A Multicenter Study of the Pharmacokinetics of Tacrolimus Ointment after First and Repeated Application to Children with Atopic Dermatitis

John Harper,* Catherine Smith,† Andris Rubins,‡ Adrian Green,* Karina Jackson,† Sanita Zigure,‡ John Bourke,§ Augustín Alomar,¶ Paul Stevenson,# Claire Foster,# and Nasrullah Undre#

*Great Ormond Street Hospital for Children, London, UK; †Skin Therapy Research Unit, University Hospital Lewisham and Kings College London, London, UK; ‡Medical Academy of Latvia, Riga, Latvia; §South Infirmary Victoria Hospital, Cork, Ireland; ¶Hospital de la Santa Creu i Sant Peu, Barcelona, Spain; #Fujisawa GmbH, Munich, Germany

The pharmacokinetics of tacrolimus after first and repeated application of 0.1% tacrolimus ointment were evaluated in 39 children, aged 6–12 y, with moderate to severe atopic dermatitis. The patients were grouped according to the size of the affected body surface area to be treated: Group 1 $\leqslant$ 1500 cm^2; Group 2 $>$ 1500 cm^2 $\leqslant$ 3000 cm^2; Group 3 $>$ 3000 cm^2 $\leqslant$ 5000 cm^2. Serial blood samples to calculate pharmacokinetic parameters taken on Day 1 (first ointment application) and Day 14 (last application) showed minimal systemic exposure to tacrolimus. Overall, 92% of the blood samples assayed contained tacrolimus concentrations below 1 ng per mL and 17% of samples were below 0.025 ng per mL, the lower limit of quantification. Systemic exposure to tacrolimus varied between patients and tended to increase proportionally as the size of the treated body surface area increased. Absorption decreased with time as the skin lesions healed and there was no evidence of systemic accumulation. The mean apparent half-life of tacrolimus ($t_{1/2,\ z}$) was 66 $\pm$ 27 h (range 19–125 h). Most patients experienced substantial clinical improvement in their atopic dermatitis. There were no clinically relevant changes in laboratory values, and the most frequently reported adverse event was skin burning, which resolved quickly as the skin condition improved.

Key words: children/clinical efficacy/pharmacokinetics/tacrolimus ointment
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Atopic dermatitis is a common chronic inflammatory skin disorder that is estimated to occur in 10%–15% of children in developed countries (Kristal and Klein, 2000). The disease is characterized by intense itching and eczematous lesions and although the exact pathogenesis is unknown, it is recognized that IgE-mediated reactions (Bos *et al*, 1994) and elevated eosinophil levels (Kapp, 1993) are involved in causing inflammation. Topical corticosteroids are the usual treatment for atopic dermatitis (Goerz and Lehmann, 1991), but prolonged application can be associated with local and systemic side effects (Lubach *et al*, 1989; Matsuda *et al*, 2000). Research in this therapeutic area has therefore focused upon finding an alternative anti-inflammatory agent that is efficacious, well-tolerated, and can be used safely in the long-term.

Tacrolimus is a calcineurin inhibitor (Dumont, 2000) that prevents the dephosphorylation of the transcription factor necessary for the expression of inflammatory T cell cytokines, such as IL-2, IL-3, TNF-α, and IL-4 (Goto *et al*, 1991). A topical formulation of tacrolimus was developed with the aim of delivering the active ingredient directly to the inflamed sites in the skin while minimizing systemic exposure to tacrolimus (Undre, 2003).

Abbreviation: AUC, area under the curve

Studies investigating the different concentrations of tacrolimus ointment (0.03%, 0.1%, 0.3%) applied to children with moderate to severe atopic dermatitis have demonstrated that the ointment is extremely efficacious (Alaiti *et al*, 1998; Boguniewicz *et al*, 1998; Kang *et al*, 2001; Paller *et al*, 2001). In a 12-wk study of 351 children treated twice daily with either 0.03% or 0.1% tacrolimus ointment, no measurable tacrolimus concentrations were detected in 90% of the blood samples collected. The mean and median tacrolimus blood concentrations were $\leqslant$2.28 ng per mL (Paller *et al*, 2001), and these blood levels are substantially lower than those measured in pediatric liver transplant patients administered tacrolimus systemically. In this study, we evaluated the systemic exposure to tacrolimus following first and repeated applications of 0.1% tacrolimus ointment to different sizes of treatment area in children with moderate to severe atopic dermatitis. The clinical efficacy and safety of the 0.1% tacrolimus ointment were also assessed during the 2-wk study period.

