Tacrolimus and pimecrolimus: From clever prokaryotes to inhibiting calcineurin and treating atopic dermatitis

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Tacrolimus ointment, a topical inhibitor of the phosphatase calcineurin, has recently been approved in the United States for use in the treatment of atopic dermatitis. It is the first topical immune suppressant that is not one of the hydrocortisone derivatives, important allies in dermatology for nearly 50 years. Although tacrolimus is less able to penetrate thick skin than glucocorticoids, it does not cause dermal atrophy, an important advantage over the hydrocortisone class. Pimecrolimus (ASM 981), a newer calcineurin inhibitor closely related to tacrolimus, is also being developed for atopic dermatitis therapy. Pimecrolimus has an altered skin penetration profile but the same mechanism of action as tacrolimus. In this review we chronicle the discovery of the calcineurin inhibitors, their presumed evolutionary role as a bacterial "smart bomb" against fungi, molecular and cellular mechanisms of action in the immune system, systemic and topical side effects, efficacy in atopic dermatitis, and future applications within the specialty of dermatology. Particular attention is given to the issues of systemic absorption of tacrolimus, the conditions in which absorption can become a concern, efficacy relative to glucocorticoids, and the choice of 0.03% or 0.1% tacrolimus ointment for use in adults and children. (J Am Acad Dermatol 2002;46:228-41.)

Tacrolimus (pronounced ta-CRO-la-miss), is the generic name for the macrolide immunosupressant previously known by its experimental name, FK506. Tacrolimus was first discovered in 1984 by Fujisawa Pharmaceutical Company while screening for antibacterial activity of a multitude of compounds. Tacrolimus is a macrolide produced by *Streptomyces tsukabaensis*, a bacterium found in the soil near Tsukuba, Japan. Tsukuba is Japan's "science city" where initial isolation and characterization of this drug was performed. This new name is derived from *T*sukuba, the location of its discovery; m*acrol*ide, its chemical class; and im*mu*ne *s*uppressant, its primary activity in humans. The chemical term *macrolide* refers to the cyclic carbon backbone of this structure (Fig 1). The macrolide class of small molecules includes well-known natural products with a variety of different mechanisms, such as the antibiotic erythromycin.

Tacrolimus has been used intravenously and orally for the prevention of organ rejection after allogeneic liver or kidney transplantation and in bone marrow transplantation. A topical formulation of tacrolimus has now been studied in more than 13,000 patients with atopic dermatitis, making it one of the most extensively investigated dermatologic therapeutics ever. Tacrolimus has been referred to as the first in a new class of topical immunomodulators (or "TIMs") by its manufacturer, Fujisawa. Although tacrolimus acts as an immunomodulator, it is surely not the first TIM, a distinction that hydrocortisone and its derivatives would hold even though they have never been referred to as such. Strictly speaking, tacrolimus is an immune suppressant and is the first in a new class of topical calcineurin inhibitors.

MECHANISM OF ACTION

An important characteristic of tacrolimus is that it has a distinct mechanism of action compared with

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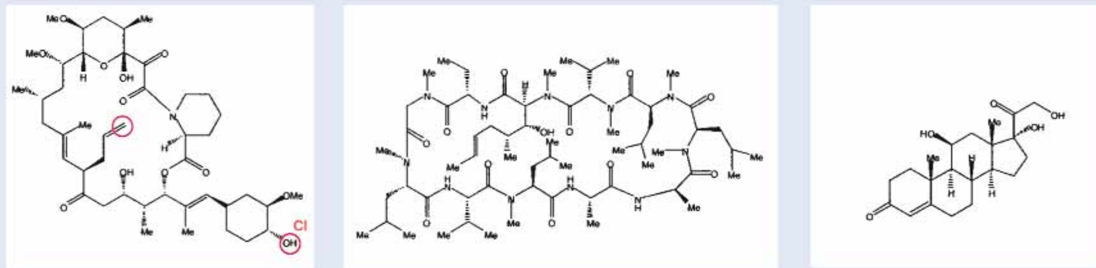

	Tacrolimus MW=804 Da Pimecrolimus* MW=809 Da	Cyclosporin A MW=1203 Da	Hydrocortisone MW= 362
Skin Penetration	Yes	No	Yes
Skin Atrophy	No	No	Yes (inhibits collagen syhthesis)
Effect	Inhibits IL-2,-3,-4 -5, GMCSF, TNF-alpha	Same as tacrolimus	Inhibits IL-1, -2, -6 IFN-alpha, TNF-alpha
Target	Calcineurin	Calcineurin	Glucocorticoid receptor
Mechanism	Binds FKBP & complex inhibits calcineurin & thus cytokine expression	Binds Cyclophilin & complex inhibits calcineurin & thus cytokine expression	Binds GC receptor, complex acts as transcription factor blocking cytokine and collagen expression

Fig 1. Chemical structures for 4 important immune suppressants are given, along with pertinent characteristics related to topical efficacy, mechanism, and side effects. Pimecrolimus* (ASM 981) is closely related to tacrolimus, with sites of changes in the tacrolimus structure shown in red (a hydroxyl group is replaced by chloride and an ethyl group is converted to a methyl). Tacrolimus and pimecrolimus share the same cellular binding targets and mechanism of action.

our current mainstay for topical immune suppression, the glucocorticoids. This alternative mechanism, as well as the size and skin permeability of tacrolimus, have important implications for its efficacy and side-effect profile relative to cyclosporine or hydrocortisone and its potent derivatives (see Fig 1).

