A Randomized, Double-Blinded Placebo Controlled Crossover Study Evaluating 0.03% Tacrolimus Ointment Monotherapy in the Treatment of Discoid Lupus Erythematosus in Dogs

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Abstract

A randomized, double-blinded placebo controlled crossover study evaluated topical 0.03% tacrolimus ointment monotherapy applied twice daily for the treatment of canine Discoid Lupus Erythematosus (DLE). Twenty-one dogs with histological confirmation of DLE were enrolled. Dogs were allowed no other therapies typically used to treat DLE for 2 weeks prior to entering the study. Time outdoors was to remain the same throughout the study. During weeks 1-4 and 7-10, dogs were randomly assigned to receive tacrolimus or placebo; one treatment type per 4 week period. Weeks 5 and 6 were the wash out period where dogs received no tacrolimus nor placebo. Dogs were evaluated by the investigators and owners on days 0, 28, 42 and 70 for depigmentation, cobblestone architecture, erythema, crusts, ulcers and overall disease severity using a 0-5 scale. Complete blood counts and serum chemistries were also evaluated at each visit. Nineteen dogs completed the study; two dogs were lost to follow up, one after the first visit and one after day 42. No statistically significant changes were noted in any lab work parameters. Investigator assessment revealed statistically significant improvement in erythema (p=0.04), crusts (p=0.04), ulcers (p=0.001) and overall disease severity (p=0.003) when dogs received tacrolimus. Owner assessment revealed statistically significant improvement in cobblestone architecture (p=0.04) when dogs received tacrolimus. A statistically significant change was noted in overall disease severity, where 13 of 18 (72%) dogs receiving tacrolimus improved whereas only 3 of 19 (17%) dogs improved in overall disease severity, where 13 of 18 (72%) dogs receiving tacrolimus. Owner assessment revealed statistically significant improvement in cobblestone architecture, erythema, crusts, ulcers and overall disease severity using a 0-5 scale. Complete blood counts and serum chemistries were also evaluated at each visit. Nineteen dogs completed the study; two dogs were lost to follow up, one after the first visit and one after day 42. No statistically significant changes were noted in any lab work parameters. Investigator assessment revealed statistically significant improvement in erythema (p=0.04), crusts (p=0.04), ulcers (p=0.001) and overall disease severity (p=0.003) when dogs received tacrolimus. Owner assessment revealed statistically significant improvement in cobblestone architecture (p=0.04) when dogs received tacrolimus. A statistically significant change was noted in overall disease severity, where 13 of 18 (72%) dogs receiving tacrolimus improved whereas only 3 of 19 (17%) dogs improved on the placebo (p=0.008). Tacrolimus ointment can be beneficial in treating dogs with DLE.

Keywords: Discoid lupus Erythematosus; DLE; Tacrolimus; Canine; Dog

Introduction

Discoid lupus erythematosus (DLE) is the second most common immune-mediated skin disease in the dog [1,2]. DLE is generally considered a benign, non-life threatening disease. However, many of the current standard systemic and/or topical therapies are not without side effects or have an impractical dosing interval. Tacrolimus is a topical immunomodulator related to cyclosporine, but more potent than cyclosporine [3]. Tacrolimus has a relatively small molecular weight and thus has relatively better epidermal penetration when compared to cyclosporine, making it ideal for topical therapy [4,5]. Most of the published clinical studies evaluating topical tacrolimus have been performed in humans with atopic dermatitis. Studies, involving more than 15000 patients, have found topical use to be highly efficacious and overall well tolerated [4,6-12].

In addition to atopic dermatitis, tacrolimus has also been used in humans with other diseases including, but not limited to alopecia areata, contact hypersensitivity, graft-versus-host disease, psoriasis, lichen planus, rosacea, pyoderma granulomatosum, cutaneous lupus erythematosus, including the subtype, DLE, and vitiligo [4,13-20].

