nasal planum. Both cases exhibited a decrease in crust and scale within the first 2 weeks, with other changes occurring more gradually. At the end of the study period, both were considered to have a partial response based on improvement in clinical scores. The first of these (case no. 1) exhibited depigmentation of approximately 80% of the dorsal nasal planum, erythema throughout the depigmented surface, and mild scale of the nasal planum and the dorsal muzzle. The clinical lesions improved slowly throughout the study and exhibited areas of repigmentation at week 8. Clinical scores for this case indicated improvement in erythema, crust, and scarring, with slight improvement in depigmentation. The owner continued to apply topical tacrolimus once daily as a sole therapy [Table 2], and the dog was reevaluated after an additional 3 months. At this time, though slight scale remained, the dorsal planum exhibited a much smaller area of depigmentation occupying approximately 40% of the dorsal surface [Figure 3]. The second of these two cases (case no. 11) had lesions confined entirely to the nasal planum with erosion, hemorrhage, and crust in many areas. This dog exhibited gradual progress, which at the end of week 8 was characterized by a decrease in erosion and ulceration with less hemorrhage of the nasal planum. The complete depigmentation was unchanged, and some erosion continued at the ventral aspect of the nares [Figure 4]. Changes in clinical scores for this case included elimination of crust, slight decrease in erythema, no change in depigmentation, and an increase in scarring from mild to moderate. The distribution of lesions at the end of the study period was unchanged.

The remaining eight cases of DLE were diagnosed 3 months to 7 years prior to beginning the study. Two of these (case nos. 3, 5) initially had seasonal clinical signs and were treated with tetracycline and niacinamide only when lesions were present. Prior to beginning the study, however, these cases were no longer responding to this therapy, even with the addition of topical glucocorticoids. Four dogs (case nos. 2, 7, 9, 12) needed topical or oral glucocorticoids in order to maintain remission of their disease. At the start of the study, two of these were under partial control (case nos. 2, 9), while two (case nos. 7, 12) continued to have active lesions.



Figures 3A, 3B—Discoid lupus erythematosus in a 4-year-old collie (case no. 1). Photograph of the dorsal muzzle on day 0 or pretreatment **(3A)** and 5 months **(3B)** following topical tacrolimus therapy.

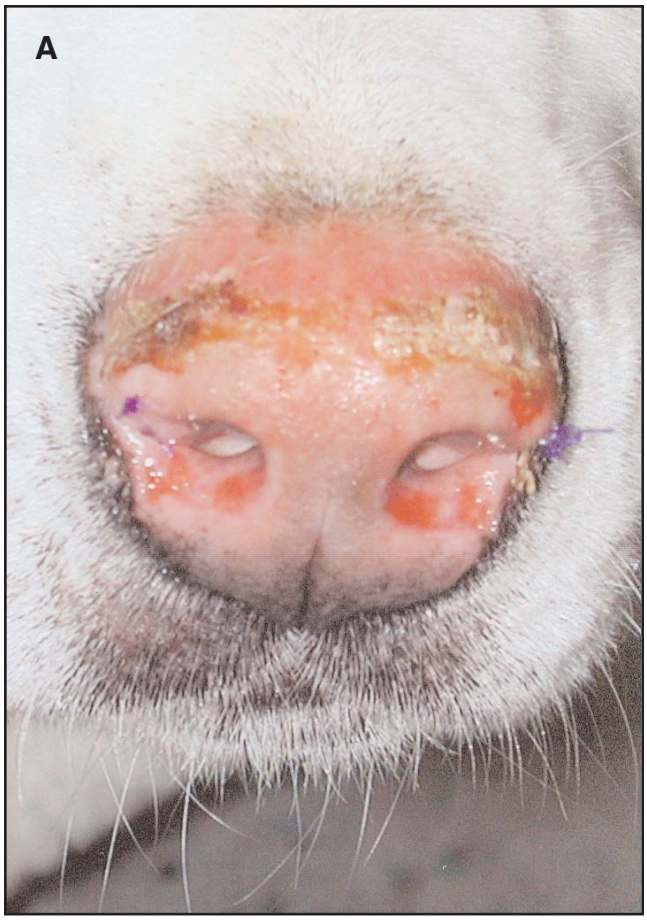
The two other cases (case nos. 4, 8) continued to have active disease with minimal response to tetracycline, niacinamide, and prednisone. Six of the eight dogs in this group exhibited improvement in their clinical disease, while two showed no clinical change. Three had an excellent response, and three had a partial response.