The mechanism of action of tacrolimus is closely related to that of cyclosporine, a drug which was developed about a decade earlier and has found systemic application in dermatology for atopic dermatitis, psoriasis, pyoderma gangrenosum, and other disorders. In the late 1980s intensive work on these potent and mysterious drugs revealed their respective binding partners, which are highly conserved proteins present in yeast through mammals. FK506 was found to bind tightly to a cellular protein later named FKBP (FK506-binding protein).[1] Analogously, cyclosporine bound a protein later named cyclophilin.[2] Interestingly, in what turned out to be something of a scientific red herring, both FKBP and cyclophilin were found to be rotamase enzymes.[1,3] Rotamases are *cis-trans* prolyl isomerases involved in protein folding, and indeed their rotamase activity was inhibited by these drugs.[3,4] The presumption was that these drugs worked by blocking the rotamase activity of their cellular binding protein. Surprisingly, subsequent study showed that rotamase activity was not relevant to immune function as other agents which bound and inhibited these rotamases had no effect on lymphocyte activation.[5]

In what became one of the most heavily cited publications from the early 1990s, Liu et al[6] reported that the physiologically relevant target of both FK506 and cyclosporine was a calcium-activated phosphatase called calcineurin. Moreover, in a surprising and unusual mechanism, calcineurin binding and inhibition required the complex of the drug bound to its intracellular receptor (either FK506 plus FKBP or cyclosporine plus cyclophilin, Fig 2). This finding led to many questions including: Why would such a com-

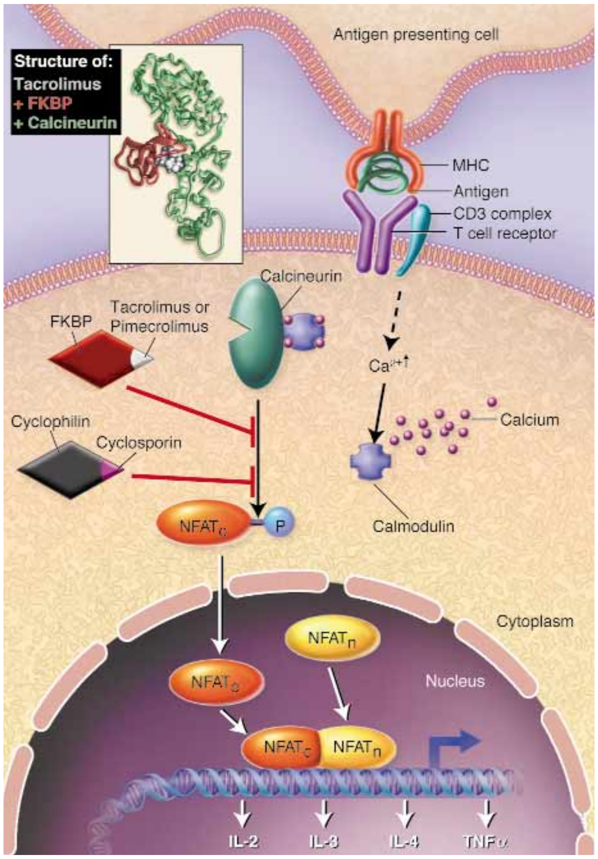

Fig 2. Molecular mechanism of inhibition of the immune response by tacrolimus, pimecrolimus, and cyclosporine. T-lymphocyte activation is initiated by interaction of antigenic peptide presented in the major histocompatibility complex *(MHC)* to the appropriate T-cell receptor. Activation signals from the CD3 complex cause an increase in intracellular calcium and induce the synthesis of the nuclear subunit of the *n*uclear *f*actor of *a*ctivated *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)*.[14] 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.[16] *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.[60] 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.

plex immunosuppressive system have evolved in two separate microbes? Indeed, the drug structures are unrelated to each other and their cellular partners FKBP and cyclophilin are also structurally unrelated. In fact, these agents did not evolve as immune suppressants but rather as inhibitors of calcineurin, a ubiquitous calcium-dependent protein phosphatase necessary for survival by regulating the response to stresses such as high salt and by controlling cell division in many eukaryotic microorganisms.[7,8]

Having no calcineurin themselves, bacterial organisms that could secrete an agent which would inhibit a neighbor's calcineurin would gain an important competitive growth advantage. This growth advantage has been verified in vitro[9] and appears to have been sufficiently powerful that convergent evolution occurred; that is, two distinct bacteria, *Streptomyces tsukubaensis* (tacrolimus) and *Beauveria nivea* (cyclosporine) evolved entirely separate mechanisms to do the same thing: inhibit calcineurin function in their fungal, eukaryotic competitors. Evolutionarily speaking, pimecrolimus is a minor variant of tacrolimus, being produced by a closely related bacterium, *Streptomyces hygroscopicus*.

In mammals, calcineurin is required for many functions in a variety of tissues: learning and memory,[10] renal function, and, of course, the immune response. The selective sensitivity of immune function to these drugs is thought to reflect the low level of expression of calcineurin in lymphocytes relative to cells in other tissues (eg, neurons) in which calcineurin is more abundant and was originally characterized. This combination of a low abundance of calcineurin and an absolute requirement for calcineurin in immune activation has led to the relatively selective immune suppression of these agents over neural and renal effects.

Glucocorticoids are well known to cause skin atrophy associated with their immunosuppressive effects. This is caused by the suppression of collagen synthesis by glucocorticoids. A major advantage of tacrolimus is that calcineurin is not required for collagen synthesis, and therefore atrophy is not caused by either cyclosporine or tacrolimus, regardless of the route of administration. Reitamo et al[11] have demonstrated in buttock skin of normal volunteers that betamethasone potently blocked collagen synthesis after a 7-day application, whereas topical tacrolimus (0.1% or 0.3% ointment) had no effect on collagen synthesis.

THE ROLE OF CALCINEURIN IN IMMUNE FUNCTION

When a T lymphocyte is activated by binding peptide antigen in the presence of a major histocompatibility protein, intracellular calcium is released and calcineurin is activated to dephosphorylate certain target proteins (see Fig 2). One critical target of calcineurin is a transcription factor called NFAT (*n*uclear *f*actor of *a*ctivated *T* cells). Upon dephosphorylation by calcineurin, the cytoplasmic subunit of NFAT translocates to the nucleus.[12,13] Once in the nucleus, it can bind its nuclear counterpart to form an active transcription factor, required for the production of a whole family of cytokines central to initiating an immune response.

