

A randomized, vehicle-controlled trial of tacrolimus ointment for treatment of atopic dermatitis in children

Mark Boguniewicz, MD,^a Virginia C. Fiedler, MD,^b Sharon Raimer, MD,^c Ira D. Lawrence, MD,^d Donald Y. M. Leung, MD, PhD,^a and Jon M. Hanifin, MD^e for the Pediatric Tacrolimus Study Group* Denver, Colo, Chicago and Deerfield, Ill, Galveston, Tex, and Portland, Ore

Background: A topical formulation of tacrolimus, an immunosuppressant currently marketed for the prevention of rejection after solid organ transplant, is a potential therapeutic agent for atopic dermatitis.

Objective: We sought to determine the safety and efficacy of tacrolimus ointment in pediatric patients with moderate-to-severe atopic dermatitis.

Methods: In this double-blind, vehicle-controlled multicenter trial, children ages 7 to 16 years were treated with twice daily application of tacrolimus ointment at 1 of 3 concentrations (0.03% [$n = 43$], 0.1% [$n = 49$], or 0.3% [$n = 44$]) or vehicle ($n = 44$) for up to 22 days, with a 2-week follow-up period.

Results: The Physician's Global Evaluation of clinical response showed that 69% (95% confidence interval: 53-82) of patients in the 0.03% tacrolimus ointment group, 67% (95% confidence interval: 52-81) in the 0.1% tacrolimus ointment group, and 70% (95% confidence interval: 54-83) in the 0.3% tacrolimus ointment group, compared with 38% (95% confidence interval: 24-54) in the vehicle group, had a marked to excellent ($\geq 75\%$) improvement or clearing of their atopic dermatitis ($P = .005$, $.007$, and $.004$, respectively for the 3 tacrolimus groups compared with the vehicle group). The mean percent improvement for a modified Eczema Area and Severity Index at end of treatment for each of the 3 tacrolimus groups (0.03%, 72%; 0.1%, 77%; and 0.3%, 81%) was significantly better than that of the vehicle group (26%; $P < .001$). The median percent reduction in pruritus was significantly greater for tacrolimus-treated patients (74% to 89%) than for vehicle-treated patients (51%,

$P = .027$). No serious systemic adverse events were noted, and systemic absorption was minimal.

Conclusion: Tacrolimus ointment appears to be safe and effective in children with atopic dermatitis. (*J Allergy Clin Immunol* 1998;102:637-44.)

Key words: Atopic dermatitis, tacrolimus, ointment, children/pediatric, eczema

Atopic dermatitis is the most common chronic skin disease in children.¹ Characterized by chronic skin inflammation, cutaneous erythema, induration, severe pruritus, overexpression of IL-10, high IgE levels, and eosinophilia, atopic dermatitis causes significant interference with school, work, and social interactions.²⁻⁶ Topical corticosteroids, by virtue of their antiinflammatory properties, are currently the most potent treatment for atopic dermatitis. However, in patients with more severe disease, the therapeutic response to these agents is not always satisfactory. In addition, chronic use may be associated with significant side effects. This is particularly true with the use of more potent topical corticosteroids on the face or intertriginous areas. In pediatric patients growth retardation and adrenal insufficiency may be associated with prolonged use of topical corticosteroids.⁷ Cyclosporine given orally has been reported to be effective for atopic dermatitis, but concerns over systemic toxicity have limited its use.⁸ Topical therapy with cyclosporine has been attempted, but patients failed to show significant improvement, probably because of insufficient penetration into the skin.⁹

Tacrolimus or FK506, a macrolide lactone isolated from *Streptomyces tsukubaensis*, is an immunosuppressive agent with a spectrum of activity similar to that of cyclosporine.¹⁰ Tacrolimus is currently used to prevent allograft rejection in liver and kidney transplantation. Its lower molecular weight and higher potency compared with cyclosporine suggest it could be effective as a topical agent. Several uncontrolled studies and 1 randomized, placebo-controlled trial in adult patients have reported that treatment of atopic dermatitis with topical tacrolimus results in markedly diminished pruritus and skin inflam-

From ^athe Division of Pediatric Allergy-Immunology, National Jewish Medical and Research Center and the Department of Pediatrics, University of Colorado Health Sciences Center, Denver; ^bthe Department of Dermatology, University of Illinois at Chicago; ^cthe Department of Dermatology, University of Texas, Galveston; ^dthe Department of Research and Development, Fujisawa USA, Inc, Deerfield; and ^ethe Department of Dermatology, Oregon Health Sciences University, Portland.

This work was supported by a grant from Fujisawa USA, Inc.

*Additional study investigators are listed in the Appendix.

Received for publication Mar 24, 1998; revised June 17, 1998; accepted for publication June 18, 1998.

Reprint requests: Jon Hanifin, MD, Department of Dermatology, Oregon Health Sciences University, 3181 SW Sam Jackson Park Rd - L468, Portland, OR 97201

Copyright © 1998 by Mosby, Inc.