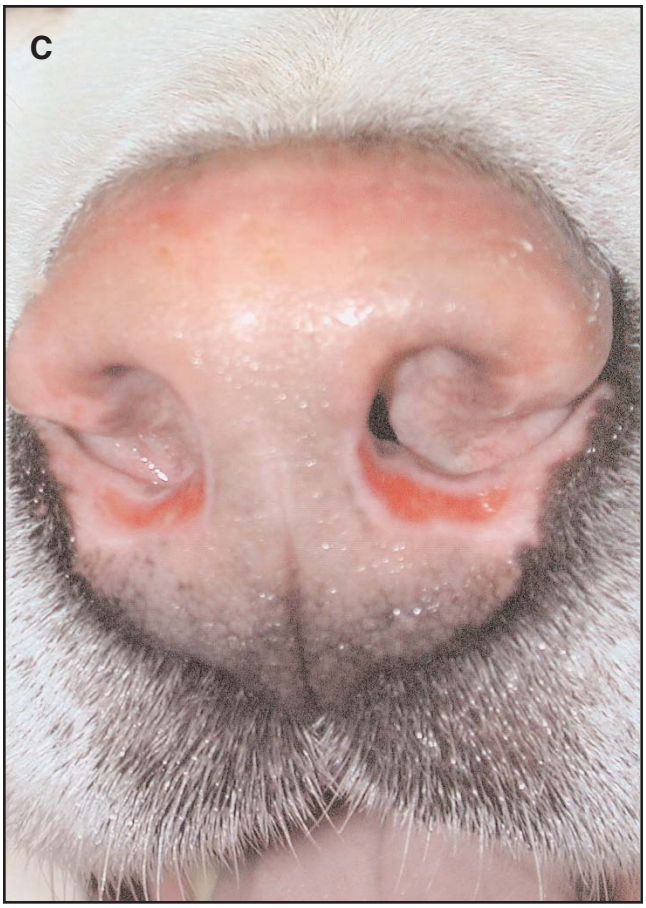
Of the excellent responders, clinical improvement was observed in 2 to 4 weeks. By week 8 in all three of these cases, all other medications had been discontinued [Table 2]. Clinical evaluation scores for these cases indicated decreases in crust and erosion from severe to mild or absent. Decreases in erythema varied from mild to severe; in depigmentation and scarring, decreases ranged from absent to mild. In case nos. 4 and 7, localized lesions on the dorsal muzzle were much smaller by week 8 [Figure 5]. Case no. 2 had a reduction in erythema, crust, and erosion after 8 weeks; and after 5 months with topical tacrolimus as a sole therapy, this case exhibited almost complete repigmentation of the nasal planum [Figure 6].

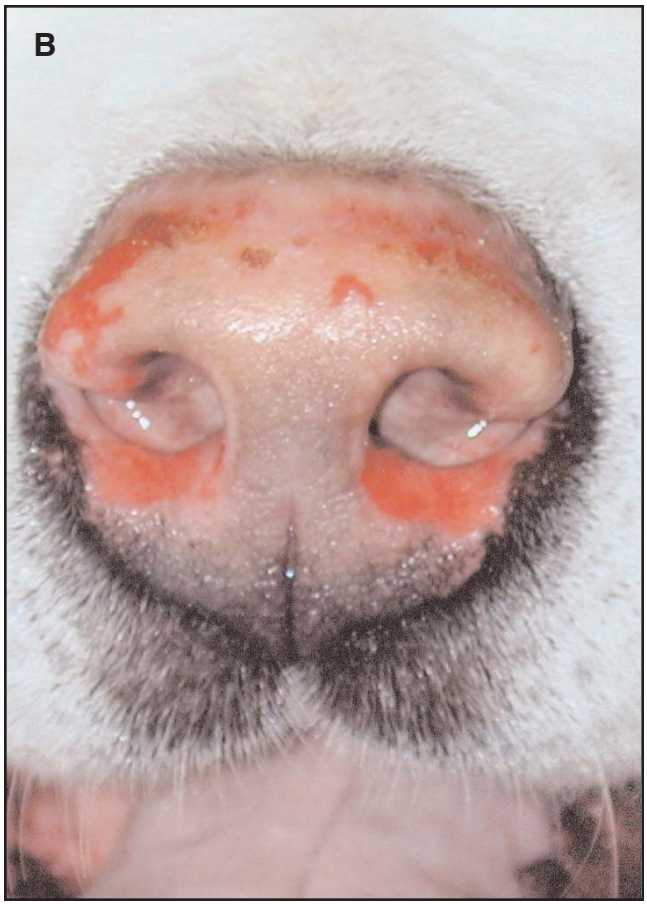
Of the three DLE cases with a partial response, two exhibited seasonal disease initially, allowing administration of tetracycline and niacinamide only when lesions were present. In both cases, lesions had been more difficult to manage in the last 12 months and were no longer responding to tetracycline and niacinamide in combination with oral (case no. 5) or topical (case nos. 3, 5) glucocorticoids. These cases exhibited focal lesions, which became moderately smaller after 8 weeks of therapy. Clinical evaluation scores indicated a one-point decrease in crust and erythema in both cases. Erosion in case no. 3 had a three-point decrease from severe to absent, as a band of erosion across the dorsal muzzle healed and was replaced by scarred epidermis. Consequently, the clinical score for scarring increased from mild to moderate. The remaining partial responder (case no. 12) had also been treated previously with a combination of tetracycline and niacinamide. Niacinamide was discontinued, however, due to the development of PU/PD, which resolved when this medication was stopped. Oral prednisone was then used with limited success, but polyphagia and PU/PD made this therapy undesirable. Partial response to topical tacrolimus in this case was characterized by decreases in erythema, crust, and depigmentation. Clinical evaluation scores indicated a one-point decrease in each of these parameters. In this case, oral prednisone was decreased and stopped by the beginning of week 4. Continued gradual improvement was seen, and the owner continued topical tacrolimus as a sole therapy.

Of the two DLE cases that exhibited no response, one (case no. 9) was diagnosed 2 years prior to beginning the study. Good response was seen initially from combination therapy with oral prednisone and tetracycline and niacinamide, but mild crust and depigmentation persisted. At the beginning of tacrolimus therapy, concurrent medications used included tetracycline and niacinamide (*tid*) and prednisone (0.4 mg/kg body weight, *q* 48 hours). Moderate depigmentation and erythema over the dorsal aspect of the nasal planum were present at day 0 with mild crust. Following 8 weeks of topical tacrolimus therapy, the mild crust was absent, but areas of depigmentation and erythema remained relatively unchanged. Clinical evaluation scores indicated a decrease in crust of 1.5, while other parameters remained unchanged. Prednisone was discontinued at week 4, and tetracycline and niacinamide decreased to once-daily administration, rather than every 8 hours, at week 4, and prednisone was discontinued at week 10. Case no. 8 had a similar course showing little change in the level of erythema and depigmentation present on the nasal planum. In this case, topical 0.1% amcinonide was discontinued when topical tacrolimus was initiated. Clinical evaluation scores indicated slight improvement in crust, but no change in other parameters measured. Distribution of lesions on the various areas of the nasal planum identified also remained unchanged.