There is a relatively little research of tacrolimus ointment therapy in dogs. To the authors’ knowledge, most of the clinical research has centered on dogs with perianal fistulas and atopic dermatitis. In preliminary studies done in dogs, topical tacrolimus appears well tolerated and safe. Marsella and Nicklin conducted a small study with 8 atopic dogs where 0.3% tacrolimus lotion was applied twice daily to the affected areas for 28 days [21,22]. Tacrolimus levels were measured at day 0 and at day 28 at 0, 2, 4, and 6 hours after application. Seven dogs were used in the statistical analyses because one dog required ear surgery and thus was unable to complete the study. Mean blood concentrations of tacrolimus were below toxic levels in all dogs. However in 2 dogs, the day 28 blood concentrations of tacrolimus were 22.17 and 27.16 ng mL-1; this level is higher than 20 ng mL-1 which is the level where an increased risk of toxicity in humans and dogs is seen. In addition, complete blood counts and chemistry panels evaluated prior to tacrolimus lotion application and at days 14 and 28 after twice daily applications showed...
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no significant changes [21,22]. Note that the concentration of tacrolimus in the study by Marsella and Nicklin was ten times the concentration used in the study described herein where the tacrolimus concentration applied was 0.03%. In another study by Marsella, et al, the efficacy and safety of 0.1% tacrolimus ointment was evaluated in atopic dogs. Here, tacrolimus blood levels were below the level of toxicity in all dogs and no adverse effects were noted [23].

In a study of topical tacrolimus involving 351 children, 84% of them had non-detectable blood levels of tacrolimus; the highest level was 2.28 ng/ml (n=1) which is still below the therapeutic range of 5-15 ng/ml for transplant recipients [5,9] [Prograf package insert]. In Marsella et al’s [21] study of 8 atopic dogs, topical 0.3% tacrolimus significantly decreased investigator scores of erythema and pruritus, but there was no difference in owner scores for pruritus between treatments or when treated with placebo or tacrolimus. Transient skin irritation after application was not seen [22]. It has been noted that atopic dogs responded to topical tacrolimus lotion or ointment where they did not respond to oral cyclosporine [Marsella R, personal communication, 2001]. Topical tacrolimus ointment therapy has been studied in dogs with perianal fistulas. Fifty percent of dogs with perianal fistulas healed fully with 0.1% tacrolimus ointment, and noticeable improvement was noted in 90% of the dogs [24].

One study conducted by Griffies, et al evaluated the use of 0.1% tacrolimus applied topically in 10 dogs with DLE and 2 dogs with pemphigus erythematosus [25]. In this study, only 2 dogs received 0.1% tacrolimus as the sole therapy, whereas the other 10 dogs received the 0.1% tacrolimus as an adjunctive therapy. Results showed that 8 of 10 dogs with DLE and the 2 dogs with pemphigus erythematosus improved (5 had a partial response and 5 had an excellent response), after 8 weeks of topical therapy. In addition, in 6 of 8 dogs with DLE that improved, the other medications could be discontinued. No adverse effects were noted either clinically or on laboratory parameters.

The main advantage of tacrolimus ointment is that it works topically and not systemically. Also, it does not cause the local side effects as topical steroids can. The purpose of this study was to evaluate the efficacy and safety of 0.03% tacrolimus ointment monotherapy in the treatment of discoid lupus erythematosus in dogs.

Methods

Dogs

Dogs of any breed, greater than 6 months of age that demonstrated historical, clinical and histological evidence of DLE were eligible to participate in the study. Histological evidence of DLE in the last 3 years was required prior to entering the study. Dogs that had no histological evidence of DLE within the previous 3 years had skin biopsies performed in a standard manner under appropriate sedation or anesthesia with appropriate pre-anesthetic medications, local anesthetics and pain control. Informed owner/patient consent was obtained prior to entering the study. Dogs with any other immune-mediated skin diseases were excluded from the study. Some dogs were treated with anti-Staphylococcal antibiotics prior to entering the study so as to rule out mucocutaneous pyoderma. No other therapies typically used to treat DLE were allowed during the study and two weeks prior to entering the study. Such therapies included systemic and topical corticosteroids, tetracycline, niacinamide, vitamin E, tattooing, fatty acids, sunblocks, azathioprine, or chlorambucil. Oral or topical cyclosporine was also not permitted for 2 weeks or more prior to entering the study. For each individual dog, their time outdoors was to remain the same throughout the study.