This cytokine activation pathway is one of the most tightly regulated (meaning truly inactive in the absence of stimulation) of all transcriptional pathways. The devastation of autoimmune disease attests to the importance of avoiding inappropriate activation of the immune system. The extraordinarily tight regulation of this pathway is possible because neither of the two subunits of the critical transcription factor, NFAT, is present in the nucleus in unstimulated cells: The nuclear subunit is newly synthesized via T-cell activation signals, whereas the cytoplasmic subunit is sequestered outside the nucleus by phosphorylation.[12] Calcineurin's essential role in the immune system is to cleave the phosphate off the cytoplasmic subunit of NFAT (NFATc) and thereby allow NFATc to enter the nucleus and promote cytokine expression. Because evolution has chosen NFAT as a critical switch for multiple cytokines, the inhibition of NFAT by tacrolimus or cyclosporine blocks the expression of multiple immune mediators and hence suppresses the immune response very effectively.[14] For example, tacrolimus and cyclosporine block degranulation of mast cells as well as the expression of genes involved in leukotriene synthesis and multiple cytokines required to activate cellular immunity.[15,16] Applied topically, tacrolimus also potently blocks Langerhans cell function 100 times more potently than betamethasone valerate, a mid-strength corticosteroid.[17]

TOXICITIES OF SYSTEMIC CALCINEURIN INHIBITION

Because calcineurin is required for multiple processes throughout the body, systemic administration of these drugs is associated with significant side effects. Systemic side effects are related to dose, blood concentrations, and duration of therapy—with problems typically arising only after weeks or months in the appropriate therapeutic range. Prolonged therapeutic blood levels of tacrolimus are associated with hypertension, nephrotoxicity (increased creatinine, hyperkalemia, hypomagnesemia, decreased glomerular filtration rate, tubular injury), psychiatric disturbances, hyperlipidemia (triglycerides and cholesterol), and immunosuppression (lymphomas and increased incidence of infections).

TOPICAL TACROLIMUS AND ATOPIC DERMATITIS

Because of the impressive efficacy of these calcineurin inhibitors systemically, there has been great interest in using these agents topically on inflamed skin. Cyclosporine, although very lipophilic and able to enter the stratum corneum, is unable to penetrate the skin[18] and has been ineffective as a topical agent despite excellent efficacy orally in psoriasis and atopic dermatitis. Tacrolimus is 30% smaller by molecular weight (see Fig 1) and is able to penetrate and inhibit skin inflammation with good efficacy in atopic dermatitis.

Topical tacrolimus has been extensively studied in children and adults with atopic dermatitis in clinical trials, and the key studies are summarized in Table I. More than 13,000 patients have participated or are currently participating in clinical trials of topical tacrolimus in atopic dermatitis. Collectively, these trials have demonstrated that tacrolimus is highly efficacious and well tolerated, with the most frequent side effects being local application site irritation which typically improves over time.

The use of topical tacrolimus in patients with atopic dermatitis was initially reported by Nakagawa et al[19] in an open-label trial comparing 3 concentrations of topical tacrolimus (0.03%, 0.1%, and 0.3%) twice daily for up to 3 weeks. All of the subjects showed a significant improvement in atopic dermatitis, and pruritus was markedly decreased by day 3. This success in treating atopic dermatitis was confirmed in another open-label study in a small series of patients with severe facial atopic dermatitis.[20]

The first rigorous, randomized, double-blind, placebo-controlled trial to demonstrate that tacrolimus ointment was effective for short-term use in patients with atopic dermatitis was conducted by the European Tacrolimus Multicenter Atopic Dermatitis Study Group.[21] The study randomized 215 patients with moderate to severe atopic dermatitis to apply tacrolimus ointment 0.3%, 0.1%, 0.03%, or vehicle. The ointment was applied twice daily to a defined area between 200 and 1000 cm^2 for 3 weeks. The study end point was a change in summary score for erythema, edema, and pruritus. The median percentage decrease in summary score, from baseline compared with the end of study, for dermatitis on the trunk and extremities was as follows: 75% for patients receiving 0.3% tacrolimus, 83.3% for the subjects who received 0.1% tacrolimus, and 66.7% for the patients who received 0.03% tacrolimus compared with 22.5% for patients receiving vehicle

Table I. Summary of the major reports of tacrolimus ointment in atopic dermatitis*

Study	Subjects (No.)	Placebo	Double-blind	Randomized	Length (days)	% Tac	% Marked improvement
Nakagawa et al[19] (1994)	33	No	No	No	7	0.30	87.8
						0.10	78.4
						0.03	78.7
	17				21	0.30	85.2
						0.10	81.9
Ruzicka et al[21] (1997)	54	Yes	Yes	Yes	21	Placebo	9.4
	51					0.30	70.6
	54					0.10	81.2
	54					0.03	58.5
Boguniewicz et al (1998)	180	Yes	Yes	Yes	N/A	Placebo	38
	(age range,					0.30	70
	7-16 y)					0.10	67
						0.03	69
Hanifin et al[23] (2001)	33	Yes	Yes	Yes	21	Placebo	12
	(age range,					0.10	54
	3-6 y)					0.03	54
Alaiti et al (1998)	39	No	No	No	8	0.30	95% had at least good improvement
Kawashima et al[32] (1996)	17	No	No	No	7	0.10	Primarily a safety study
Reitamo et al (2000)	316	No	No	No	365	0.10	86 at 1 y
Hanifin et al[23] (2001) & Soter et al[60] (2001)	632	Yes	Yes	Yes	84	Placebo	19.8
						0.03	61.6
						0.10	72.7
Paller et al[22] (2001)	351	Yes	Yes	Yes	84	Placebo	26.7
	(age range,					0.03	72.6
	2-15 y)					0.10	78
Kang et al[30] (2001)	255 (age range, 2-15 y)	No	No	No	365	0.10	Patients improved over first week and tended to stay clear

*Medication was applied twice daily in all studies. Study design differed between these reports, but "marked improvement" was taken as >50% improved as determined by the Physician's Global Evaluation of Clinical Response or the Eczema Area and Severity Index.

alone. These changes for all treatment groups were statistically significant ($P < .001$) by day 3 of treatment and remained so for the duration of the study.