The two remaining cases (case nos. 10, 6) were diagnosed with PE 9 months and 4 years, respectively, prior to beginning topical tacrolimus. The initial presentation of case no. 10 consisted of depigmentation, erosion, and crusting of the nasal planum with crusted papules extending to the dorsal muzzle. Papules and occasional pustules were also present at the medial pinnae. After 3 months of therapy with tetracy-

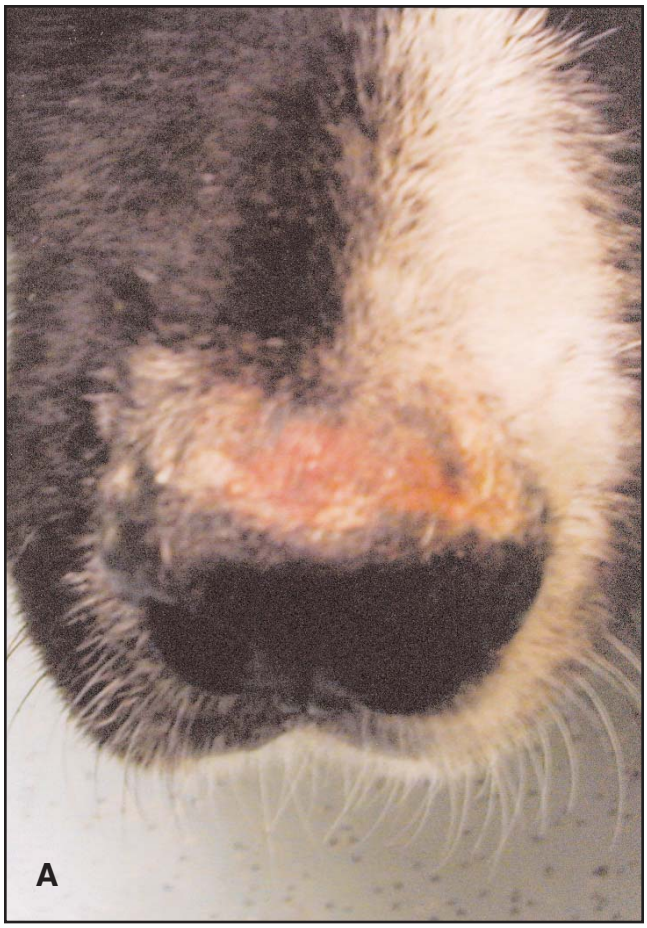


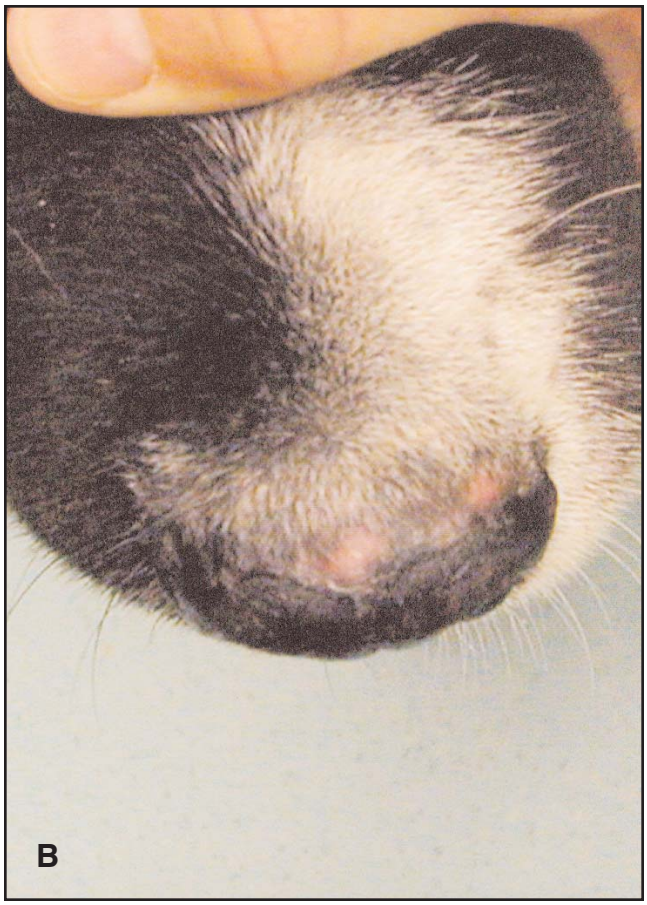




Figures 4A-4C—Discoid lupus erythematosus in a 4-year-old Akita (case no. 11). Photograph of the nasal planum on day 0 or pretreatment **(4A)**, and at 4 **(4B)** and 8 weeks **(4C)** following topical tacrolimus therapy.