0091-6749/98 \$5.00 + 0 1/1/92702

mation.¹¹⁻¹³ No studies have investigated the effects of tacrolimus in children, the population of greatest interest. The current randomized, double-blind, vehicle-controlled, multicenter study is the first trial designed to evaluate the efficacy and applicability of topically applied tacrolimus ointment in childhood atopic dermatitis.

METHODS

Patients and study design

Subjects were children ages 7 to 16 years with 5% to 30% body surface area involvement and moderate-to-severe atopic dermatitis according to the criteria of Hanifin and Rajka.² Before enrollment, patients had to stop topical and inhaled corticosteroids for 1 week and systemic corticosteroids for 6 weeks. Patients receiving ultraviolet light therapy and immunotherapeutic agents had to stop such therapy at least 1 month before enrollment. Patients requiring anti-infective drugs were excluded. Nonsedating antihistamines were discontinued before enrollment. Menstruating female patients had to have a negative pregnancy test result and practice effective birth control. The protocol was approved by the Institutional Review Boards at all participating centers, and written informed consent was obtained from the parents or legal guardians of all patients.

Patients were randomized within each center by using permuted blocks of size 8 by means of a centralized computer-generated randomization schedule to treatment with twice daily applications of either tacrolimus ointment at 1 of 3 concentrations (0.03% [$n = 43$], 0.1% [$n = 49$], or 0.3% [$n = 44$] or vehicle ($n = 44$) for up to 22 days, with a 2-week follow-up period. Tacrolimus ointment and vehicle were identical in appearance and were dispensed in identical coded tubes. The study drug was blinded to all investigators, study coordinators, patients, and the sponsor except for the Department of Pharmaceutical Sciences, Fujisawa USA, which prepared the study medication. The 3 concentrations of drug used in this study were selected because they had been shown to be safe in studies conducted in Japan and Europe in adults with atopic dermatitis.¹²⁻¹⁴ Medication tubes were weighed before dispensing and on return to the investigator to assess compliance. Patients were limited to a maximum of 10 g of study medication per application. Patients were given a non-medicated emollient to use as needed on nontreatment areas of the body and all over during the follow-up period.

Patients were evaluated at 8 visits: prestudy, baseline, day 4, day 8, day 14, day 22/end of treatment, day 29, and day 36/termination. If a new skin lesion appeared during the study or an existing lesion increased in size, the physician could increase the treatment area at the next study visit. If an area affected by atopic dermatitis decreased in size or cleared, the physician could decrease the treatment area at the next study visit. If a patient's atopic dermatitis cleared completely during the study, the physician could discontinue treatment with the study drug, but the patient could not resume treatment if the atopic dermatitis flared during the follow-up period.

Outcome variables

The Physician's Global Evaluation of clinical response, with improvement between less than 0% (worse) and 100% (cleared) compared with baseline, was prospectively defined as the primary response parameter. Other outcome measures included a modified Eczema Area and Severity Index (a composite index of area involved, the physician's assessment of clinical signs, and the patient's assessment of pruritus, with a maximum score of 120) and a Head and Neck Total Score (the sum of the physician's assessment of individual signs in this body area, with a maximum score of 18). The modified Eczema Area and Severity Index was calculated as follows: for each of the 4 body regions (head/neck, upper limbs, trunk, and lower limbs), 3 clinical signs of atopic dermatitis were graded on

a scale of 0 to 3 and totaled (erythema grade + induration grade + excoriation grade = total grade). Each total grade was then multiplied by the affected area score for each body region, with an adjustment based on the relative contribution of each region to total body surface area. The resultant value for each body region was summed and added to a total pruritus score. The total pruritus score was calculated by summing the adjusted affected area scores for each body region and multiplying the sum by the pruritus score (adjusted to a scale of 0 to 3). The Head and Neck Total Score was the sum of the physician's assessment of erythema, edema-induration-papulation, excoriations, oozing-weeping-crusting, scaling, and lichenification, with each sign rated on a scale of 0 (absent) to 3 (severe).

The patient's assessment of overall treatment effect was based on the patient or parent/guardian rating the atopic dermatitis compared with baseline from "much better" to "much worse." In addition, the patient's assessment of pruritus was measured with a visual analog scale from 0 to 10 cm. Duration of remission of atopic dermatitis during the follow-up period was also assessed.

Safety variables

Safety variables included adverse events reported by patients or parents/legal guardians or observed by the physician at all study visits. Blood was obtained at baseline, day 4, and day 22/end of treatment for determination of complete blood count with differential and biochemistry panel.