Results

Demographic and baseline characteristics Demographic and baseline characteristics were similar for all three treatment groups (Table I). In Group 1, 12 patients had moderate atopic dermatitis at baseline and four patients had severe

Table I. Patient demographics

	Treatment group 1 $\leqslant$1500 cm^2 (N = 16)	Treatment group 2 >1500 and $\leqslant$3000 cm^2 (N = 14)	Treatment group 3 >3000 and $\leqslant$5000 cm^2 (N = 9)
Male:female (%)	43.8:56.2	64.3:35.7	66.7:33.3
Ethnic group – number of patients (%)			
Caucasian	13 (81.3%)	10 (71.4%)	8 (88.9%)
Black	1 (6.2%)	1 (7.1%)	0 (0.0%)
Others	2 (12.5%)	3 (21.4%)	1 (11.1%)
Age in years (mean ± SD)	9.8 ± 1.6	8.9 ± 2.2	8.7 ± 1.9
Weight (kg) (mean ± SD)	35.8 ± 7.9	31.6 ± 10.7	32.1 ± 7.9
Height (cm) (mean ± SD)	141.8 ± 12.8	133.7 ± 16.4	132.2 ± 14.6
Treatment area (cm^2) (mean ± SD)	1001 ± 443	2317 ± 466	3903 ± 534
% Total body surface area (mean ± SD)	24.1 ± 14.8	50.0 ± 14.9	52.8 ± 22.9
% Total body surface area (range)	11–66	24–74	28–94

atopic dermatitis. In Groups 2 and 3, two patients in both groups had moderate atopic dermatitis whereas 12 and seven patients, respectively, had severe atopic dermatitis at baseline.

Patient disposition Two patients withdrew from the study prior to active treatment. Fifteen patients did not apply the ointment as defined in the protocol and were consequently excluded from the study. A further five patients were withdrawn because of incomplete pharmacokinetic profiles. The evaluable population therefore comprised 39 patients: Group 1 $\leqslant$1500 cm^2 (N = 16); Group 2 >1500 to $\leqslant$3000 cm^2 (N = 14); Group 3 >3000 to $\leqslant$5000 cm^2 (N = 9).

Treatment area and tacrolimus dosage The mean sizes of treatment areas are shown in Table I. On Day 1 (Profile 1), the mean dose of tacrolimus administered was 0.007, 0.013, and 0.016 mg per kg, for Groups 1, 2, and 3, respectively, whereas on Day 14 (Profile 2), the respective mean tacrolimus doses administered were 0.006, 0.012, and 0.014 mg per kg.

Pharmacokinetic parameters In general, the systemic exposure to tacrolimus following application of 0.1% tacrolimus ointment was low and highly variable (Fig 1*a* and *b*). Overall, 92% of all the blood samples assayed contained tacrolimus concentrations below 1 ng per mL and 17% of samples were below 0.025 ng per mL, the lower limit of quantification of the assay. Compared with pediatric liver transplant patients administered an oral dose of 0.15 mg per kg per 12 h, the tacrolimus blood levels measured on Days 1 and 14 in the pediatric atopic dermatitis patients were minimal (Fig 2).

There was a trend for the systemic exposure to tacrolimus to increase as the size of the treated affected body surface area increased (Table II). On Day 1, the mean area under the curve (AUC_{0-24}) values for Groups 1, 2, and 3 were 5.17, 17.48, and 11.03 ng h per mL, respectively. The corresponding mean values on Day 14 were 3.34, 15.44, and 11.35 ng h per mL. A similar trend was observed with respect to the mean maximum concentration values (C_{max}), which increased as the size of the treatment area increased.