cline and niacinamide, little progress was observed. Immunosuppressive therapy with oral prednisone and azathioprine was then prescribed with good response. After 8 weeks, the dog's lesions had decreased to only mild crust at the dorsal muzzle and the pinnal apices. However, serum biochemical profiling at this time indicated increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (SAP), and gamma glutyl transferase (GGT). These changes were attributed to azathioprine therapy, which was then discontinued. Despite the continuation of oral prednisone (1.5 mg/kg body weight, *q* 48 hours), lesions began to recur within 2 weeks. At this point, the dog was entered into the topical tacrolimus study. At day 0 there was erythema, erosion, and moist crust at the dorsal muzzle and nasal planum and crust at the medial pinnae. Previously elevated liver values returned to within reference ranges, except for ALT (199 μ/L; reference range, 12 to 118 μ/L) and SAP (340; reference range, 5 to 131). After 2 weeks of topical tacrolimus therapy, crust and erosion had decreased from moderate to absent. Further tapering of the dose of oral pred-





Figures 5A, 5B—Discoid lupus erythematosus in a 6-year-old Akita (case no. 4). Photograph of the dorsal muzzle on day 0 or pretreatment **(5A)** and 8 weeks **(5B)** following topical tacrolimus therapy.

nisone was initiated, resulting in discontinuation at week 6. Tacrolimus was applied to the nasal planum, the dorsal muzzle, and the medial surface of the pinnae in this case. At week 8, the dog continued to do well with only mild erythema remaining. Clinical evaluation scores indicated a two-point decrease in crust, erosion, and depigmentation, which was classified as an excellent response.

Case no. 6 was diagnosed with PE 4 years prior to entering the study. Lesions consisted of erythema, depigmentation, erosion, and crust of the nasal planum and dorsal muzzle. For 3 years following the initial diagnosis, these were relatively mild, and treatment consisted of tetracycline and niacinamide during the spring and summer months only. One year prior to beginning the tacrolimus study, lesions were no longer controlled with this regimen and had increased in severity, warranting the addition of oral prednisone. Though some improvement was seen, remission of clinical disease was not achieved. Following minimal progress and increases in liver enzyme values (AST, 184 U/L; reference range, 15 to 66 U/L; ALT, 1,380 U/L; reference range, 12 to 118 U/L; SAP, 1,937; reference range, 5 to 131; GGT, 161 U/L; reference range, 1 to 12 U/L), azathioprine was added (53 mg/m^2, *sid*), and the glucocorticoid was changed to oral triamcinolone. After 5 weeks of glucocorticoid and azathioprine combination therapy with minimal progress, the dog was started on topical tacrolimus. Lesions at day 0 consisted of severe erythema, erosion, and crust of the nasal planum and dorsal muzzle with an area of scarring, depigmentation, and crust extending from the dorsal nasal planum onto the muzzle approximately 6 cm. Triamcinolone (0.16 mg/kg body weight, per os [PO] *q* 48 hours) and azathioprine (53 mg/m^2, PO) were continued. After 2 weeks, there was much less crust over the dorsal planum and muzzle. A large, erythematous area of erosion and depigmentation remained at this stage. Triamcinolone was reduced (0.055 mg/kg body weight, PO *q* 48 hours), and azathioprine was continued at the previous dose. Over the study period, gradual progress continued with a diamond-shaped area of depigmentation, scarring, and reepithelialization remaining at week 8. Oral medications at this time were further decreased and included triamcinolone (0.05 mg/kg body weight, PO *q* 72 hours) and azathioprine (50 mg, *q* 48 hours).

Laboratory Abnormalities

No abnormalities in laboratory parameters were noted at any time in the two cases treated with topical tacrolimus alone (case nos. 1, 11), in one of the two dogs with no response (case no. 9), and in one dog with an excellent response (case no. 2) [Table 3]. In the remaining eight cases, abnormalities consisted of increases in ALT, SAP, and serum cholesterol (CHOL). In seven of these eight cases,

Table 1

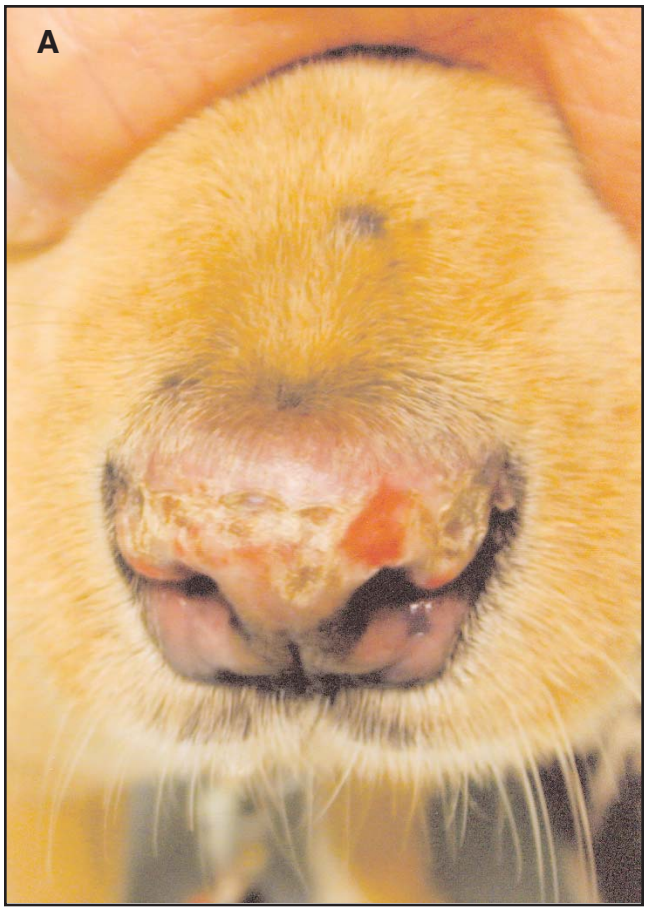
Response to Topical 0.1% Tacrolimus in 12 Dogs With Discoid Lupus Erythematosus (DLE) or Pemphigus Erythematosus (PE)