Three pivotal, multicenter phase III, double-blind, randomized, vehicle-controlled clinical trials were conducted in the United States and reported recently in 984 children and adults with atopic dermatitis.[22,23] The pediatric trial was a multicenter, randomized, double-blind, 3-arm, parallel-group, vehicle-controlled study.[22] This study evaluated the use of tacrolimus ointment at two concentrations (0.03% and 0.1%) to vehicle in children aged 2 to 15 years with moderate to severe disease involving greater than 10% body surface area. Three hundred fifty-one children were randomized to receive study medication: 118 received 0.1% tacrolimus ointment, 117 received 0.03% tacrolimus ointment, and 116 received vehicle. Patients applied study medication twice daily to all affected areas of the skin and were assessed at baseline, at weeks 1, 2, 3, 6, 9, and 12 and at the end of study. The primary efficacy end point in this study was defined as 90% or more reduction in the Physician's Global Assessment (physician's global evaluation of clinical response). As shown in Fig 3, excellent improvement in the areas treated was seen in 40.7% and 35.9% of those that received 0.1% and 0.03% concentration, respectively, versus 6.9% of vehicle-treated patients ($P < .001$). An improvement in the Physician's Global Assessment of 50% or more by the end of treatment corresponded to a moderate improvement in atopic dermatitis, and those that received 0.1% and 0.03% concentrations were judged to have a moderate improvement of 78.0% and 72.6%, respectively, compared with 26.7% of

Blood levels	Side effects
0.4-0.9 ng/mL	⅓ had mild skin irritation
0.09-0.7 ng/mL	⅓ had mild skin irritation
Most <0.25 ng/mL, highest 4.9 ng/mL	Burning at application site
7/254 were >1 ng/mL	None systemic; burning, pruritus in first 4 d
8 had peak ≥1 ng/mL	One subject had skin burning
<0.5% Bioavailability	Burning, flushing
2 had 20 ng/mL; blood levels correlated with disease severity	None
<1 ng/mL in 76%	Burning, pruritus
80% had no blood levels detected	25.8% burning
	45.6% burning
	57.7% burning
Minimal absorption	29.0% burning
	42.7% burning
	33.7% burning
No changes in basic hematologic and metabolic profiles	25.8% burning

vehicle-treated patients. Patients were also evaluated for efficacy by means of the Eczema Area and Severity Index (EASI). The EASI is a composite score based on physician assessment of clinical signs of atopic dermatitis (erythema, edema/induration/papulation, excoriation, oozing/weeping/crusting, scaling, and lichenification) as well as the percent body surface area affected. Statistically significant improvement in the overall EASI score, as well as each individual sign of atopic dermatitis and percent body surface area, was noted for either tacrolimus treatment group compared with vehicle ($P < .001$). No significant difference in efficacy was noted between the 0.1% and 0.03% tacrolimus ointment concentrations.[22]

In adult patients two identically designed, randomized, double-blind, multicenter studies were

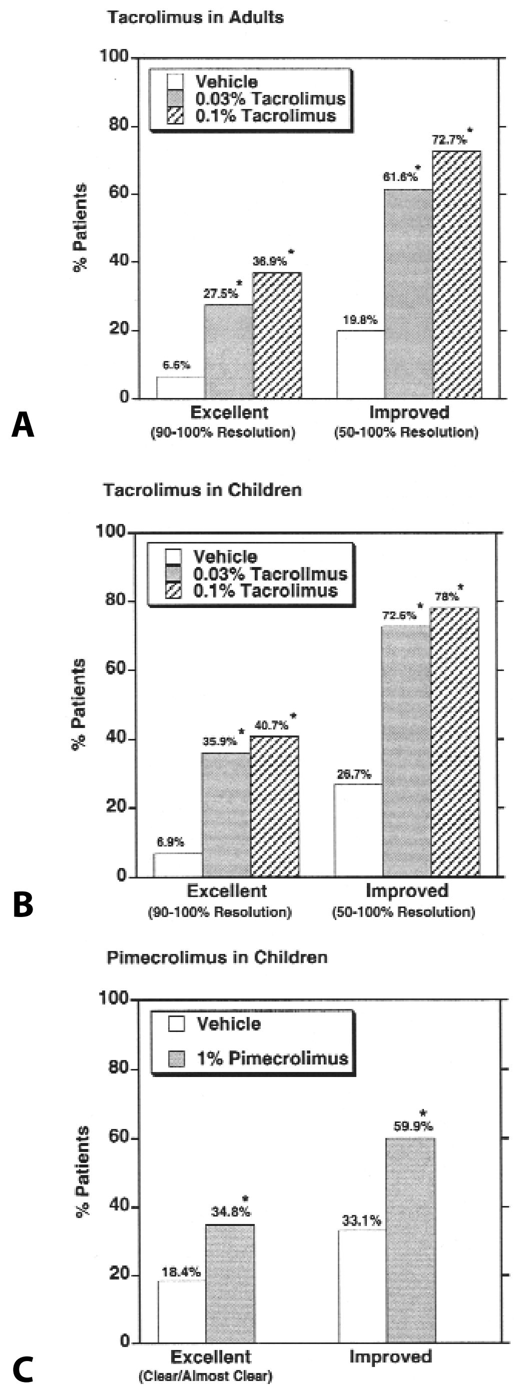

Fig 3. Efficacy of tacrolimus in adults and children. **A** and **B**, These panels show improvement ratings in the physician global evaluations for the recently published, randomized, double-blinded 12-week trials of tacrolimus and vehicle for adults (**A**)[23] and children (**B**).[22] The percentage of patients with excellent improvement (90%-100% improvement, *shaded bars*) and at least moderate improvement (>50% improvement, *cross-hatched bars*) are depicted. **C,** In this panel the investigator's global assessment scores are shown at the conclusion of the 6-week trial of 1% pimecrolimus.[28] *Asterisk, P* < .001 for each category of therapy relative to vehicle.

