Pharmacokinetics of 0.1% Tacrolimus Ointment After First and Repeated Application to Adults with Moderate to Severe Atopic Dermatitis

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The systemic exposure to tacrolimus after first and repeated application of 0.1% tacrolimus ointment was investigated in 32 adults with moderate to severe atopic dermatitis. Patients were allocated to treatment groups according to the size of the affected area to be treated: Group 1 < 3000 cm² (N = 11); Group 2 > 3000 cm² ≤ 6000 cm² (N = 12); Group 3 > 6000 cm² ≤ 10,000 cm² (N = 9). Ointment was applied twice daily for 13 d and once daily on Day 14; the size of application area remained the same irrespective of healing. Blood samples were collected on Days 1 (first application), 4, and 14 (last application) and analyzed by a validated HPLC-MS/MS method. Systemic exposure to tacrolimus was generally low with 96% of blood samples assayed containing concentrations below 1 ng per mL and 23% of samples below the lower limit of quantification (0.025 ng per mL). Peak concentrations after first ointment application were ≤ 2.8 ng per mL, and the mean area under the concentration–time curve between 0 and 12 h using the trapezoidal rule (AUC_{0–12}) values were 1.1, 1.6, and 4.8 ng h per mL for Groups 1, 2, and 3, respectively. The corresponding mean values on Day 14 were similar indicating negligible systemic accumulation of tacrolimus after repeated ointment applications. Both the rate and extent of topical absorption decreased as the skin lesions healed.

Key words: adults/atopic dermatitis/pharmacokinetics/tacrolimus ointment


Atopic dermatitis is a common, chronic, inflammatory skin disease characterized by intense itching and eczematous lesions. Emollients afford some symptomatic relief by helping prevent water loss and painful cracking of the skin, but disease exacerbations often require treatment with topical corticosteroids, the prolonged application of which can be associated with dermal atrophy and other side effects (Furue et al, 2003). Therefore there is a need for an alternative treatment that is efficacious, and free of the long-term side effects associated with corticosteroids.

Tacrolimus is a calcineurin inhibitor and reduces inflammation by suppressing T-lymphocyte responses (Chher and Plosker, 2001). Clinical trials in adults (Ruzicka et al, 1997; Hanifin et al, 2001; Soter et al, 2001) and children (Boguniewicz et al, 1998; Kang et al, 2001; Paller et al, 2001) have shown that tacrolimus ointment is extremely effective in treating and controlling moderate to severe atopic dermatitis. Systemic exposure to tacrolimus following ointment application is low and absorption decreases as the skin lesions heal (Paller et al, 2001; Soter et al, 2001).

To gain additional knowledge of the systemic exposure to tacrolimus following first and repeated ointment application, 0.1% tacrolimus ointment was applied for 14 d to different sizes of treatment area in adults with moderate to severe atopic dermatitis. The main study objective was to investigate the pharmacokinetics of tacrolimus in relation to increasing treatment surface area. Clinical efficacy was assessed by measuring the improvement in the body surface areas treated.

Results

Demographic and baseline characteristics The treatment groups were comparable with respect to age, weight, height, and estimated total body surface area. In Group 3, there were more patients with a grading of severe atopic dermatitis at baseline compared with the other two treatment groups.

Patient disposition Two patients withdrew from the study prior to active treatment and three patients did not provide complete pharmacokinetic profiles and were excluded from the study. Therefore complete sets of data for pharmacokinetic analyses were obtained for 32 patients (Group 1, N = 11; Group 2, N = 12; Group 3, N = 9).

Treatment area and tacrolimus dosage The mean size of treatment areas were 2410 ± 454 cm² (Group 1), 4104 ± 872 cm² (Group 2) and 8553 ± 1308 cm² (Group 3). On Day 1, the patients in Groups 1 and 2 both received a mean tacrolimus dose of 0.05 mg per kg while in Group 3, the mean tacrolimus dose was 0.11 mg per kg. As the

Abbreviations: AUC_{0–12}, area under the concentration–time curve between 0 and 12 h using the trapezoidal rule
condition of the skin improved, less ointment was required to cover the treatment area and the tacrolimus dose decreased; on Day 14, the respective mean doses were 0.03, 0.04, and 0.07 mg per kg.