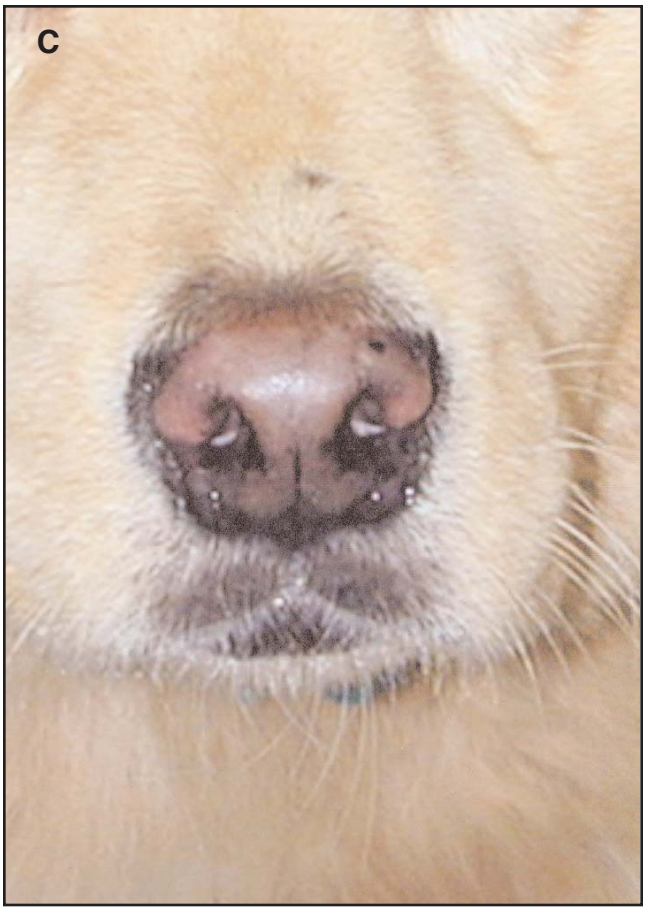
Case No.	Signalment*	Diagnosis	Response	Description
1	4-yr, 29-kg, FS collie	DLE	Partial	Rapid decrease in crust/scale; gradual, partial return of pigment
2	3-yr, 29.5-kg, MN chow chow mix	DLE	Excellent	Rapid improvement with decreased crust and erosion within a few days; almost complete repigmentation over 3 months
3	4-yr, 27.3-kg, MN Jindo	DLE	Partial	Decreased ulceration with gradual contraction of scarred lesion
4	6-yr, 49.5-kg, MN Akita	DLE	Excellent	Rapid improvement
5	10-yr, 27.3-kg, MN German shepherd mix	DLE	Partial	Gradual decrease in size of lesions
6	6-yr, 28.6-kg, MN chow chow	PE	Excellent	Marked decrease in erosion, crust, and area involved; also on triamcinolone and azathioprine
7	5-yr, 28.6-kg, FS border collie	DLE	Excellent	Gradual improvement
8	7-yr, 30-kg, FS Australian shepherd	DLE	No response	No change
9	7-yr, 25.9-kg, MN springer spaniel	DLE	No response	Only minor decrease in crust, but able to maintain without glucocorticoids (week 10)
10	3-yr, 26.3-kg, FS Akita	PE	Excellent	Crust, papules, and erosion resolved
11	4-yr, 30.5-kg, FS Akita	DLE	Partial	Persistent erythema and depigmentation; less erosion and hemorrhage
12	5-yr, 22.7-kg, MN springer spaniel	DLE	Partial	Marked improvement in crust and ulceration