Study design, Treatments and Blood Analysis

The study was a prospective, randomized, double-blinded placebo controlled cross-over study. During weeks 1-4, dogs were randomly assigned to receive the placebo (Aquaphor Healing Ointment (Eucerin)) or study drug (0.03% tacrolimus ointment (Protopic, AstellasPharma US)). Aquaphor was used as the placebo per the Protopic manufacturer’s recommendation. The ointments were placed in similar containers and marked ointment A or ointment B. Following the initial treatment period, dogs received either the study drug or placebo for a two week period (weeks 5 and 6) washout period. During weeks 7-10, dogs that initially received the study drug received the placebo and dogs that initially received the placebo received the study drug [21-24]. The investigators and all but one veterinary technician were blinded with respect to what the dogs were receiving. One technician was responsible for dispensing the placebo/study drug to the dog owner. Owners were instructed to apply the study drug or placebo to the affected area(s) twice daily (approximately every 12 hours). Owners were instructed to attempt to keep the dog from licking the ointment for 1 minute or more after each application. This was accomplished by distracting the dog with toys or food, holding the dog’s muzzle closed or placing a hand between the nasal planum and rostroventral lips. Owners were instructed to wear gloves and/or wash their hands after each application (gloves were supplied to the owners). The owners recorded in a log, each time the ointments were applied and where on the dog the ointments were applied. Owners also recorded any difficulty in applying the ointments and any adverse events noted during the study.

Dogs were evaluated on days 0, 28, 42, and 70. At each examination, each dog was evaluated by the investigators and the dog owner for each of the following: depigmentation, loss of cobblestone architecture of the nasal planum, erythema, crusts, ulcers and overall disease severity. A scale of 0 (normal), 1 (mild disease), 2 (mild to moderate disease), 3 (moderate disease), 4 (moderate to severe disease and 5 (severe disease) was utilized to assess each of these parameters. A complete blood count and biochemical analysis were performed at each visit (days 0, 28, 42 and 70).

The Animal Care and Use Committee at the Veterinary Referral Center of Colorado approved this study.

Statistical Analysis

Logistic regression (the GLIMMIX procedure in SAS, SAS Institute, Cary NC) was used to evaluate treatment effects
Results

Twenty-one dogs were entered into the study. Nine of the dogs were spayed females, one dog was an intact female and 11 of dogs were neutered males. Breeds of dogs in the study included German shepherd mixes (n=4), German shepherd Dogs (n=2), Labrador retriever mixes (n=2), Labrador retrievers (n=2), Australian shepherd mixes (n=2), Australian shepherd (n=1), American foxhound (n=1), basenji (n=1), malamute (n=1), collie (n=1), corgi (n=1), keeshond (n=1), Siberian husky (n=1), border collie/great Dane mix (n=1). Mean age of dogs entering in the study was 6.3 years (range 2.2-14.0 years). Approximate mean age of onset of DLE of dogs in the study was 5.0 years (range 1.3-8.3 years).

Seven dogs had received tetracycline and one dog had received doxycycline at some point for at least 2 weeks prior to entering the study. Other antibiotics administered to the dogs prior to entering the study included, amoxicillin in one dog, cephalaxin in five dogs and clindamycin in one dog. Two dogs had received enrofloxacin; one of these dogs had also received cephalaxin just prior to the enrofloxacin. One owner also applied mupirocin ointment to the dog's nasal planum concurrently while the dog received cephalaxin. The dogs were minimally responsive or unresponsive to these antibiotic therapies, except for one dog who on tetracycline at three times daily dosing may have shown some improvement.

Other medications for DLE that the dogs received prior to entering the study (with adequate withdrawal times) included neomycin-polymyxin-dexamethasone ophthalmic ointment, neomycin-polymyxin-bacitracin ophthalmic ointment, nystatin-neomycin-thiostrepton-triamcinolone ointment (Panalog®), betamethasone dipropionate ointment, fluocinolone-DMSO (Synotic®), neomycin sulfate-isoflupredone acetate-tetracaine hydrochloride ointment (Trito®), prednisolone acetate ophthalmic solution, vitamin E (both orally and topically), niacinamide, prednisone, omega-3 fatty acids, and carprofen.

Randomization via a coin toss resulted in 12 dogs starting with 0.03% tacrolimus ointment and 9 dogs starting with the placebo ointment; statistically, there was no sequence effect. This indicates that the order in which the dogs received tacrolimus and placebo had no effect on the results. Twenty dogs had DLE of the nasal planum and one dog had DLE of the vulva. Dogs started the study in the following months: (number of dogs entered in that month is in parenthesis following the month) were January (2), February (2), March (4), May (6), June (3), July (2), October (1), November (1). Nineteen dogs completed the study and were used in the statistical analyses, with one exception noted below. One dog was lost to follow up after the initial visit. A second dog was lost to follow up after the third visit (day 42). Both of these dogs were receiving tacrolimus when they were lost to follow up.