Fig 4. Atopic dermatitis before (left panels) and 3 weeks after (top right) or 1 week after (bottom right) 0.1% tacrolimus ointment. **A,** Arms of a subject (see orienting patches of previous hypopigmentation) and on **(B)** face and torso of 6-year-old boy. Of note, at the time of the initial photograph of the boy, he had received long-term treatment with emollients, courses of oral antibiotics, and mid-strength topical steroids with poor control.

conducted in parallel, and jointly reported.[23] In total, 632 adults with moderate to severe atopic dermatitis, aged 15 to 79 years, were randomized to receive topical tacrolimus ointment (0.03% or 0.1%) or vehicle. The study design was essentially similar to the pediatric study; the primary efficacy end point in this study was defined as 90% or more reduction in the Physician's Global Assessment, and other efficacy measures included EASI, physician assessment of individual signs of atopic dermatitis, the percent body surface area involved, and the patient's assessment of pruritus. An excellent improvement in the areas treated, based on the Physician's Global Assessment, was seen in 36.8% and 27.5% of those who received 0.1% and 0.03% concentration, respectively, versus 6.6% of vehicle-treated patients ($P <$ .001, Fig 3).

Although the impact of these improvements in skin disease is apparent in the clinical examination (Fig 4), they have also been more formally documented in terms of quality of life changes for tacrolimus ointment in patients with atopic dermatitis. In a double-blind, placebo-controlled, quality-of-life study of 985 adult and pediatric patients, stan-

Table II. Side-effect profiles for 0.03%, 0.1%, and vehicle ointments in patients with atopic dermatitis*

	Vehicle (n = 212)	0.03% (n = 210)	0.1% (n = 209)
Skin burning	26%	**46%**	**58%**
Flu-like symptoms	19%	23%	**31%**
Headache	11%	**20%**	**19%**
Skin tingling	2.4%	3.4%	**7.6%**
Folliculitis	0.5%	**6.2%**	**4.3%**
Alcohol intolerance	0.0%	**3.4%**	**6.9%**
Skin infection	11%	12%	**4.7%**†
Acne	1.8%	4.3%	**7.1%**
Hyperesthesia	0.5%	3.0%	**6.5%**
Cyst	0.0%	**1.1%**	**3.1%**

*Boldface type indicates that the adverse effect incidence was statistically significantly different for the treatment compared with the vehicle control.

†The skin infection incidence for 0.1% tacrolimus is significantly lower than that for vehicle control. Two significant digits are shown. (Data adapted from Soter NA, Fleischer AB Jr, Webster GF, Monroe E, Lawrence I. J Am Acad Dermatol 2001;44(Suppl):S39-46.)

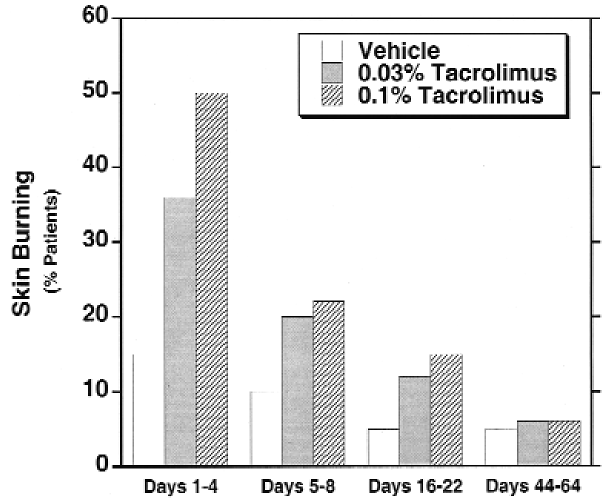

Fig 5. Incidence of skin burning during 6 weeks of therapy with tacrolimus ointment demonstrates a marked decrease in this adverse effect over the course of therapy. (Data adapted from Soter NA, Fleischer AB Jr, Webster GF, Monroe E, Lawrence I. J Am Acad Dermatol 2001;44:S39-46.)

dard instruments such as the Dermatology Life Quality Index demonstrated significant improvements in quality of life for patients using either 0.03% or 0.1% ointment in adults, children, and toddlers.[24]

PIMECROLIMUS AND ATOPIC DERMATITIS

Although there are currently far fewer studies of pimecrolimus than of tacrolimus in the treatment of atopic dermatitis, this newer calcineurin inhibitor has also been shown not to induce skin atrophy[25] and to be similar in anti-inflammatory potency to clobetasol-17-propionate (0.05% ointment) in a pig skin model.[26] A recent double-blinded randomized human trial of 260 patients compared pimecrolimus 0.2%, 0.6%, 1.0%, vehicle cream and betamethasone-17-valerate 0.1% cream. The 1.0% formulation of pimecrolimus was the most effective of the 3 but proved less potent than the betamethasone cream in this trial.[27]

Two independent 6-week, randomized, multicenter studies evaluated the safety and efficacy in children of 1% pimecrolimus versus vehicle ointment. Among 403 children with mostly mild to moderate atopic dermatitis, 1% pimecrolimus was significantly more effective than vehicle, with 59.9% improved in the pimecrolimus group versus 33.1% improved in the placebo group (Fig 3).[28] A remarkable difference between pimecrolimus and tacrolimus is that the rate of skin burning is much lower with pimecrolimus (10.9% of patients) compared with vehicle (12.5%).[28] This surprising difference in skin burning between tacrolimus and pimecrolimus could be an important therapeutic difference for a subset of patients with severe tacrolimus-associated burning.