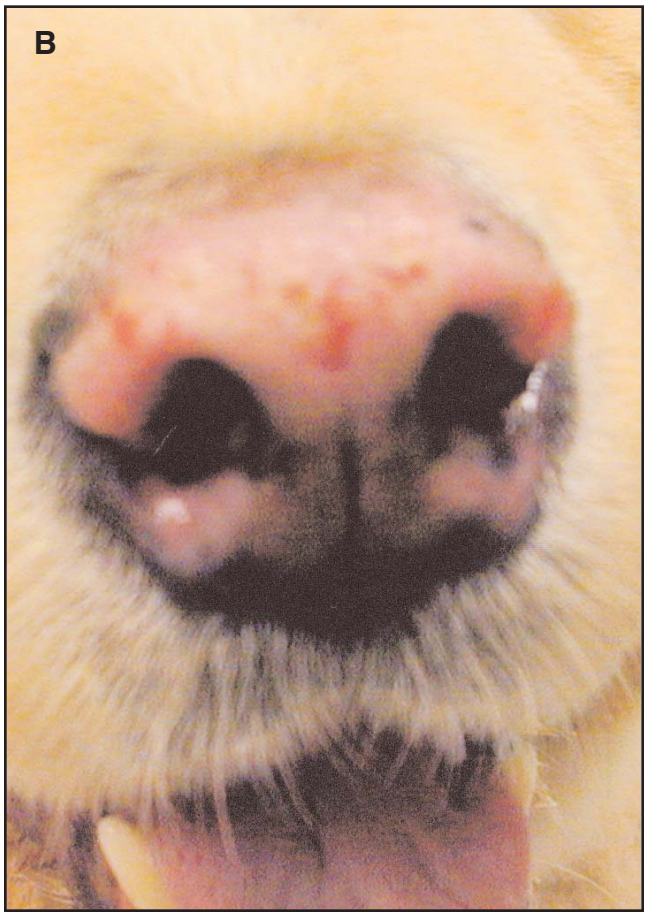
* FS=female spayed; MN=male neutered

abnormalities were present at day 0 prior to beginning the study. The one case without abnormalities at day 0 (case no. 8) had a single elevation at week 2 (CHOL, 352 mg/dL; reference range, 92 to 324 mg/dL) that was not identified on subsequent evaluation. In the remaining seven cases, abnormalities observed at day 0 included elevated SAP (n=7; range, 145 to 2,234 U/L; mean, 533.1 U/L; median, 334 U/L; reference range, 5 to 131 U/L) and increased CHOL (n=2 [case no. 3, 355 mg/dL; case no. 7, 369 mg/dL]; reference range, 92 to 324 mg/dL). Two of these cases were receiving tetracycline and niacinamide with topical glucocorticoid applied to lesions twice weekly. Three of the remaining five were receiving oral glucocorticoids, while two were receiving glucocorticoids and azathioprine. The two dogs receiving azathioprine also had an increased ALT (case no. 6, 201 U/L; case no. 10, 199 U/L; reference range, 12 to 118 U/L) at day 0.

At week 8, five cases continued to exhibit laboratory abnormalities [Table 3]. These consisted of increased SAP (n=5; range, 209 to 1,297 U/L; mean, 446.6 U/L; median,







Figures 6A-6C—Discoid lupus erythematosus in a 3-year-old chow chow mixed-breed dog (case no. 2). Photograph of the nasal planum on day 0 or pretreatment **(6A)**, and at 2 **(6B)** and 5 **(6C)** months following topical tacrolimus therapy.

237 U/L; reference range, 5 to 131 U/L), with two cases (case nos. 3, 7) also having increased CHOL. Case no. 6, which had the highest SAP, also had an elevated ALT (424 U/L; reference range, 12 to 118 U/L). When reevaluated at 12 weeks, all but case nos. 5, 6, and 7 had returned to within normal limits.

Tacrolimus Levels

Tacrolimus levels measured varied from <1.5 to 10.7 ng/mL (mean, 5.9 ng/mL; median, 5.8 ng/mL). Fluctuations observed in levels among patients did not correlate with apparent improvement of clinical lesions, adverse clinical effects or laboratory abnormalities, or frequency of application. When all levels were compared over the 8-week study, there was a slight downward trend from week 2 to week 8 [Figure 7]. However, when tacrolimus levels were compared to packed cell volume (PCV), a trend was identified in which levels increased with a higher PCV, independent of any other factors [Figure 8]. To verify this trend, whole blood was obtained from a small population of normal dogs (n=6) not receiving tacrolimus therapy. Tacrolimus levels measured were similar in these dogs when compared to those receiving the therapy despite no drug being administered.

Table 2

Concurrent Drugs

Case No.	Week 0		Week 8
1	None		None
2	Tetracycline/niacinamide	500 mg *q* 12 hrs	Discontinued week 4
3	Tetracycline/niacinamide Topical 0.1% amcinonide	500 mg *q* 8 hrs Replaced with tacrolimus	Discontinued week 8 Discontinued week 0
4	Tetracycline/niacinamide Prednisone	500 mg *q* 12 hrs 0.8 mg/kg *q* 48 hrs	Discontinued week 2 Discontinued week 6
5	Tetracycline/niacinamide Topical 1% hydrocortisone	500 mg *q* 12 hrs Replaced with tacrolimus	Discontinued week 2 Discontinued week 0
6	Azathioprine Triamcinolone	53 mg/m^2 *q* 24 hrs 0.16 mg/kg *q* 24 hrs	50 mg *q* 48 hrs 0.05 mg/kg *q* 72 hrs
7	Tetracycline/niacinamide Topical 0.1% amcinonide	500 mg *q* 8 hrs *q* 72 hrs; replaced with tacrolimus	500 mg ea *q* 24 hrs; discontinued after week 8 Discontinued week 0
8	Topical 0.1% amcinonide	*q* 72 hrs; replaced with tacrolimus	Discontinued week 0
9	Tetracycline/niacinamide Prednisone	500 mg *q* 24 hrs 0.4 mg/kg *q* 48 hrs	500 mg *q* 24 hrs 0.4 mg/kg *q* 48 hrs; discontinued week 10
10	Prednisone	1.5 mg/kg *q* 48 hrs	Discontinued week 6
11	None		None
12	Tetracycline/niacinamide*	500 mg *q* 8 hrs	Discontinued week 2

* Niacinamide discontinued due to polyuria/polydipsia that resolved once stopped

Discussion

Dermatological uses of oral and parenteral tacrolimus in human medicine include treatment of psoriasis, Bechet's disease, pyoderma gangrenosum, and difficult cases of systemic lupus erythematosus (SLE).[25,27,28] More recently, topical formulations have received widespread attention as a therapy for adults and children with atopic eczema. In long-term studies of up to 12 months in children and adults, topical tacrolimus has been found to be an effective treatment for atopic dermatitis with decreases in pruritus, percent body surface area affected, and overall severity of disease within 2 weeks that was maintained for the entire study. Adverse events from this therapy were most commonly confined to burning or pruritus at the site of application. The duration of burning or pruritus in the majority of cases was <10 minutes and generally occurred for only the first few days.[19,20,29]

The use of tacrolimus in dogs has been predominantly as an experimental model for human transplantation. In canine studies, systemic tacrolimus has been identified as a potent immunosuppressive drug that requires cautious