Assessment by clinicians showed a statistically significant improvement in erythema (p=0.04), crusts (p=0.04), ulcers (p=0.001), and overall disease severity (p=0.003) (Figures 1,2). Owner assessment showed statistically significant improvement in cobblestone architecture (p=0.04). Owner assessment also showed trending towards statistically significant changes in depigmentation (p=0.11), erythema (p=0.13), and overall disease severity (n=0.16). When evaluating overall disease severity, 13 of 18 (72%) dogs receiving tacrolimus improved whereas only 3 of 19 (17%) dogs receiving the placebo improved (p=0.008). Eighteen and nineteen dogs were statistically evaluated for overall disease severity after tacrolimus and placebo applications, respectively. The difference in number of dogs statistically evaluated is because one owner failed to complete the “overall disease severity” data point on day 70. This dog had received placebo from days 0-28 and tacrolimus from days 42-
The standard therapies have included sun avoidance, the use of sunscreen (SPF 15 or greater), vitamin E, fatty acids, as well as immune-modulators in diseases such as DLE has is falling out of favor. Vitamin E and fatty acids are safe, however as sole therapies are often ineffective; hence these therapies are typically used as adjunctive therapies. Tetracycline or doxycycline with niacinamide, vitamin E and fatty acids may take up to 2 months to be effective.

There is one report of hydroxychloroquine used with success in a dog with generalized discoid lupus erythematosus; this dog also had 0.1% tacrolimus ointment applied intermittently. There is also a report of three German shorthaired pointers with exfoliative cutaneous lupus erythematosus where hydroxychloroquine may have helped stabilize the disease, but remission was not achieved. Antimalarials, along with photoprotection topical corticosteroids and/or tacrolimus, are commonly used in humans with DLE. To the authors’ knowledge, there are no reports of using antimalarials in dogs with localized DLE. However, one author (im) has used hydroxychloroquine in a dog with DLE confined to the nasal planum that responded to oral corticosteroids, however was refractory to many other standard therapies. This dog had no response or steroid sparing effect while receiving hydroxychloroquine.

Anecdotal reports indicated that topical 1% cyclosporine was effective in treating dogs with DLE. Rosychuk observed that topical 1% cyclosporine was most effective when ulcerated lesions were present; when the ulcers resolved, the response topical cyclosporine therapy seemed to plateau. In contrast, tacrolimus ointment appeared to be effective in the various types of DLE lesions, i.e. with or without ulcers. (Rosychuk R, personal communication, 2004) Tacrolimus is related to cyclosporine, but more potent than cyclosporine. Tacrolimus is smaller in molecular weight; this likely contributes to better epidermal penetration. These properties, and possibly others, likely result in its improved efficacy over topical cyclosporine. Tacrolimus diffuses across cell membranes and binds to FK506 (tacrolimus)-binding proteins that inhibit calcineurin and thus interleukin (IL)-2 release and cytokine expression. Tacrolimus is typically reserved for moderately to severely affected cases refractory to other therapies and/or where sun avoidance is not possible as these therapies often have undesirable side effects. Topical corticosteroids are considered safer than systemic corticosteroids, however cutaneous atrophy, alopecia and/or poor hair growth are typical side effects. In a study by White et al, tetracycline and niacinamide combination therapy was found to be significantly effective in up to 70% of the dogs with DLE. However, Rosenkrantz found tetracycline and niacinamide combination therapy to be effective in only 25% of dogs with DLE. Tetracycline/niacinamide therapy is safe, however must be given every 8 hours, which makes this therapy impractical for many dog owners. In general, due to the prevalence of resistant bacterial infections, the use of antibiotic therapy used as immune-modulators in diseases such as DLE has is falling out of favor. Vitamin E and fatty acids are safe, however as sole therapies are often ineffective; hence these therapies are typically used as adjunctive therapies. Tetracycline or doxycycline with niacinamide, vitamin E and fatty acids may take up to 2 months to be effective.
also inhibits the transcription and release of other cytokines, including IL-3, IL-4, IL-5, interferon-gamma, granulocytemacrophage colony stimulating factor and tumor necrosis factor-alpha [4-6,17,33]. In mammals, calcineurin is involved in many functions including renal and brain function and the immune response. Lymphocytes have a relatively low level of calcineurin expression when compared to neurons or nephrons. The low abundance and absolute requirement of calcineurin in the activation of the immune system has led to the relatively selective sensitivity of immune function to tacrolimus, resulting in immunosuppression [5]. Similarly, tacrolimus down-regulates the expression of cytokines in basophils, eosinophils, keratinocytes, mast cells and Langerhans’ cells [21,34,35].