ADVERSE EFFECTS OF TOPICAL TACROLIMUS

The principal adverse events reported to date in phase III clinical trials with topical tacrolimus are primarily those of local application site reactions (see Table II for a summary). Burning at the site of application has been the most frequent adverse event reported, occurring in approximately 33% to 45% of those treated with 0.03% tacrolimus ointment and 31% to 61% of those treated with 0.1% tacrolimus ointment, depending on the study. This burning sensation is typically mild to moderate in intensity and is self-limited. For the 0.1% ointment, 90% of the skin burning events lasted between 2 minutes and 3 hours (median, 15 minutes).[29] In addition, the incidence of burning decreased roughly 10-fold over the course of therapy with 0.1% tacrolimus (Fig 5). It is intriguing to consider the possibility that cutaneous nerves are sensitive to calcineurin inhibition by tacrolimus and that this sensitivity underlies this common and uncomfortable sensation. However, it is difficult to reconcile such a hypothesis with the low rate of pimecrolimus-induced skin burning because it too would inhibit cutaneous nerve calcineurin.[28]

A long-term safety and efficacy study involving 255 children was also recently reported. Children applied

0.1% tacrolimus ointment (vehicle and 0.03% treatments were not included) twice daily for an average of 279 days during the 12-month period.[30] Transient skin burning (25.9% of children), pruritus (23.1%), and skin infection (11.4%) were the most common adverse events associated with the treatment sites. Importantly, the authors noted no increase in infections or other serious side effects relative to age-matched historical control populations.

A concern for any topical preparation is whether it may interact with sunlight. Extensive safety studies have found that tacrolimus ointment is not phototoxic, photosensitizing, or photoallergenic.[31] For an immunomodulating agent, an additional concern is that tacrolimus may promote photocarcinogenesis. Indeed, there was a detectable decrease in the latency to skin tumor formation in a hairless mouse model when tacrolimus ointment was added to daily ultraviolet (UV) irradiation (6 hours a day, equivalent to noonday sun in Phoenix, Ariz) over a 40-week period.[29] The medical significance of this finding is unclear, but it is probably prudent to suggest that patients avoid excessive UV exposure while receiving tacrolimus therapy, as the package insert suggests.

SYSTEMIC ABSORPTION OF TOPICAL TACROLIMUS

Although topical tacrolimus can penetrate the skin adequately to suppress local inflammation, it is only minimally absorbed into the blood. Only about 0.5% of the tacrolimus applied to the skin can be detected in blood. Thus, even when large areas of skin are treated, blood levels are either typically undetectable or subtherapeutic. Low blood concentrations of tacrolimus have been measured in many studies for atopic and nonatopic patients, and the main studies have been summarized in Table I. In organ transplant recipients, the ideal therapeutic range is 5 to 15 ng/mL, and the lower limit of detection in the blood is 0.5 ng/mL. In the two largest clinical trials in adults reported, involving 632 adults, the majority of patients (78%-83.6%) using topical tacrolimus had no detectable levels. In the largest pediatric study to date involving 351 atopic children, 84% of the children in this study had no detectable blood levels of tacrolimus, and the highest detectable level was 2.28 ng/mL, found in one subject.[22] The highest blood levels reported after topical tacrolimus therapy were by Kawashima et al[32] in which 2 of 17 subjects had blood levels of 20 ng/mL. Importantly, this was a short trial of only 7 days, large amounts (10-20 g) were applied daily, and patients with the most severe disease at onset had the highest levels. These levels have not been observed in other studies but do suggest that patients applying large amounts of tacrolimus on severely affected skin may attain significant serum levels of drug at least transiently.

A particularly important condition in which increased absorption has been documented is Netherton syndrome.[33] Netherton syndrome is a rare autosomal recessive disease that often presents with erythroderma, failure to thrive, and in some cases with ichthyosis linearis circumflexa (characteristic migratory serpiginous erythematous plaques with double-edged scale along the margins). Netherton syndrome can be misdiagnosed as uncomplicated atopic dermatitis and has been shown to be alleviated by tacrolimus. These patients, however, absorb tacrolimus from the skin much more effectively than patients with atopic dermatitis and can develop serum levels well into the therapeutic range. The authors strongly recommend that Netherton syndrome be excluded from the differential and, if a patient with Netherton syndrome is treated, that blood levels of tacrolimus be followed closely.

CHOOSING 0.03% OR 0.1% OINTMENT FOR ADULTS AND CHILDREN

Two concentrations of tacrolimus (0.03% and 0.1%) are now available, and a prescribing physician must choose which to use for each patient. In children, 0.03% is the only concentration approved by the Food and Drug Administration. How should the choice be made between these concentrations?

Evidence of a dose-response relationship was seen in adults for the two tacrolimus concentrations in that patients in the 0.1% ointment group had a higher success rate (36.8% vs 27.5% with ≥ 90% improvement) than patients in the 0.03% ointment group in Physician's Global Assessment ($P < .04$).[23] This effect can be seen graphically in Fig 3, as a trend in both children and adults toward improved efficacy of the 0.1% concentration. Subgroup analyses revealed that the superiority of 0.1% ointment was more statistically significant for patients with more severe disease at baseline ($P < .009$) or those with extensive involvement of body surface area (>75%) ($P < .004$).[23] In African Americans, the 0.03% ointment was quite ineffective—not statistically significantly different from vehicle; thus in this population it is especially critical to use the higher 0.1% concentration, which was much more effective than vehicle ointment (29% vs 7% of patients with >90% improvement in disease).[23] Aside from these cases of African American skin or severe or extensive disease, the difference between the two concentrations was less marked.