Table 3

Laboratory Abnormalities

Case No.	Diagnosis*	Week 0†	Concurrent Medications	Week 8	Concurrent Medications
1	DLE	None	None	None	
2	DLE	None	Tetracycline/niacinamide	None	
3		SAP, 145 CHOL, 355	Tetracycline/niacinamide, topical amcinonide	SAP, 209‡ CHOL, 358‡	
4	DLE	SAP, 157	Tetracycline/niacinamide, prednisone	None	
5	DLE	SAP, 334	Tetracycline/niacinamide, topical 1% hydrocortisone	SAP, 237	
6	PE	SAP, 2234 ALT, 201	Triamcinolone, azathioprine	SAP, 1,297 ALT, 424‡	Triamcinolone, azathioprine
7	DLE	SAP, 352 CHOL, 369	Tetracycline/niacinamide, topical amcinonide	SAP, 333 CHOL, 371	Tetracycline/niacinamide
8	DLE	None	Topical amcinonide	None	
9	DLE	None	Tetracycline/niacinamide, prednisone	None	Tetracycline/niacinamide
10	PE	SAP, 340 ALT, 199	Prednisone, azathioprine discontinued 2 weeks prior	SAP, 157‡	
11	DLE	None	None	None	
12	DLE	SAP, 170	Prednisone (discontinued after week 0), tetracycline/niacinamide§	None	

* DLE=discoid lupus erythematosus; PE=pemphigus erythematosus
† SAP=alkaline phosphatase; CHOL=serum cholesterol; ALT=alanine aminotransferase
‡ Rechecked; normal at 12 weeks
§ Niacinamide discontinued due to polyuria/polydipsia that resolved once stopped

administration and careful drug monitoring.[30,31] These findings were similar to those seen in humans.

The use of topical tacrolimus in dogs has been previously reported by two groups. The first report described clinical experience in the treatment of canine perianal fistulas with a topical 0.3% tacrolimus ointment formulated from the oral product.[32] In this series of 10 dogs, five of 10 had complete resolution of perianal lesions, four of 10 had partial response, and one showed no improvement. No adverse effects associated with medication were seen during this trial. Blood levels of tacrolimus were not measured.[32]

The second report is a randomized, double-blind, placebo-controlled, crossover study in which a 0.3% lotion was formulated from the oral drug and applied daily to the skin and coat of six atopic dogs for 28 days.[33] At the end of the study period, there was a decrease of erythema in the tacrolimus group, but no decrease of pruritus. In this study, tacrolimus levels (measured by ELISA methodology) were

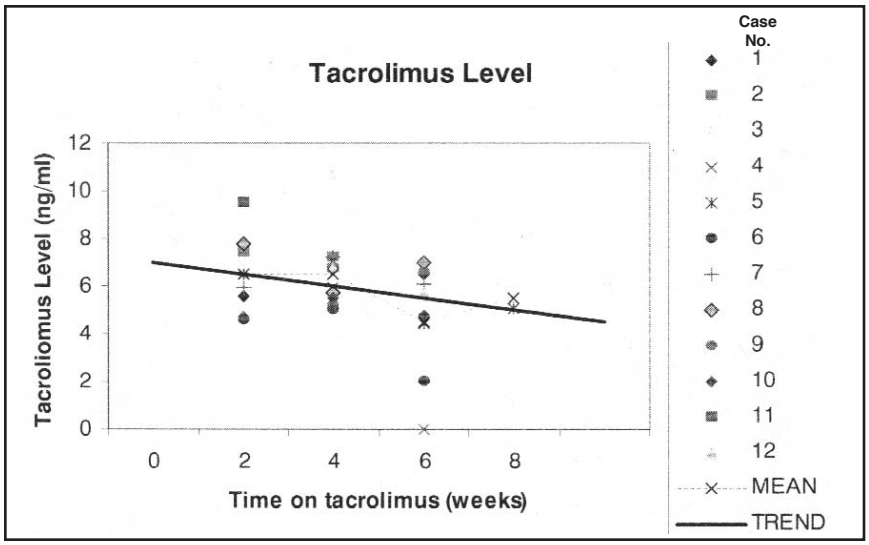


Figure 7—Whole blood tacrolimus levels of treated dogs during the study period (Abbot IMx microparticle enzyme immunoassay).

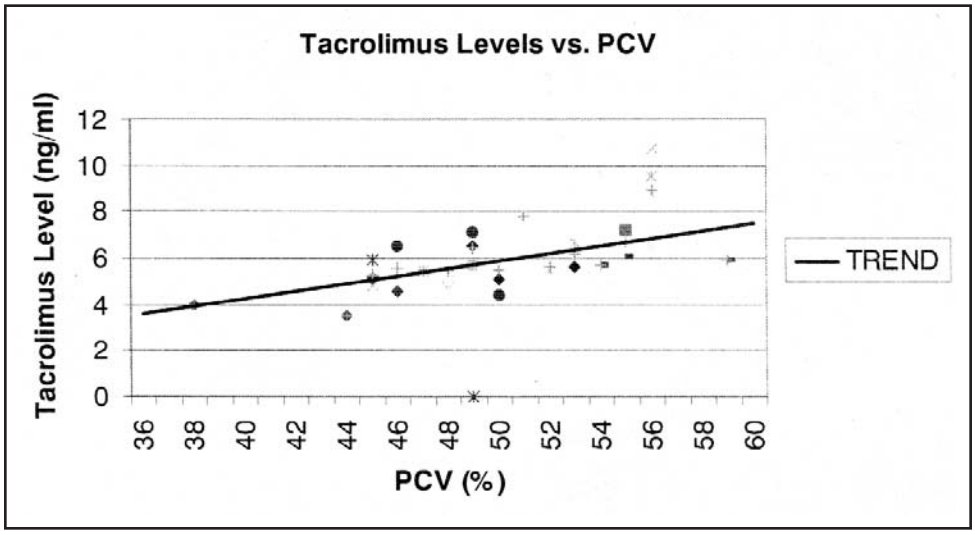


Figure 8—Whole blood tacrolimus levels versus packed cell volume (PCV) during the study period (Abbot IMx microparticle enzyme immunoassay).