Tacrolimus blood concentrations

Blood was obtained at baseline, day 4, and day 22/end of treatment for determination of tacrolimus levels. Whole blood tacrolimus concentrations were measured with an ELISA assay, with a lower level of quantitation of 0.05 ng/mL.¹⁵

Sample size and statistical analyses

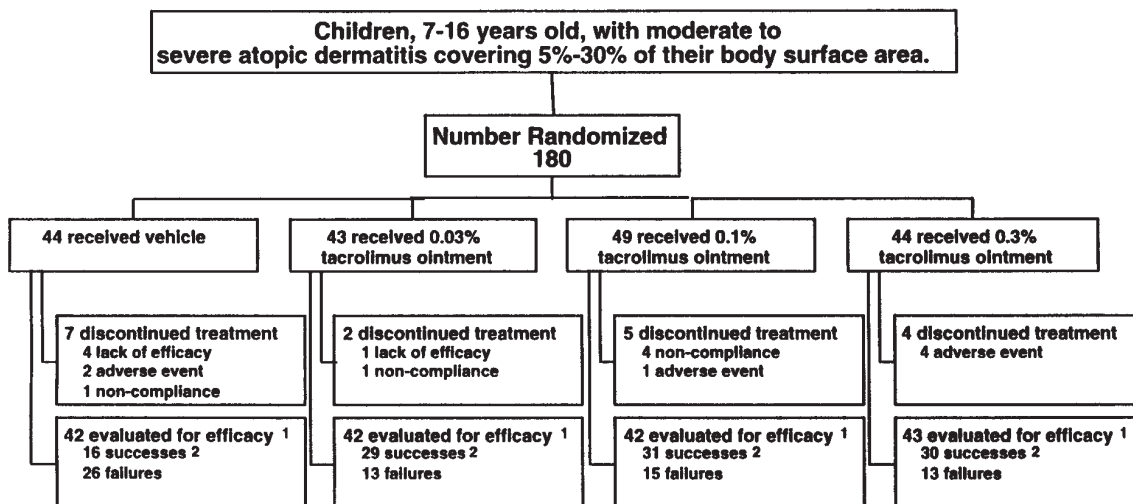
Calculation of the sample size was determined by using standard methods for binomial data. The effective rate (marked improvement or better) of Physician's Global Evaluation of clinical response at the last day of treatment was the determining factor for sample size calculation. Assuming the estimated effective rate (marked improvement or better) in the vehicle group and tacrolimus 0.1% group are 50% and 80%, respectively, 40 patients per each group are necessary to detect the statistically significant difference between 2 groups using a 2-sided test at 80% power and $\alpha = 0.05$.

As defined in the protocol, the primary analysis for efficacy involved data from all patients receiving at least 3 consecutive days (minimum of 5 applications) of study drug. The ratings of the Physician's Global Evaluation of clinical response and the patient's assessment of treatment effects were dichotomized into success (Physician's Global Evaluation rating $\geq$ marked improvement or patient's assessment rating $\geq$ better) and failure (others) and then analyzed with logistic regression models. The modified Composite Eczema Area and Severity Index and Head and Neck Total Score were analyzed with general linear models. For other efficacy variables, 1-way ANOVA, chi-square test, and Kruskal-Wallis test were used to compare groups according to the scale and property of the data. All patients were included in the safety analyses.

RESULTS

Patient characteristics

Each of 180 children at 18 study centers with atopic dermatitis were randomized to 1 of the 4 treatment arms (Fig 1). Patient characteristics at baseline in each treatment group were similar with regard to mean age, gender, racial distribution, duration of atopic dermatitis, and



¹ All randomized patients who received at least 3 days (minimum 5 applications) of treatment were evaluated for efficacy.

² Success was defined as $\geq 75\%$ improvement based on the Physicians Global Evaluation of Clinical Response.

FIG 1. Trial profile.



FIG 2. Left ear of 12-year-old boy who entered the study with moderate atopic dermatitis at baseline (A) and day 15 (B). Improvement was noted by physician beginning on day 4 of treatment with 0.03% tacrolimus ointment.