Pharmacokinetic parameters Pharmacokinetic parameters were determined for all three profiles and are shown in Table I.

In general, the systemic exposure to tacrolimus was low and highly variable in all three treatment groups. Overall 96% of all the blood samples assayed contained tacrolimus concentrations below 1 ng per mL and 23% of samples were below the lower limit of quantification of the assay (0.025 ng per mL). The mean blood concentration-time profiles (Fig 1a–c) show that the exposure to tacrolimus decreased between Day 4 and 14 in all three treatment groups as the condition of the skin improved.

Systemic exposure to tacrolimus tended to increase as the size of the treated body surface area increased. The higher tacrolimus blood levels observed, however, were still very low, especially when compared to the tacrolimus blood levels present in adult transplant recipients administered tacrolimus systemically (Fig 2). The mean area under the concentration–time curve between 0 and 12 h using the trapezoidal rule (AUC$_{0–12}$) values for Groups 1, 2, and 3 following the first application of ointment were 1.1, 1.6, and 4.8 ng h per mL, respectively. The mean AUC$_{0–12}$ values measured on Day 14 were comparable to those on Day 1 (Table I), indicating a decrease in percutaneous absorption and no accumulation of tacrolimus.

A similar trend was observed with the mean maximum concentration values (C$_{max}$). There were no clear trends discernible for the times to maximum concentration (t$_{max}$) among the three treatment groups.

The mean disposition half-life (t$_{1/2}$) estimated from Day 1 blood-concentration data was similar in the three treatment groups (8.6, 9.4, and 8.5 h for Groups 1, 2, and 3, respectively). There was high individual variation in this parameter with values ranging from 2.7 to 18.0 h. Blood samples were collected for up to 7 d after the last application of ointment and an apparent slower elimination half-life was observed (mean: 76 ± 22 h; range: 51–136 h).

Clinical improvement Most patients experienced considerable clinical improvement during the 14-d-treatment period (Table II). By Day 14, there was a large reduction in the mean size of the affected body surface area in each of the treatment groups. The patients in Group 1 experienced the largest reduction (66.3%) followed by Group 3 (42.0%) and Group 2 (26.3%).

The improvement in clinical condition achieved with the 0.1% tacrolimus ointment is also reflected in the physician’s global evaluation of clinical response. Seven patients (21.9%) obtained a rating of “moderate” improvement (defined as ≥50% improvement); 14 patients (43.8%) received

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**Table I. Pharmacokinetic parameters of tacrolimus in adults after first and repeated application of 0.1% tacrolimus ointment (N = 32)**

<table>
<thead>
<tr>
<th></th>
<th>C$_{max}$ (ng per mL) Mean ± SD</th>
<th>t$_{max}$ (h) Median</th>
<th>AUC$_{0–12}$ (ng h per mL) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>0.14 ± 0.16</td>
<td>4</td>
<td>1.1 ± 1.4</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.25 ± 0.42</td>
<td>4</td>
<td>1.6 ± 2.8</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.66 ± 0.85</td>
<td>4</td>
<td>4.8 ± 6.3</td>
</tr>
<tr>
<td>Day 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>0.21 ± 0.24</td>
<td>2</td>
<td>2.1 ± 2.3</td>
</tr>
<tr>
<td>Group 2</td>
<td>1.10 ± 2.78</td>
<td>7</td>
<td>3.9 ± 5.0</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.96 ± 0.80</td>
<td>4</td>
<td>10.2 ± 9.2</td>
</tr>
<tr>
<td>Day 14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>0.60 ± 1.62</td>
<td>2</td>
<td>1.7 ± 2.3</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.19 ± 0.22</td>
<td>3</td>
<td>1.5 ± 1.5</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.65 ± 0.38</td>
<td>4</td>
<td>5.4 ± 2.8</td>
</tr>
</tbody>
</table>

AUC$_{0–12}$, area under the concentration–time curve between 0 and 12 h using the trapezoidal rule.
a rating of “marked” improvement (≥75%–89%) while seven patients (21.9%) experienced “excellent” improvement (90%–99%) and one patient (3.1%) achieved clearance. Only one patient had no appreciable improvement during the study.