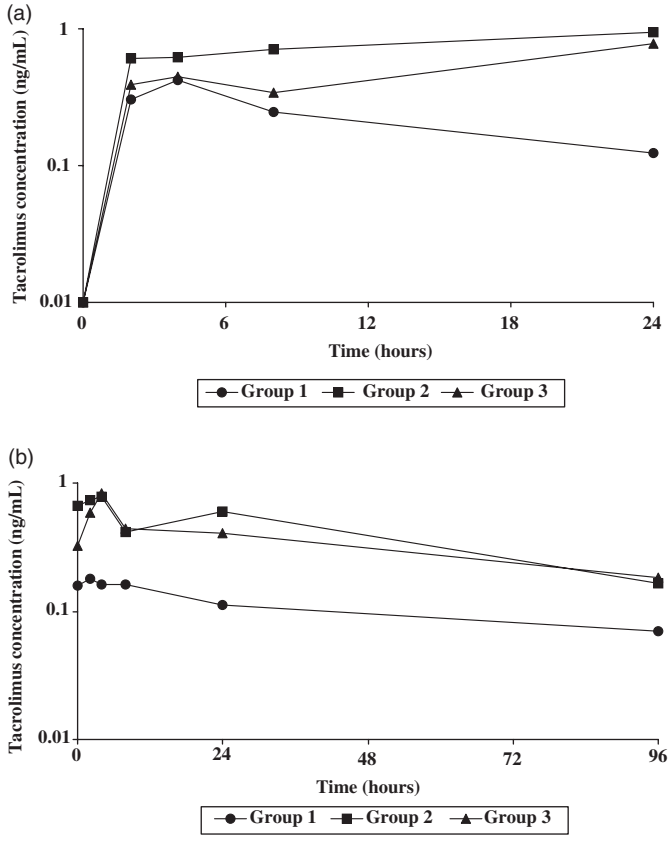

Figure 1
Systemic exposure to tacrolimus. (*a*) Mean tacrolimus blood concentration–time profile (Day 1). (*b*) Mean tacrolimus blood concentration–time profile (Day 14).

Evaluation of the mean pre-dose and minimum concentration measurements (Table III) showed that in all three treatment groups, the concentrations of tacrolimus absorbed decreased with time as the skin lesions healed, indicating no systemic accumulation of tacrolimus. There was no apparent trend in times to maximum concentration (t_{max}) among the treatment groups or between first and last ointment application. The elimination half-life measured after

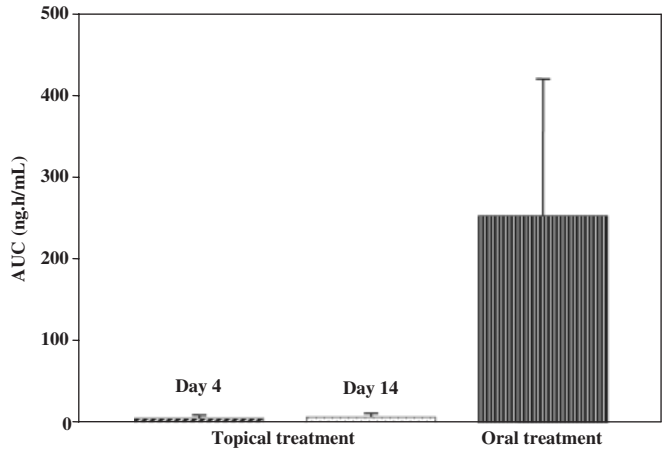

Figure 2
Systemic exposure to tacrolimus in pediatric atopic dermatitis patients compared with pediatric transplant patients. Mean area under the curve of tacrolimus in pediatric patients with moderate to severe atopic dermatitis treated with 0.1% tacrolimus ointment compared with pediatric liver transplant patients treated with tacrolimus systemically.

the last application of 0.1% tacrolimus ointment was highly variable among the patients with an overall mean of 66 ± 27 h (range 19–125 h).

Clinical efficacy Most patients experienced substantial improvement in their clinical condition during the 14-d treatment period. In Group 1, the percentage decrease between baseline and Day 14 in the mean size of affected body surface area was 39.8%, and 36.6% and 40.0%, in Groups 2 and 3, respectively. With regard to the physician's assessment of individual signs and symptoms, the mean percentage decrease between baseline and Day 14 was 58.3% for Group 1, 48.9% for Group 2, and 55.3% for Group 3. The physician's global evaluation of clinical response was also very favorable, with 34 of the 39 patients (87.2%) receiving a rating of at least "moderate improvement" (i.e., $\geqslant$50% improvement).