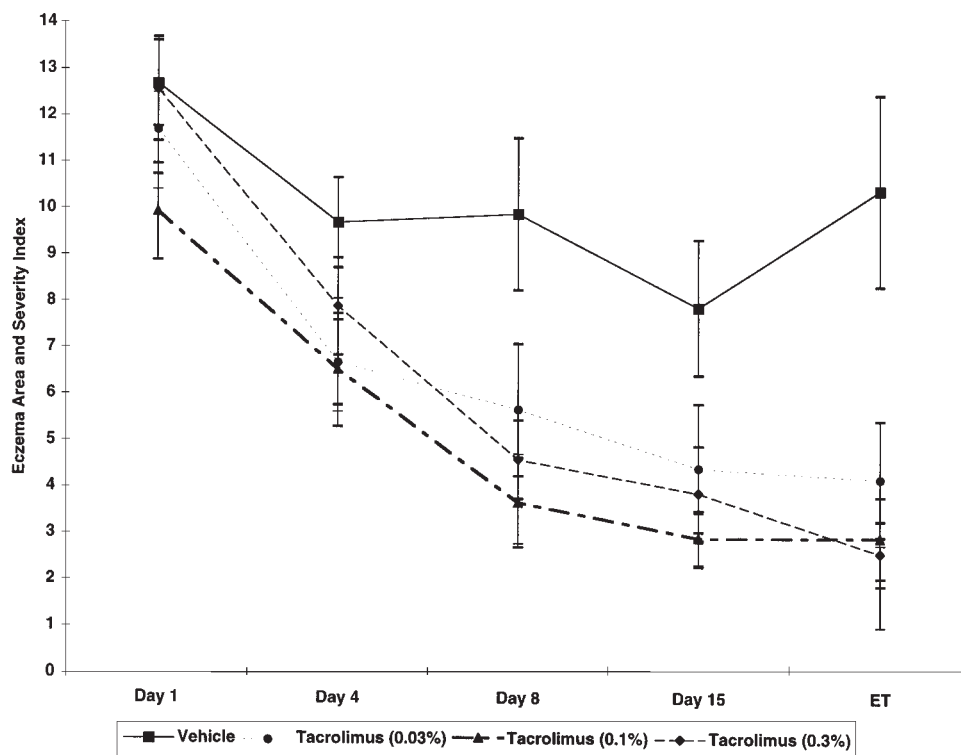


FIG 3. Modified Eczema Area and Severity Index (mean $\pm$ SEM). At end of treatment, each of 3 tacrolimus groups had significantly greater ($P < .001$) reduction in modified Eczema Area and Severity Index compared with vehicle group.

TABLE I. Baseline characteristics of 180 children with atopic dermatitis randomly assigned to receive tacrolimus ointment or vehicle

Characteristic	Tacrolimus concentrations				P value
	0% (Vehicle) (n = 44)	0.03% (n = 43)	0.1% (n = 49)	0.3% (n = 44)	
Age at entry (yrs)	10.4 (2.9)	10.1 (2.2)	10.8 (2.7)	10.5 (2.8)	.669
Sex (M/F)	18/26	18/25	21/28	23/21	.687
Race					
White	27	24	38	32	—
Black	14	12	10	11	—
Other	3	7	1	1	—
Duration of atopic dermatitis (yrs)	8.7 (3.7)	8.1 (3.5)	7.8 (3.5)	8.8 (3.4)	.468
Body surface area involved (%)	19.7 (7.4)	17.7 (7.7)	15.5 (7.9)	19.3 (8.6)	.049
Severity index					
Moderate	32	38	42	39	—
Severe	12	5	7	5	—

Data are mean $\pm$ SD, except gender and race, which are expressed as number of patients. Severity index is based on Rajka and Langeland criteria.²⁷ P values are from a chi-square test for gender, race, and severity and from ANOVA for age, duration of atopic dermatitis, and body surface area involved. —, Not applicable.

skin severity index (Table I). The only significant difference at baseline between treatment groups occurred in the percent of body surface area involved. In addition, although not statistically significant, there were numerically more patients with severe disease in the vehicle group than in any of the tacrolimus groups. However, examination of either percent of involved body surface area or severity of disease as a covariate in the logistic

analysis of the Physicians's Global Evaluation and the ANOVA of the modified Eczema Area and Severity Index score did not result in any substantive change in the primary conclusions.

Patients applied study medication to all affected areas except in rare cases where small areas of atopic dermatitis close to the eyes were omitted. A similar total amount of ointment was used in each of the treatment groups

TABLE II. Tacrolimus blood concentrations, adverse events, and withdrawals in the treatment groups

Variable	Tacrolimus concentrations				P Value
	0% (Vehicle) (n = 44)	0.03% (n = 43)	0.1% (n = 49)	0.3% (n = 44)	
Mean total amount of ointment used (g)	98	94	86	91	—
Mean tacrolimus blood levels (Day 4) (ng/mL ± SD)	—	0.10 ± 0.17	0.21 ± 0.32	0.31 ± 0.41	.005*
Mean tacrolimus blood levels (Day 22) (ng/mL ± SD)	—	0.07 ± 0.10	0.09 ± 0.31	0.18 ± 0.21	<.001*
No. of patients with adverse events					
Increased serum creatinine	0	1† (2.3%)	0	0	.361‡
Increased pruritus at application site	7 (15.9%)	11 (25.6%)	10 (20.4%)	13 (29.5%)	.445‡
Skin burning at application site	3 (6.8%)	9 (20.9%)	5 (10.2%)	10 (22.7%)	.092‡
Increased erythema at application site	2 (4.5%)	0	1 (2.0%)	3 (6.8%)	.309‡
No. of drop outs					
Lack of efficacy	4 (9.1%)	1 (2.3%)	0	0	.027‡
Patient noncompliance	1 (2.3%)	1 (2.3%)	4 (8.2%)	0	.148‡
Adverse events	2§ (4.5%)	0	1 (2.0%)	4¶ (9.1%)	.143‡

—, Not applicable.

*Kruskal-Wallis test.

†Transient elevation to 1.4 mg/dL, resolved without change in study drug application.

‡Chi-square test.

§Pruritus at application site (1 patient) and asthma (1 patient).

||Ingrown toenail infection.

¶Pruritus/skin burning at application site (1 patient), middle ear infection (1 patient), elevated liver enzymes (1 patient), and asthma/sinusitis (1 patient).

Head and Neck Total Score (mean ± SEM). At end of treatment, each of 3 tacrolimus groups had significantly greater (P < .001) reduction in Total Score of head and neck region compared with vehicle group.

TABLE III. Patient's assessment of pruritus*

Variable	Tacrolimus concentrations				P value
	0% (Vehicle) (n = 44)	0.03% (n = 43)	0.1% (n = 49)	0.3% (n = 44)	
Mean pruritus rating at baseline	5.4	5.7	4.9	5.2	—
Mean pruritus rating at the end of treatment	3.6	1.8	1.7	1.8	—
Mean percent improvement from baseline to end of treatment	3.6	64.7	47.1	47.8	.027†
Median percent improvement from baseline to end of treatment	50.5	88.7	73.6	77.1	—

*Pruritus based on 10-cm visual analog scale.