Skin irritation (skin burning, itching) was the most frequently reported adverse event. Altogether 10 patients (31.2%) experienced transient skin irritation (Group 1: two patients; Group 2: five patients; Group 3: four patients) which, in most cases, was mild to moderate in severity and normally resolved within 10–15 min. None of the patients discontinued treatment because of skin irritation, and the sensation of skin burning resolved completely after the first four days of treatment. There were no serious adverse events or adverse events leading to treatment discontinuation. There were no clinically relevant changes in laboratory assessments.

Discussion

The data presented here are consistent with those published previously (Paller et al., 2001; Soter et al., 2001) and show that although tacrolimus applied topically is absorbed, the overall systemic exposure to the drug as measured by AUC₀–₁₂ is low and highly variable. There was a tendency for systemic exposure to increase proportionally as the size of treatment area increased, but exposure was still substantially lower than for transplant patients. Although a direct measure of systemic bioavailability following the topical application of the ointment was not made in this study, the highest mean value of AUCᵢ₀–₁₂, 10.2 ng h per mL measured during the study was only 3% of the corresponding value for kidney transplant patients (346 ng h per mL) administered an oral tacrolimus dose of 0.2 mg per kg per 12 h (Undre et al., 1998). In addition to the observed low systemic exposure, the pre-dose and minimum concentration measurements indicate that, despite repeated applications of 0.1% tacrolimus ointment to the same treatment area, there is no systemic accumulation of tacrolimus.

The patients with the larger treatment areas had more severe atopic dermatitis and a higher number of open lesions, which probably led to an initially higher absorption of tacrolimus. The blood concentration–time profiles show that as the skin lesions healed, the systemic exposure to tacrolimus decreased. A decrease in the percutaneous penetration of tacrolimus over the dosing time interval also becomes evident when the other pharmacokinetic data are considered. The mean disposition t₁/₂ on Day 1 of 8–9 h is shorter than the elimination half-life of 40 h measured in healthy subjects (Möller et al., 1999) and therefore reflects absorption and elimination processes occurring concurrently. Furthermore, the mean t₁/₂,α after the last application of ointment was longer than the systemic half-life of tacrolimus in healthy subjects; this apparent longer half-life is probably attributable to the rate of absorption being slower than the rate of elimination. This observation, combined with the fact that the AUCᵢ₀–₁₂ values on Day 14 and 1 were similar, indicates that both the extent and rate of topical absorption of tacrolimus decreases as the condition of the skin improves. These data suggest therefore that in this patient population, due to the decrease in percutaneous absorption, 0.1% tacrolimus ointment should be applied twice daily.

Although the study was of short duration as it was designed primarily to determine the pharmacokinetics of 0.1% tacrolimus ointment, we observed considerable improvement in the clinical condition of most patients. There were no clinical safety concerns associated with the ointment.¹

In conclusion, these pharmacokinetic data confirm that patients applying 0.1% tacrolimus ointment have low systemic exposure to tacrolimus and there was no evidence of systemic accumulation. Furthermore, 0.1% tacrolimus ointment is efficacious and well-tolerated by adults with moderate to severe atopic dermatitis.

¹The FDA is currently considering whether to add a black box warning about the theoretical risk of malignancies in atopic dermatitis patients applying topical treatments containing calcineurin inhibitors.