Adverse events Pruritus and skin burning were the most frequently reported adverse events. In Group 1, four patients (25%) reported skin burning and pruritus, respectively, whereas in Group 2, one patient (7%) experienced skin burning and two patients (14%) had pruritus. None of the patients in Group 3 reported skin burning, but two patients (7%) did experience pruritus. In all of these patients, skin irritation was mild to moderate in severity, resolved quickly, and did not result in the discontinuation of treatment. No clinically relevant changes in laboratory assessments were observed.

Discussion

Despite the potential concern of applying an ointment containing a calcineurin inhibitor to children with atopic dermatitis, these data indicate clearly that although tacrolimus is absorbed into the systemic circulation following topical application, the amount absorbed as measured by AUC_{0-24} is low and highly variable. The extent of systemic exposure tended to increase as the size of the treatment area increased, but the highest blood level recorded in the atopic dermatitis patients during the study was only around 3% of that measured in liver transplant patients administered tacrolimus orally (Wallemacq *et al*, 1998).

As the study was conducted with children and the number of blood samples that could be taken was limited, there is no pharmacokinetic profile available for Day 4 and

Table II. Pharmacokinetic parameters of tacrolimus in pediatric patients treated with 0.1% tacrolimus ointment

	AUC_{0-24} (ng h per mL) (mean ± SD)	C_{max} (ng per mL) (mean ± SD)	t_{max} (h) (median)	C_{min} (ng per mL) (mean ± SD)
Day 1				
Group 1 (N = 16)	5.17 ± 8.82	0.44 ± 0.76	4	0.12 ± 0.17
Group 2 (N = 14)	17.48 ± 25.74	0.99 ± 1.37	23	0.95 ± 1.37
Group 3 (N = 9)	11.03 ± 11.88	1.03 ± 1.13	4	0.78 ± 1.18
Day 14				
Group 1 (N = 16)	3.34 ± 2.50	0.20 ± 0.19	6	0.11 ± 0.08
Group 2 (N = 14)	15.44 ± 28.80	0.83 ± 1.34	4	0.59 ± 1.08
Group 3 (N = 9)	11.35 ± 8.66	0.98 ± 1.03	2.5	0.41 ± 0.40

AUC, area under the curve.

Table III. Mean pre-dose concentrations and minimum concentrations of tacrolimus (ng per mL) in children treated with 0.1% tacrolimus ointment

	Day 1 (24 h) (mean ± SD)	Day 4 (0 h) (mean ± SD)	Day 14 (0 h) (mean ± SD)	Day 14 (24 h) (mean ± SD)
Group 1 (N = 16)	0.12 ± 0.17	0.29 ± 0.28	0.16 ± 0.17	0.11 ± 0.08
Group 2 (N = 14)	0.95 ± 1.37	0.96 ± 0.90	0.67 ± 1.12	0.59 ± 1.08
Group 3 (N = 9)	0.78 ± 1.18	0.96 ± 1.58	0.32 ± 0.30	0.41 ± 0.40

the pre-dose concentration on Day 4 was considered as a measure of exposure on that day. Examination of the pre-dose concentrations of tacrolimus measured throughout the study showed that the systemic exposure to tacrolimus decreased as the skin lesions healed, and that there was no systemic accumulation despite repeated applications of ointment over the 14-d treatment period.

The apparent elimination half-life measured after the last application of ointment was extremely variable and longer than the systemic half-life measured in healthy subjects administered tacrolimus either orally or intravenously. This longer elimination half-life is more indicative of a slower rate of absorption rather than the actual rate of elimination. This observation, when considered together with the fact that the AUC_{0-24} values on Days 1 and 14 were similar, indicating that there was no accumulation with repeated application, implies that both the extent and rate of absorption of tacrolimus decrease as the skin lesions heal.

The clinical condition of the patients improved considerably during the short treatment period and if the study had been longer, it is likely we may have seen further improvement and perhaps even disease clearance in several children. Although some patients experienced discomfort because of the burning sensation upon application of the ointment, the treatment was generally well-accepted by the children, which is an important aspect with regard to long-term compliance.

In conclusion, this study confirms that pediatric patients applying 0.1% tacrolimus ointment have very low systemic exposure to tacrolimus, and there appears to be little risk of immunosuppression. Treatment with 0.1% tacrolimus ointment resulted in considerable clinical improvement and is a viable alternative to corticosteroids in children with moderate to severe atopic dermatitis.