†P value calculated with a general linear model method (vehicle vs tacrolimus pooled).

—, Not applicable.

(Table II). Eighteen of the 180 patients discontinued their treatment early. This included 7 patients in the vehicle group, 2 in the 0.03% tacrolimus ointment group, 5 in the 0.1% tacrolimus ointment group, and 4 in the 0.3% tacrolimus ointment group (Fig 1). Four of 44 (9.1%) patients in the vehicle group discontinued treatment because of lack of efficacy compared with 1 of 136 (0.7%) patients receiving tacrolimus ointment (Table II). Discontinuation because of adverse events occurred in 2 of 44 (4.5%) vehicle-treated patients and 5 of 136 (3.7%) tacrolimus ointment-treated patients. Noncompliance with the study protocol resulted in discontinuation of treatment in 1 (2.4%) patient in the vehicle group and 5 (3.3%) patients in the tacrolimus ointment groups.

Efficacy outcome

Clinical improvement was generally noticeable for tacrolimus-treated patients within the first 1 to 2 weeks of treatment. Fig 2 shows the response in an individual patient after twice daily application of 0.03% tacrolimus ointment for 2 weeks. This patient showed excellent improvement beginning as early as day 4 of treatment.

The Physician's Global Evaluation of clinical response reported at end of treatment showed that 69% (95% confidence interval: 53-82) of patients in the 0.03% tacrolimus ointment group, 67% (95% confidence interval: 52-81) in the 0.1% tacrolimus ointment group, and 70% (95% confidence interval: 54-83) in the 0.3% tacrolimus ointment group, compared with 38% (95%

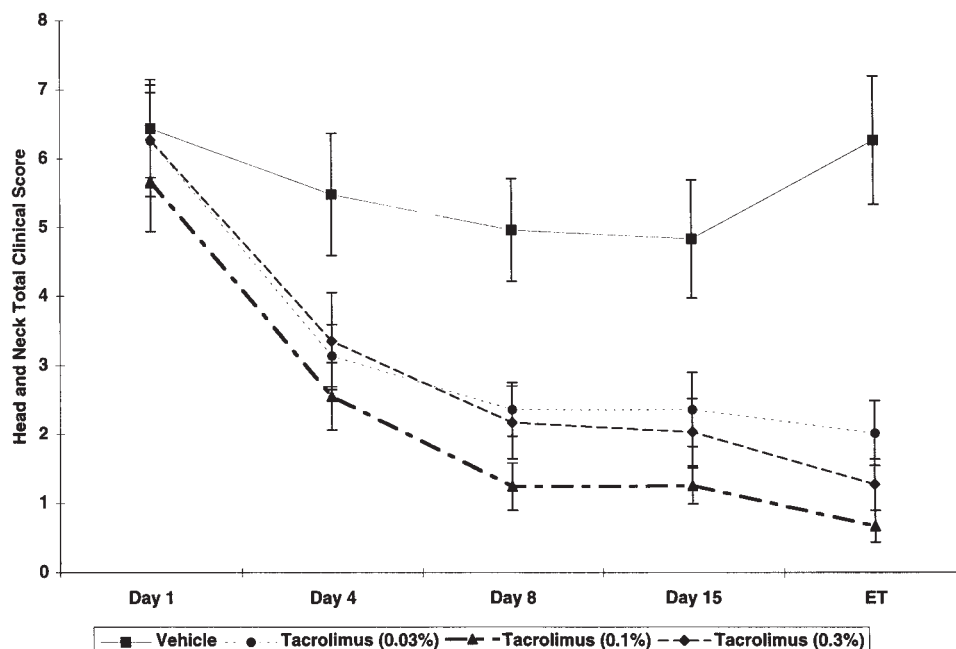


FIG 4. Head and Neck Total Score (mean $\pm$ SEM). At end of treatment, each of 3 tacrolimus groups had significantly greater ($P < .001$) reduction in Total Score of head and neck region compared with vehicle group.

confidence interval: 24-54) in the vehicle group, had a marked to excellent ($\geq 75\%$) improvement or clearing of their atopic dermatitis ($P = .005$, $.007$, and $.004$, respectively, for the 3 tacrolimus groups compared with the vehicle group). Sixteen of 42 (38.1%) vehicle-treated patients compared with 7 of 131 (5.3%) tacrolimus-treated patients had no appreciable improvement or worsened at end of treatment. Of these 7 tacrolimus-treated patients, 5 were treated with the 0.03% concentration.

The mean percent improvement for a modified Eczema Area and Severity Index at the end of treatment for each of the 3 tacrolimus groups (72%, 0.03%; 77%, 0.1%; and 81%, 0.3%) was significantly better than that of the vehicle group (26%; $P < .001$, Fig 3). Differences between the tacrolimus- and vehicle-treated groups were significant by day 8 of treatment. An analysis of the Head and Neck Region Total Score showed significant improvement for each of the tacrolimus groups compared with the vehicle group ($P < .001$, Fig 4). The mean percent improvement was 65% for the 0.03% group, 83% for the 0.1% group, and 81% for the 0.3% group compared with a 2% worsening in the vehicle group.