As described above, the incidence of significant blood levels of tacrolimus is minimal, even in children using 0.1%. Moreover, the incidence of adverse events was lower in children using 0.1% ointment than in those using 0.03%, which certainly does not support a need to limit the dosing in children to the 0.03% concentration.[22] In terms of price, the cost of the two concentrations differed by only about 5% based on pricing by a major US retail pharmacy ($71 for 30 g of 0.03% ointment and $75 for 30 g of 0.1% ointment; CVS Pharmacy, August 2001). In terms of usage, in the blinded study, patients used the same amount of the two concentrations, and significantly less than vehicle ointment (4.5 g/d for 0.03% ointment, 4.7 g/d for 0.1%, and 6.3 g/d on average for vehicle ointment).[23]

In summary, 0.1% ointment is somewhat more effective than 0.03% ointment, does not have a worse side-effect profile, is not particularly more expensive, and has been demonstrated to be safe in children despite the decision of the Food and Drug Administration to assign a pediatric indication for only the 0.03% ointment. With rare exceptions, therefore, it seems reasonable to prescribe the 0.1% ointment.

EFFICACY OF TACROLIMUS VERSUS GLUCOCORTICOIDS

Currently there are very few data directly comparing the efficacy of tacrolimus with that of glucocorticoids. Three studies published in Japanese and in abstract form have been summarized and are illuminating.[31] In one of these reports, 0.1% tacrolimus compared favorably with a mid-potency steroid, betamethasone valerate (0.12%) ointment, with 100% of 41 tacrolimus-treated patients showing at least moderate improvement in atopic dermatitis versus 72% of 40 betamethasone-treated patients. A second Japanese study with approximately 80 patients in each arm showed equivalent results between 0.1% tacrolimus (94% with moderate improvement) and betamethasone valerate (90%). A third study compared 0.1% tacrolimus (97% with at least moderate improvement) with alclometasone dipropionate 0.1% (70% moderate improvement).[31] In vitro, the ability of tacrolimus to inhibit T-lymphocyte cytokine production was shown to be stronger than that of alclometasone dipropionate and equal to or stronger than that of betamethasone valerate,[34] although such studies would not take into account the likely superior penetration of glucocorticoids into the site of inflammation.

In general, tacrolimus will have an upper hand therapeutically because it does not cause atrophy of thin skin, which it readily penetrates, whereas glucocorticoids will be preferred in thicker areas where atrophy is less of a worry. A particular advantage of tacrolimus is safety in treating facial dermatoses because of the lack of atrophy and improved safety for the eye. Unlike steroids, with concerns about their contribution to glaucoma, tacrolimus appears to be safe because there was no evidence of increased intraocular pressure when applied to the eyelids (Krupnick A, Lebwohl M, personal communication, October 2001). Although the data are currently scant, it appears that tacrolimus ointment will have efficacy similar to mid-strength glucocorticoids. Like hydrocortisone and its numerous derivatives, later generations of calcineurin inhibitors will no doubt have improved efficacy relative to tacrolimus ointment.

OFF-LABEL USES FOR TOPICAL TACROLIMUS

As the first major topical immune suppressant since the hydrocortisone class, no doubt tacrolimus will be investigated for a wide variety of inflammatory skin diseases. The following disorders are some of the first to be investigated beyond atopic dermatitis.

Psoriasis

The similar mechanism of action of cyclosporine and tacrolimus raises the possibility that dermatoses responsive to systemic cyclosporine (eg, psoriasis) might benefit from topical tacrolimus. Systemic tacrolimus was first reported to be effective in the treatment of psoriasis in an open-label study of 7 patients.[35] Subsequently, a European multicenter study of patients with severe psoriasis treated with systemic tacrolimus was reported.[36] In this randomized, double-blind, placebo-controlled trial, 53 patients received systemic tacrolimus or placebo and by week 9, 63% of oral tacrolimus-treated patients had responded versus 25% of placebo patients. The most common side effects included diarrhea, paresthesia, insomnia, pharyngitis, and headache.[36]

Topical tacrolimus, however, has been ineffective in a small study of psoriatic patients,[37] most likely because of inability to penetrate the thick hyperkeratotic skin lesions of chronic plaque-type psoriasis. Indeed, topical tacrolimus has been shown to be effective in facial lesions of psoriasis[38] where skin is thinner and more readily penetrated. Tacrolimus has even been shown to be safe on eyelids, with no observed increase in intraocular pressure (Krupnick A, Lebwohl M, personal communication, October 2001), unlike glucocorticoids. Inverse psoriasis will likely be an important application for topical tacrolimus because penetration should be excellent in occluded intertriginous sites where glucocorticoid atrophy would be a concern. Indeed, initial observations in inverse psoriasis appear encouraging. Pimecrolimus under occlusion has been reported to be effective in psoriasis with comparable efficacy to clobetasol-17-propionate ointment (0.05%).[39] There is relatively little advantage, however, of calcineurin inhibitors over glucocorticoids in areas of thick skin because concern for atrophy is minimal on sites such as knees and elbows.

Pyoderma gangrenosum

Topical and systemic tacrolimus have been successfully used to treat pyoderma gangrenosum. Successful use of systemic tacrolimus was initially described using a dose of 0.3 mg/kg per day in 4 patients with steroid- and cyclosporine-resistant pyoderma gangrenosum.[40] Subsequently, others described single case reports of additional patients with pyoderma gangrenosum responsive to systemic tacrolimus.[41,42] Topical tacrolimus 0.3% in carmellose sodium paste (Orabase) has also been successfully used as a second-line treatment in 5 patients with parastomal pyoderma gangrenosum.[43] In this report, 4 patients healed within 8 weeks, with the fifth patient having partial improvement. In a patient treated with tacrolimus 0.1%, there was no improvement. No serum levels of tacrolimus were detected at 7 days after initiation of treatment. No side effects were reported in this small series of patients.