Materials and Methods

Study design This was a multicenter, open, phase II pharmacokinetics study performed in Ireland, Latvia, Spain, and the UK. The study was conducted in accordance with the ethical principles described in the Declaration of Helsinki, and the ethics committee of each study center reviewed the protocol and granted approval before the start of the study. A screening visit was carried out 7 d before the baseline visit (Day 1, ointment application).

Patients Following written informed consent from the parent or guardian, 61 patients, aged 6–12 y with a diagnosis of atopic dermatitis based on the criteria of Hanifin and Rajka (1980), were enrolled into the pharmacokinetics study. The patients all had a grading of moderate to severe atopic dermatitis as defined by the scoring system of Rajka and Langeland (1989). This technique separately rates the extent, course, and intensity of the disease on a scale between one and three and then adds the three individual scores to determine overall severity (maximum = 9). A score of 3–4 represents mild atopic dermatitis, 4.5–7.5 represents moderate atopic dermatitis, and 8–9 denotes severe disease.

At the Day 1 visit, the body surface area to be treated was defined by the physician and the patients were allocated to one of three groups depending upon the size of the affected area to be treated: Group 1 $\leqslant$1500 cm^2; Group 2 $>$1500 to $\leqslant$3000 cm^2; and Group 3 $>$3000 to $\leqslant$5000 cm^2. The designated treatment area remained the same throughout the study irrespective of any clinical improvement in the skin condition.

Treatment The patients received two applications of 0.1% tacrolimus ointment on each day, except for Days 1 and 14 when the ointment was applied only once. On Days 1, 4, and 14, the ointment was applied in the morning by the investigator whereas all of the other ointment applications took place at home.

Assessments Pharmacokinetic profiling took place on Days 1, 4 (pre-dose blood sample), and 14. Blood concentration–time profiles were obtained by collecting serial blood samples (2.5 mL into EDTA monovettes). The blood samples were immediately frozen and stored at $-20^{\circ}C$ before being shipped to the contract research laboratory. Profile 1 comprised a pre-dose blood sample (0 h) followed by samples taken 2, 4, 8, and 24 h after the first application of ointment on Day 1. Profile 2 consisted of a pre-dose sample and then samples taken 2, 4, 8, 24, and 96 h after the last ointment application on Day 14.

The tacrolimus blood concentration was assayed using high-pressure liquid chromatography with tandem mass spectrometry detection. Internal standard (32-0-acetyl derivative of tacrolimus (Fujisawa, Osaka, Japan)) and protein precipitation reagent (aqueous zinc sulfate solution (Sigma, Dorset, UK):methanol (Romil, Cambridge, UK):acetonitrile (Romil) 50:30:20) were added to the samples. Solid phase extraction (using ^{18}C cartridges (Anachem, Luton, UK)) of analytes was performed before injection onto the liquid chromatography mass spectrometer (Sciex API III Plus, Perkin-Elmer, Beaconsfield, UK). The lower limit of quantification for this method was 0.025 ng per mL with a precision, based on the co-efficient of variation, of less than 21.3% for the lowest QC sample and less than 14.7% for all other concentrations. All of the procedures were performed according to OECD-GLP guidelines.

The maximum blood concentration (C_{max}), time to attain C_{max} (t_{max}), minimum concentration (C_{min}), and pre-dose blood concentration (C_0) were derived directly from the individual blood concentration–time profiles. Non-compartmental analysis, using the computer program PCModfit v1.60, was used to determine the AUC concentration time curve between 0 and 24 h using the trapezoidal rule (AUC_{0-24}), between 0 and 96 h using the trapezoidal rule (AUC_{0-96}), and the terminal half-lives ($t_{1/2}$). As there were no 12 h samples taken, truncated AUC were calculated for the time period 0–12 h by assuming a log-linear relationship between the 8 and 24 h concentration points. For the calculation of AUC, concentrations less than the lower limit of quantification were set to zero.

The physician's global evaluation of clinical response and the physician's assessment of individual signs and symptoms were performed on Days 1, 4, 14, and 18 (follow-up visit) to measure the clinical efficacy of the 0.1% tacrolimus ointment. Safety was assessed by the monitoring of clinical adverse events reported by the patient or parent or observed by the physician, and any changes from baseline in the required physical examinations and laboratory blood tests.

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Address correspondence to: Nasrullah Undre, Fujisawa GmbH, Neumarkter Strasse 61, 81673 Munich, Germany. Email: nas.undre@fujisawa.de

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