In humans, the most commonly reported adverse event associated with 0.03% tacrolimus ointment use was skin burning, albeit generally transient [8,17]. In a study by Soter, et al., skin burning was reported in 45.6% and 25.8% of the atopic humans treated with 0.03% tacrolimus ointment and the placebo, respectively (a significant difference; \( p < 0.001 \)) [8]. In addition, in studies conducted in humans, little to no significant systemic absorption was noted [8,9,33]. Tacrolimus applied to approximately 45% of body surface area (mean) was found to be safe and effective when compared to a placebo in atopic humans [8,9]. In March, 2005, a black box warning was issued by the United States Food and Drug Administration which stated that the long-term safety of topical calcineurin inhibitors has not been established and that although a causal relationship was not been shown, there have been rare reports of cancer (for example, lymphoma) in patients who had been treated with tacrolimus and the related topical immune-modulator, pimecrolimus.

This study showed 0.03% tacrolimus to be safe and effective in the treatment of dogs with DLE, showing 72% of the dogs receiving tacrolimus improved whereas only 17% of the dogs receiving the placebo improved. In addition, no significant changes in any laboratory parameters were noted in dogs treated with 0.03% tacrolimus ointment or the placebo ointment. In this study, assessments by clinicians showed a statistically significant improvement in erythema, crusts, ulcers, and overall disease severity, whereas the parameters assessed by the owners only showed statistically significant improvement in cobblestone architecture. Further review of the results would suggest that although the owner assessment of response due to tacrolimus was similar to that reported by the clinicians, dogs in the placebo group were considered to be improved at a higher rate when assessed by the owner as compared to the clinician.

Topical tacrolimus ointment has an advantage over other therapies in being safe and well tolerated by dogs. For example, cutaneous atrophy, alopecia and poor hair regrowth, as can be seen with topical corticosteroid use, are not seen with topical tacrolimus. Also, systemic side effects as can be seen with systemic corticosteroids, tetracycline, doxycycline, niacinamide, azathioprine and chlorambucil, are not seen with topical tacrolimus. Tacrolimus as a sole therapy is also more effective than vitamin E and fatty acid therapy. A disadvantage of tacrolimus ointment is that some dogs do not lend themselves to topical therapies; however none of the dog owners in this study found the topical applications to be difficult and all dogs tolerated the applications well. Another disadvantage to some clients may be the cost of the brand name tacrolimus (Protopic®). In this study, the 0.03% tacrolimus ointment was used instead of the 0.1% tacrolimus ointment because the lower concentration was found to be less irritating in people.

A retrospective study by Wiemelt et al that compared the histopathological features of DLE and mucocutaneous pyoderma (MCP) affecting the nasal planum was presented in the midst of the current study [36]. Due to some of the overlapping clinical and histopathological features of DLE and MCP, the study by Wiemelt et al evaluated 27 nasal planum biopsies to determine if DLE and MCP were histopathologically distinguishable. Then 15 of the cases were followed to determine if they were responsive to immunomodulatory therapies or antibiotic therapy (excluding tetracycline and doxycycline). Of those 15 cases, 11 were immunomodulatory responsive and 4 were antibiotic responsive. The authors found that response to treatment was not predictable based on histopathological features [36]. Because of this study, it has been recommended by some that the dogs be treated with appropriate antibiotic therapy for 2 or more weeks prior to the biopsy procedure. If the dog’s nasal dermatitis shows good improvement, then continued antibiotic therapy (until maximum or complete improvement is noted) may be warranted prior to considering biopsies. If there is little or no improvement of the nasal dermatitis, then biopsies are warranted to pursue a more definitive diagnosis of the various causes of nasal planum disease. For this reason, alter the Wiemelt et al study was presented, several study candidates were placed on antibiotic therapy prior to biopsies or prior to starting the study; these dogs had little or no response to antibiotic therapy. Because not all dogs were treated with antibiotics prior to entering the study, it is conceivable that some dogs entered into this study had MCP rather than DLE. If this were the case, then one could conclude that a higher success rate might have been noted as one would not expect dogs with MCP to respond to topical tacrolimus ointment or that perhaps both DLE and MCP do respond to topical tacrolimus ointment.

In summary, the authors believe 0.03% tacrolimus to be safe, well tolerated and effective in the treatment of dogs with DLE, although safety with long-term use of 0.03% topical tacrolimus is unknown. It may be used as the sole therapy for dogs with DLE, or if needed as an adjunctive therapy with other DLE therapies.

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**Declarations**

**Ethical Approval:** The Animal Care and Use Committee at the Veterinary Referral Center of Colorado approved this study.

**Clinical Trial Registration:** not applicable

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