increased on day 28 compared to day 0. No clinically important changes were observed in CBC and serum biochemical values during the trial, and no adverse effects were noted.[33]

While the pathogenesis of lupus erythematosus has not been fully elucidated, it has been suggested that B cell hyperactivity caused by immunologically abnormal T cell activation plays an important role. The reported ability of tacrolimus to inhibit mixed lymphocytic reaction by interfering with the action and production of T cell-derived soluble mediators, inhibit the induction of cytolytic T lymphocytes, and inhibit the activation of cloned T cells makes it a logical choice for the therapy of this disease. Previous studies in mice known to develop a spontaneous and progressive autoimmune disease, which resembles human SLE, found that those animals treated with tacrolimus had a significantly decreased incidence of lupus nephritis, lymphadenopathy, splenomegaly, and autoantibody production.[34] In human patients, tacrolimus may be a useful additional immunosuppressive agent in some patients whose SLE is not well controlled by conventional treatments.[28] Though topical use for cutaneous lesions associated with lupus has yet to be reported, these mechanisms and a wide safety margin make topical tacrolimus an attractive choice for localized lesions.

Since PE is proposed as a potential crossover disease sharing features of both lupus erythematosus and the pemphigus complex, these mechanisms may also be important. Though the antigen has not been identified, autoantibody production is an important feature of PE, which has also been categorized as a more benign form of pemphigus foliaceous. The ability of tacrolimus to inhibit T cell-dependent immunoglobulin production *in vitro* in a manner more potent than methylprednisolone, cyclosporine, or 6-mercaptopurine, is an added consideration in selecting this drug for therapy of pemphigus diseases.[35]

Of dogs in the present study, 10 of 12 exhibited improvement in clinical lesions associated with PE or DLE. Of the dogs that showed progress over the 8-week trial, eight of 10 were treated with tacrolimus alone by the end of the study and remained in remission at 12 weeks. The two PE cases treated

had more severe lesions than did the DLE cases and required more aggressive therapy. The progress of case no. 6 in particular, while most notable after beginning topical therapy, may have represented a lag phase associated with azathioprine therapy, making it difficult to determine if the benefit was from topical or systemic therapy. The ability to manage the relapse in clinical lesions in case no. 10 following the discontinuation of azathioprine due to elevated liver values, illustrated the ability of topical tacrolimus to assist in managing this case. Further benefit was seen as oral glucocorticoids were gradually discontinued and the dog was maintained in remission with topical therapy alone at 12 weeks. In all cases, the largest reduction in clinical evaluation scores was seen in crust and erosion. In the two cases with no response, DLE-associated lesions were restricted primarily to erythema and depigmentation. One of these cases (case no. 9), while exhibiting no real improvement on topical therapy, was able to discontinue oral glucocorticoids, which had been a necessary part of the dog's treatment regimen for the previous 12 months. After 8 weeks of topical therapy, this dog's clinical disease was maintained in remission by a combination of tacrolimus and once-daily tetracycline and niacinamide.

The repigmentation of the nasal planum in case nos. 1 and 2 at 5 months and 3 months, respectively, and the contraction of the depigmented, scarred dorsal muzzle lesion in case no. 7 suggest that long-term evaluation may be necessary to appropriately judge results. Photoaggravation has been previously reported in cases of DLE and PE and may have contributed to the apparently seasonal nature of some of the cases in the present study.[1] However, progression of previously seasonal cases to nonseasonal disease prior to beginning the trial would make improvement due to restricted sunlight exposure less likely.

Measurement of tacrolimus levels by the assay selected in this study was of little benefit. The false-positive measurements of tacrolimus levels in dogs not receiving the medication indicate apparent cross-reactivity with one or more components in the samples submitted. The trend of increasing levels that corresponded to increasing PCV suggests that increasing erythrocyte concentrations may contribute to the interference observed. Falsely elevated levels in humans with higher hematocrits have also been observed.[36] Interference of concurrent medications might also be considered. However, medications used in the dogs of this study have not been reported to alter the assay in humans. Similar values observed here in dogs on no systemic medications would also make this less likely.

Despite poor quantification of drug levels, the lack of laboratory abnormalities in dogs treated with topical tacrolimus alone, and the improvement in abnormal laboratory values as other medications were discontinued confirmed that there were no adverse systemic effects associated with this therapy. Though systemic absorption cannot be entirely ruled out, the amount of drug needed in the current formulation to reach levels used as an immunosuppressant in previous studies, even if the topical medication were ingested, would approach 30 g.[30,31] Typical amounts per application were 0.2 to 0.5 g.

Conclusion

The results of the current study indicate that topical 0.1% tacrolimus may be a safe and effective therapy for the treatment of DLE and PE. While further investigation is needed, this therapy may provide a better alternative with fewer systemic and topical adverse effects than current treatment modalities. Due to a lack of useful data from this study regarding tacrolimus blood levels in dogs, caution is warranted until further information on systemic absorption is determined. The absence of adverse clinical signs or laboratory parameters attributable to tacrolimus would suggest that its use should be as safe as that seen in human patients. However, until that information is further verified, careful monitoring of CBC, serum biochemical profiles, and adverse clinical effects is warranted, especially within the first few months of therapy.

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