The percent of patients reporting feeling "much better" or "better" at the end of treatment was 76% in the 0.03% tacrolimus ointment group, 91% in the 0.1% tacrolimus ointment group, and 91% in the 0.3% tacrolimus ointment group ($P = .025$, $<.001$, and $<.001$, respectively) compared with 52% in the vehicle group. Table III is a summary of the pruritus rating on the basis of the patient's assessment. The pruritus rating was recorded on a 10-cm visual analog scale where 0 = "No Itch" and 10 = "Worst Itch Imaginable." The percent reduction in pruritus, from baseline to the end of treat-

ment, was significantly greater for tacrolimus-treated patients than for vehicle-treated patients ($P = .027$).

Twelve of 42 patients in the vehicle group compared with 25 of 42 in the 0.03% tacrolimus ointment group, 21 of 46 patients in the 0.1% tacrolimus ointment group, and 24 of 43 patients in the 0.3% tacrolimus ointment group were rated as having "excellent improvement" or "cleared" (ie, $>90\%$ improvement on the basis of the Physician's Global Evaluation of clinical response). These patients were evaluated for the time to recurrence of atopic dermatitis during the 2-week follow-up period. Of these, 9 (75%) in the vehicle group, 18 (72%) in the 0.03% tacrolimus ointment group, 17 (81%) in the 0.1% tacrolimus ointment group, and 21 (88%) in the 0.3% tacrolimus ointment group had recurrence of their atopic dermatitis. The recurrence rate of the 13 patients rated as "cleared" was 77%.

Adverse events and tacrolimus blood concentrations

No serious systemic adverse events were noted during this study. The most common adverse events reported were local increased pruritus and burning during the first 4 days of the study drug application. Overall, there was a greater number of patients in each of the tacrolimus-treated groups complaining of increased burning or pruritus at the application site as compared with the vehicle-treated group (Table II). However, these adverse effects were not significantly different between the vehicle and tacrolimus groups ($P = .445$ for pruritus; $P = .092$ for burning). Furthermore, the prevalence of both pruritus and burning decreased after day 4 of treatment and was similar in all treatment groups after this initial treatment period.

Table II shows the whole blood tacrolimus concentrations obtained during the course of this study. Only 7 (2.8%) of the 254 blood samples evaluated contained more than 1 ng/mL of tacrolimus. Six patients had a tacrolimus blood level greater than 1 ng/mL on day 4. The highest blood level of tacrolimus measured on day 4 was 1.66 ng/mL in a patient treated with 0.3% tacrolimus ointment. This patient had an asymptomatic elevation of liver function tests on day 4, which resulted in her termination from the study. The elevation may have been related to alcohol consumption by this patient. Within 2 days after discontinuation of study drug, her liver function tests had essentially normalized. A single patient had a blood level of tacrolimus of 2.06 ng/mL on the day 22 visit while receiving 0.1% tacrolimus ointment. This patient had no concurrent abnormalities in her blood chemistries and no symptoms.

Overall, mean and median hematology and chemistry parameters, including those measuring renal and hepatic function, remained within the normal range throughout the study. However, 1 patient receiving 0.03% tacrolimus ointment had a transient elevation of her serum creatinine to 1.4 mg/dL (normal, <0.8 mg/dL) on treatment day 4, which normalized (0.6 mg/dL) by day 7 despite continued treatment (Table II). She was noted to have a concurrent viral illness with diarrhea (days 4 to 8), suggesting a component of dehydration. Her tacrolimus blood levels were consistently below 1 ng/mL (0.06 ng/mL on day 22).

DISCUSSION

This study demonstrates that topically applied tacrolimus ointment is effective in reducing the signs and symptoms associated with atopic dermatitis in children. Each of the 3 tacrolimus concentrations studied was significantly better than vehicle in all of the efficacy parameters evaluated. Improvements were frequently observed within the first week of treatment. No statistical differences were found among the 3 tacrolimus-treated groups. However, evaluation of individual signs and symptoms in the head/neck region suggest that the 2 higher concentrations may yield better response (data not shown). As shown in Fig 3, tacrolimus ointment is an effective alternative therapy for atopic dermatitis involving the face and neck, whereas chronic use of topical corticosteroids can result in cutaneous atrophy and telangiectasias.