Table II. Measurement of clinical efficacy of 0.1% tacrolimus ointment

<table>
<thead>
<tr>
<th>Mean affected BSA (cm²)</th>
<th>Percentage decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 ≤3000 cm²</td>
<td>2344</td>
</tr>
<tr>
<td>Group 2 &gt;3000 cm² ≤6000 cm²</td>
<td>4104</td>
</tr>
<tr>
<td>Group 3 &gt;6000 cm² ≤10,000 cm²</td>
<td>8609</td>
</tr>
</tbody>
</table>

Physician’s assessment of global response—number of patients (%)

<table>
<thead>
<tr>
<th></th>
<th>Clear</th>
<th>Excellent</th>
<th>Marked</th>
<th>Moderate</th>
<th>Slight improvement</th>
<th>No appreciable improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>1 (3.1%)</td>
<td>7 (21.9%)</td>
<td>14 (43.8%)</td>
<td>7 (21.9%)</td>
<td>2 (6.3%)</td>
<td>1 (3.1%)</td>
</tr>
</tbody>
</table>

BSA, bovine serum albumin.
Materials and Methods

Study design This was a single-center, open, phase II pharmacokinetics study. The study was conducted in accordance with the ethical principles described in the Declaration of Helsinki, and the Ethics Committee of the study center (Zalu klinikas izpetes Etikas komiteja, Riga, Latvia) reviewed the protocol and granted approval before the start of the study. A screening visit was carried out seven days before the baseline visit (Day 1, ointment application).

Patients Following written informed consent from the patient, 37 male and female patients aged 18 y or older with a diagnosis of atopic dermatitis based on the criteria of Hanifin and Rajka (1980) were enrolled into the study. The patients were required to have a grading of moderate to severe atopic dermatitis (i.e., score at least 4.5) as defined by the scoring system of Rajka and Langeland (1989) which rates extent, course and intensity of the disease separately before summing up the three scores to determine overall severity. At the Day 1 visit, the area to be treated was defined by the physician and the patients were allocated to one of three groups according to the size of treatment area: Group 1 ≤3000 cm²; Group 2 >3000 cm² to ≤6000 cm²; Group 3 >6000 cm² to ≤10,000 cm².

Treatment The patients received two applications of 0.1% tacrolimus ointment on each day, except for Day 14 when the ointment was applied only once so that a full pharmacokinetic profile to 168 h could be obtained. The size of the application area remained the same irrespective of healing. On Days 1 and 4, the first of the two ointment treatments was applied by the investigator as was the single application on Day 14. All other ointment applications were performed by the patient at home.

Assessments Pharmacokinetic profiling, using the tacrolimus assay as described by Hill et al (1997), took place on Days 1, 4, and 14. Blood concentration–time profiles were attained by collecting serial blood samples (2.5 mL into EDTA monovettes). The blood samples were immediately frozen and stored at −20°C before being shipped to the contract research laboratory that performed the analyses. Profile 1 comprised a pre-dose blood sample (0 h) followed by samples taken 2, 4, 6, 8, 10, and 12 h after first application of 0.1% tacrolimus ointment while Profile 2 consisted of samples taken pre-dose, 2, 4, 8, and 12 h following ointment application on Day 4. Profile 3 comprised a pre-dose sample and samples taken 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 h after the last application of ointment. 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 13C 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 12.3%. All of the procedures were performed according to OECD-GLP guidelines.

The maximum blood concentration (Cmax), time to attain Cmax (tmax), minimum concentration (Cmin), and pre-dose blood concentration (C0) were read directly from the individual blood concentration-time profiles. Non-compartmental analysis was used to determine the AUC0–12, the disposition half-life (t1/2,d), and terminal half-life (t1/2,z). For calculation of AUC0–12, Concentrations less than the lower limit of quantification were set to zero.

Safety assessments were based on laboratory blood tests, clinical adverse events and the changes from baseline of the physical examinations. Clinical efficacy was evaluated according to the ratings of the physician’s global evaluation of clinical response made on Days 1 and 14, and the decrease in total affected body surface area. For the physician’s global evaluation of clinical response, the investigator rated the change from baseline in the patient’s overall clinical status. The investigator reported “cleared” to indicate improvement of 100%, “excellent” for improvement of 90%–99%, “marked” for 75%–89%, “moderate” for 50%–74%, “slight” for 30%–49%, “no appreciable improvement” for 0%–29%, and “worse” for worsening of the condition. Total affected body surface area was calculated by multiplying the percentage affected area of each body region (head/neck, upper limbs, trunk and lower limbs) by the following factors (head/neck 0.1; upper limbs 0.2; trunk 0.3, and lower limbs 0.4) to give the sum of the total affected area.

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