Tacrolimus may improve the lesions of pyoderma gangrenosum through its inhibition of neutrophil chemotaxis, as neutrophils have been implicated in the pathogenesis of this condition.[44,45] The mechanism by which tacrolimus inhibits neutrophils may relate to suppression of proinflammatory cytokines such as granulocyte-macrophage colony-stimulating factor[40] or interleukin 8 and its receptor,[46] thus leading to diminished chemotaxis of neutrophils.

Lichen planus

Oral lichen planus is responsive to topical cyclosporine and appears to be effectively treated with topical tacrolimus. Tacrolimus has been used topically to treat erosive mucosal lichen planus in 6 patients with complete resolution in 3 and improvement in the other 3.[47] In addition, an excellent outcome was noted by one of us (P. N.), using tacrolimus powder from capsules compounded at 0.1% in olive oil and applied twice daily by the finger to affected buccal mucosa. A case of lichen planus of the lip was successfully treated (by G. P.) with 0.1% tacrolimus ointment with rapid resolution over a week.

Graft-versus-host disease

Skin is the most commonly affected target for graft-versus-host disease (GVHD), and the incidence of GVHD is rapidly rising along with the annual 10% to 15% increase in bone marrow transplantations carried out in the United States.[48,49] Because GVHD is a T-cell–mediated process that is effectively blocked by calcineurin inhibition, this disorder is another possible application for topical tacrolimus. In a case series of 18 patients, more than 70% had rapid alleviation of erythema and pruritus after applying 0.1% tacrolimus ointment.[50] All patients with GVHD went on to require additional systemic therapy such as photophoresis or psoralens plus ultraviolet A (PUVA). The authors concluded that in GVHD, tacrolimus will not be an adequate therapy but rather may be a useful bridging agent to rapidly control symptoms while slower therapies are initiated.[50]

Alopecia areata

For alopecia areata, a single case report of no efficacy[51] together with failures in two of our own patients suggest that tacrolimus may not be effective in this disorder when applied as an ointment. These early reports are disappointing as cyclosporine and tacrolimus are known to have stimulatory effects directly on the follicle,[52] inducing anagen as well as promoting hair growth and indeed characteristic hirsutism when cyclosporine is taken orally.[54] We have also tried compounding tacrolimus in a clobetasol scalp application to attempt to promote penetration and possibly synergize with the potent steroid, again without success in 3 additional cases of alopecia areata (P. N., unpublished results).

Allergic contact dermatitis

In human studies, dinitrochlorobenzene-induced allergic contact dermatitis was prevented by pretreatment with tacrolimus.[54] In this study, 5 patients received pretreatment with 0.01%, 0.1%, and 1.0% topical tacrolimus, and a fourth area was untreated and skin biopsies were performed. No histologic evidence of inflammation was evident in the tacrolimus-treated skin; however, the areas that were not pretreated with tacrolimus did show inflammation histologically. In a murine model, topical tacrolimus suppressed experimental oxazolone-induced local draining lymph node proliferation, and proinflammatory cytokine suppression was observed.[55] Tacrolimus ointment may be especially useful for treatment of contact dermatitis on the face where glucocorticoid atrophy is a worry.

Rosacea

Acne rosacea is an inflammatory dermatosis, which is typically not optimally treated with glucocorticoids because of potential atrophy of facial skin and "steroid addiction" that can severely complicate rosacea therapy. There is thus a great need to have a safe, anti-inflammatory agent for topical use in rosacea. Goldman[56] has described 3 patients with steroid-induced rosacea who had excellent resolution of their erythema after 7 to 10 days of 0.075% tacrolimus ointment.

Other applications

Topical tacrolimus has been used in a number of case reports, including ichthyosis linearis circumflexa[57] and recalcitrant leg ulcers associated with rheumatoid arthritis.[58] This latter application is especially intriguing as tacrolimus acts as an immunosuppressant that does not inhibit collagen synthesis or epidermal growth, which are critical in regrowth of skin over an ulcer. As already noted, topical tacrolimus is effective in treating skin involvement with Netherton syndrome. In this disease, however, great caution must be exercised as these patients have markedly increased systemic absorption of tacrolimus, which can easily reach therapeutic levels in the blood.[33] Currently ongoing studies include seborrheic dermatitis, dyshidrotic eczema, hand eczema, and vitiligo. Over time, tacrolimus will be investigated in all inflammatory dermatoses, and the results of such research will be of broad interest to dermatologists.

FUTURE DIRECTIONS

There is a paucity of research evaluating commonly used treatments in a comparative manner, or in combination therapy, in general in atopic dermatitis. Such trials are of special interest with tacrolimus, particularly combination or sequential trials with topical steroids. Combination trials with topical steroids would be of interest to explore the possibility of synergy while minimizing the side effects of steroids by reducing their frequency of use. In addition, one can speculate there may be a benefit of minimizing the initial local irritation associated with tacrolimus use by coadministration of topical steroids. Such research should be a priority to improve our evidence-based approach to this condition and to further define the role of tacrolimus, especially in combination with well-established therapies.

The introduction of topical calcineurin inhibitors has ushered in a new era in the treatment of atopic dermatitis and clearly will be viewed as a milestone in dermatology because of their efficacy and safety profiles. Extensive, well-organized, clinical trials have demonstrated that topical tacrolimus is both safe and effective in the treatment of atopic dermatitis. Local irritation is the predominant side effect, and this tends to decrease after repeated applications. To date systemic toxicity has not been apparent in clinical studies with topical tacrolimus. However, at least in the near future topical steroids will continue to be used as the preferred agent for most cases of atopic dermatitis. This is likely for several reasons. First, topical steroids will remain more economical than tacrolimus because in many cases they are available as generics. Second, practitioners will remain more familiar with topical steroids for a few years. Finally, the current ointment formulation has limitations because many patients prefer a cream and ointment cannot be easily applied to sites such as the scalp where gels or lotions are typically preferred. New formulations, including pimecrolimus, are in progress and will be initiated into clinical studies shortly guaranteeing that the number of calcineurin inhibitors will continue to grow for many years to come.

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