The blood tacrolimus concentrations measured in this study were all substantially below concentrations that have been associated with an increased risk of toxicity (>20 ng/mL) in transplant patients.¹⁶ Only 2.8% of the blood tacrolimus values obtained exceeded 1 ng/mL, and mean values decreased over time. These findings are consistent with previous reports that topical tacrolimus is minimally absorbed through affected skin, and systemic absorption decreases as skin lesions heal.¹⁴

No safety concerns were apparent when tacrolimus ointment was applied for up to 22 days. The mean and median values for laboratory parameters (other than IgE,

eosinophil, and LDH, which are usually elevated in atopic dermatitis patients) were within normal range throughout the study. No serious systemic adverse events were observed during this study. Although there was no statistical difference among the 4 treatment groups in adverse events at the application sites, a greater number of patients treated with tacrolimus ointment reported pruritus and skin burning during the study. For the tacrolimus-treated patients, the prevalence of adverse events at application sites decreased after the first 4 days. These findings are likely due to rapid healing of skin lesions associated with continued use of this medication. Treatment with tacrolimus ointment did not result in an increase in skin infections because only 2 were reported in the study, 1 of which occurred in a vehicle-treated patient.

The exact mechanism or mechanisms by which tacrolimus reduces the skin inflammation associated with atopic dermatitis requires further study. However, it is well established that tacrolimus is a potent immunosuppressive agent that interacts with a cyclophilin-like cytoplasmic protein, FK506 binding protein, and this complex in turn inhibits the ability of calcineurin to dephosphorylate the transcription factor, NFAT, required for activation of IL-2 and IL-4 gene transcription.^{17,18} Consequently, IL-2 synthesis and T-cell proliferation is inhibited. Tacrolimus also inhibits the transcription and release of other T cell-derived cytokines, such as IL-3, IL-4, IFN- γ , TNF- α , and GM-CSF, which are known to contribute to allergic inflammation.^{19,20} Of note, other cell types important in allergic skin inflammation, including mast cells, basophils, eosinophils, keratinocytes, and Langerhans' cells, have tacrolimus (FK506)-binding proteins and downregulate their mediator or cytokine expression after treatment with tacrolimus.²¹⁻²³

Relevant to atopic dermatitis, in a study by Nakagawa et al¹² skin biopsy specimens from patients with atopic dermatitis taken after 3 and 7 days of treatment with topical tacrolimus showed markedly diminished T-cell and eosinophilic infiltrates. It has been suggested that the therapeutic effects of this agent may be due to inhibition of IL-4 and IL-5 gene transcription.¹¹ However, recent studies indicate that the T-cell activation in atopic dermatitis is biphasic with activation of the T_{H2}-like cytokines, IL-4, IL-5, and IL-13 during the acute phase of the eruption.^{5,6,24} In contrast, the chronic inflammatory atopic dermatitis skin lesion is associated with increased expression of the T_{H1}-cytokines interferon- γ and IL-12.^{5,25,26} Thus the capacity of tacrolimus to inhibit the activation of multiple cell types and different cytokines may account for its ability to effectively reduce skin inflammation in atopic dermatitis.

In conclusion, we have shown that tacrolimus ointment at 3 different concentrations (0.03%, 0.1%, and 0.3%) was significantly more effective than vehicle in the treatment of children with moderate-to-severe atopic dermatitis. Its rapid onset of action, minimal systemic absorption, safe and efficacious application to the

head/neck region, and few adverse effects support the therapeutic potential of tacrolimus ointment. Long-term studies up to 1 year in duration are currently in progress.

REFERENCES

- Schultz-Larsen F, Holm NV, Henningsen K. Atopic dermatitis. A genetic-epidemiologic study in a population-based twin sample. *J Am Acad Dermatol* 1986;15:487-94.
- Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol* 1980;92:44-7.
- Rasmussen JE. Management of atopic dermatitis. *Allergy* 1989;44(suppl)9:108-13.
- Leung DYM. Atopic dermatitis: the skin as a window into the pathogenesis of chronic allergic diseases. *J Allergy Clin Immunol* 1995;96:302-18.
- Ohmen JD, Hanifin JM, Nickoloff BJ, Rea TH, Wyzkowski R, Kim J, et al. Overexpression of IL-10 in atopic dermatitis: contrasting cytokine patterns with delayed-type hypersensitivity reactions. *J Immunol* 1995;154:1956-63.
- Hamid Q, Boguniewicz M, Leung DYM. Differential in situ cytokine gene expression in acute vs. chronic atopic dermatitis. *J Clin Invest* 1994;94:870-6.
- Hanifin J. Atopic dermatitis in infants and pediatric patients. *Pediatr Clin North Am* 1991;38:763-89.
- Sowden JM, Berth-Jones J, Ross JS, Motley RJ, Marks R, Finlay AY, et al. Double-blind, controlled, crossover study of cyclosporin in adults with severe refractory atopic dermatitis. *Lancet* 1991;338:137-40.
- De Rie MA, Meinardi MHM, Dos J. Lack of efficacy of topical cyclosporin A in atopic dermatitis and allergic contact dermatitis. *Acta Derm Venereol* 1991;71:452-4.
- Kino T, Hatanaka H, Hashimoto M, Nishiyama M, Goto T, Okuhara M, et al. FK-506, a novel immunosuppressant isolated from a Streptomyces I. Fermentation, isolation, physico-chemical and biological characteristics. *J Antibiot* 1987;40:1249-55.
- Mori A, Suko M, Nishizaki Y, Kamoinuma O, Matsuzaki G, Ito K, et al. Regulation of interleukin-5 production by peripheral blood mononuclear cells from atopic patients with FK506, cyclosporin A and glucocorticoid. *Int Arch Allergy Immunol* 1994;104:32-5.
- Nakagawa H, Etoh T, Ishibashi Y, Higaki Y, Kawashima M, Torii H, et al. Tacrolimus ointment for atopic dermatitis [letter]. *Lancet* 1994;344:883.
- Ruzicka T, Bieber T, Schöff E, Rubins A, Dobozy A, Bos JD, et al. A short-term trial of tacrolimus ointment for atopic dermatitis. *N Engl J Med* 1997;337:816-21.
- Kawashima M, Nakagawa H, Ohtsuki M, Tamaki K, Ishibashi Y. Tacrolimus concentration in blood during topical treatment of atopic dermatitis [letter]. *Lancet* 1996;348:1240-1.
- Alak AM, Cook M, Bekersky I. A highly sensitive enzyme-linked immunosorbent assay for the determination of tacrolimus in atopic dermatitis patients. *Ther Drug Monit* 1997;19:88-91.
- Kershner RP, Fitzsimmons WE. Relationship of FK506 whole blood concentrations and efficacy and toxicity after liver and kidney transplantation. *Transplantation* 1996;62:920-6.
- O'Keefe SJ, Tamura J, Kincaid RL, Tocci JM, O'Neill EA. FK-506- and CsA-sensitive activation of the interleukin-2 promoter by calcineurin. *Nature* 1992;357:692-4.
- Chan SC, Brown MA, Wilcox TM, Li S-H, Stevens SR, Tara D, et al. Abnormal IL-4 gene expression by atopic dermatitis T lymphocytes is reflected in altered nuclear protein interactions with IL-4 transcriptional regulatory element. *J Invest Dermatol* 1996;106:1131-6.
- Yoshimura N, Matsui S, Hamashima T, Oka T. Effect of a new immunosuppressive agent, FK506, on human lymphocyte responses in vitro. II. Inhibition of the production of IL-2 and gamma-IFN, but not B cell-stimulating factor 2. *Transplantation* 1989;47:356-9.
- Schreiber SL, Crabtree GR. The mechanism of action of cyclosporin A and FK506. *Immunol Today* 1992;13:136-42.
- de Paulis A, Stellato C, Cirillo R, Ciccarelli A, Oriente A, Marone G. Anti-inflammatory effect of FK-506 on human skin mast cells. *J Invest Dermatol* 1992;99:723-8.
- Kaplan A, Matsue H, Shibaki A, Kawashima T, Kobayashi H, Ohkawara A. The effects of cyclosporin A and FK506 on human skin mast cells. *J Dermatol Sci* 1995;10:130-8.
- Thomson AW, Nalesnik M, Abu-Elmagd K, Starzl T. Influence of FK 506 on T lymphocytes, Langerhans' cells and the expression of cytokine receptors and adhesion molecules in psoriatic skin lesions: a preliminary study. *Transplant Proc* 1991;23:3330-1.
- Van der Heijden F, Wierenga EA, Bos JD, Kapsenberg JL. High frequency of IL-4 producing CD4+ allergen-specific T lymphocytes in atopic dermatitis lesional skin. *J Invest Dermatol* 1991;97:389-94.
- Hamid Q, Naseer T, Minshall EM, Song YL, Boguniewicz M, Leung DYM. In vivo expression of IL-12 and IL-13 in atopic dermatitis. *J Allergy Clin Immunol* 1996;98:225-31.
- Grewe M, Gyufko K, Schopf E, Krutmann J. Lesional expression of interferon- γ in atopic eczema. *Lancet* 1994;343:25-6.
- Rajka G, Langeland T. Grading of the severity of atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 1989;144:13-4.

APPENDIX

Pediatric Study Group: Mark Boguniewicz, National Jewish Medical and Research Center, Denver, Colo; Debra L. Breneman, University of Cincinnati College of Medicine, Cincinnati, Ohio; Lynn A. Drake, Massachusetts General Hospital, Boston, Mass; Frank E. Dunlap, Argus Research, Tucson, Ariz; Virginia Fiedler, University of Illinois, Chicago, Ill; Jon M. Hanifin, Oregon Health Sciences University, Portland, Ore; Teri A. Kahn, Cleveland Clinic, Cleveland, Ohio; Sewon Kang, University of Michigan, Ann Arbor, Mich; Steven E. Kemper, Minnesota Clinical Study Center, Fridley, Minn; Gerald T. Kilpatrick, Hill Top Research, West Palm Beach, Fla; Donald Y. M. Leung, National Jewish Medical and Research Center, Denver, Colo; Mark R. Ling, Emory University School of Medicine, Atlanta, Ga; Nicholas J. Lowe, Clinical Research Specialist, Santa Monica, Calif; Anne W. Lucky, Dermatology Research Associates, Cincinnati, Ohio; Amy Paller, Children's Memorial Hospital, Chicago, Ill; Daniel J. Piacquadio, University of California, San Diego, Calif; Sharon A. S. Raimer, University of Texas Medical Branch, Galveston, Tex; Gerald D. Weinstein, University of California, Irvine, Calif; David G. Wilson, The Education and Research Foundation, Inc, Lynchburg, Va.