Pharmacokinetics of Tacrolimus (FK506) Ointment after a Single Dermal Application to the Rat

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Summary: The pharmacokinetics of tacrolimus (FK506) were studied in the male rat after a single dermal application of FK506 ointment over an application period of 24 hours. In the typical experiment, the amount of ointment applied was 100 mg per each rat, and application area was 10 cm². The pharmacokinetic parameters estimated were maximal blood concentration (Cmax), time to reach Cmax (Tmax), and area under the blood concentration-time curve (AUC).

1. Blood concentrations of FK506 were determined after a single dermal application of FK506 ointment under the occlusive or non-occlusive dressing condition. After respective application of 0.5 and 0.1% FK506 ointment to the intact and damaged skin, Cmax and AUC values did not differ significantly between either dressing conditions. Blood concentrations of FK506 after a single dermal application of FK506 ointment to the damaged skin were much higher than those applied to the intact skin. The bioavailability of FK506 after application of 0.5% FK506 ointment to the intact skin was 5% and that to the damaged skin was 62%.

2. After a single dermal application of 0.03, 0.1 and 0.5% FK506 ointment to the intact skin, the Cmax and AUC values increased in proportion to the strength of the ointment. After a single dermal application of 0.3% FK506 ointment to 2.5, 5 and 10 cm² of the intact skin, the Cmax and AUC values increased proportionally to the area of the skin. The Cmax and AUC values did not the differ significantly after single dermal application of 10, 30, 100 and 300 mg of 0.3% FK506 ointment to the intact skin.

Key words: Tacrolimus, FK506, Ointment, Absorption, Blood concentrations, Single dermal application, Rats

Introduction

Tacrolimus (FK506) is a 23-membered macrolide with very potent immunosuppressive actions and has been marketed as an immunosuppressive agent for the prevention and control of rejection in organ transplantation. Clinical trials of FK506 ointment are in progress for the treatment of atopic dermatitis.

The absorption, distribution, metabolism and excretion study of FK506 ointment is essential for estimating the results of pharmacological and toxicological studies of the ointment. In this study, we investigated the absorption of FK506 after a single dermal application of FK506 ointment to male rats.

Materials and Methods

Materials

FK506 and FK506 ointment were prepared at Fujisawa Pharmaceutical Co., Ltd. Other reagents were obtained as described previously.

Animals, Treatment, Dosage and Application

Male Sprague-Dawley strain rats, 6 to 7 weeks old, were purchased from Clea Japan Inc. Hair clipping and shaving, and application under occlusive and non-occlusive dressing condition were described in the previous study. In the typical experiment, the amount of ointment applied was 100 mg per each rat and application area was 10 cm² on the dorsal skin. To prepare the damaged skin, the stratum corneum was removed by tape-stripping ten times with adhesive tape.

FK506 dissolved in PEG400 was injected intravenously to the rats as described previously.

Sample Collection

Blood samples were collected from the tail vein as described in the previous study.

Assay of FK506

FK506 in the whole blood was measured by enzyme immunoassay as described previously.

Calculation of Pharmacokinetic Parameters

Model independent analysis was used for the pharmacokinetic analysis of blood concentrations of FK506 as described previously. The pharmacokinetic parameters calculated were maximal blood concentration (Cmax), time to reach Cmax (Tmax), area under the
blood concentration-time curve (AUC), elimination half life ($T_{1/2}$), total body clearance (Cl), and distribution volume at steady state (Vdss).

**Results**

The pharmacokinetic properties of tacrolimus (FK506) were investigated in the male rat after a single dermal application of FK506 ointment. In the typical experiment, the amount of ointment applied to the dorsal skin was 100 mg/rat and application area was 10 cm², and application period was 24 hours.

To study the effect of skin condition on the absorption of FK506, blood concentrations of FK506 after a single dermal application of FK506 ointment were measured under occlusive and non-occlusive dressing condition and pharmacokinetic parameters are shown in Table I. After application of 0.5% FK506 ointment to the intact skin, Cmax, Tmax and AUC values did not differ significantly between either dressing conditions. After application of 0.1% FK506 ointment to the damaged skin, the Tmax of the non-occlusive group was significantly earlier than that of the occlusive group but the Cmax and AUC values were not significantly different in either dressing conditions.

<table>
<thead>
<tr>
<th>FK506 concentration (skin condition)</th>
<th>Skin dressing</th>
<th>Pharmacokinetic parameters</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cmax (ng/mL)</td>
</tr>
<tr>
<td>0.5% (intact)</td>
<td>Occlusive</td>
<td>2.85±1.30</td>
</tr>
<tr>
<td></td>
<td>Non-occlusive</td>
<td>2.92±1.08</td>
</tr>
<tr>
<td>0.1% (damaged)</td>
<td>Occlusive</td>
<td>17.44±1.29</td>
</tr>
<tr>
<td></td>
<td>Non-occlusive</td>
<td>15.05±1.04</td>
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</table>

Amount of FK506 ointment applied was 100 mg/rat, and the application area was 10 cm². Values represent mean±S.E. of 6 to 7 animals.

*; AUC value from the initiation of application to the final measurable time point.

Table I: Effect of skin dressing on pharmacokinetics of FK506 after single dermal application of FK506 ointment to rats

The blood concentrations of FK506 after a single dermal application of FK506 ointment to the damaged skin were much higher than those when the ointment was applied to the intact skin (Table II, Fig. 1). Blood concentrations of FK506 after a single intravenous injection of FK506 are also shown in Fig. 1. The bioavailability of FK506 after application of 0.5% FK506 ointment to the intact skin was 5% and 62% after application to the damaged skin.

After a single dermal application of 0.03, 0.1 and 0.5% FK506 ointment to the intact skin, the Cmax and AUC values increased in proportion to the strength of the ointment as depicted in Fig. 2. After a single dermal application of 0.3% FK506 ointment to 2.5, 5 and 10 cm² of the intact skin, the Cmax and AUC values increased proportionally to the applied area of the ointment (Fig. 3). As shown in Table III, the Cmax and AUC values did not differ significantly after single dermal application of 10, 30, 100 and 300 mg of 0.3% FK506 ointment to the intact skin.

**Discussion**

The blood concentrations of FK506 were determined after single dermal application of FK506 ointment to the...
rat under different experimental conditions, and the pharmacokinetic properties of FK506 ointment were estimated.

The percutaneous absorption of target compounds from the transdermal formulation is affected by the condition of the skin dressing. After a single dermal application of FK506 ointment to the intact and damaged dorsal skin of rats, $T_{\text{max}}$ and AUC values did not differ significantly between the occlusive and non-occlusive dressing conditions. These results indicate that the absorption of FK506 from the skin is similar or very nearly so between both skin dressings. We also reported similar results in the experiment measuring the radioactivity of $^{14}$C-FK506 ointment.

The percutaneous absorption of drugs is classified into two pathways; the transepidermal route through the stratum corneum and the transappendageal route through the pilosebaceous unit or eccrine gland. Physico-chemical properties of compounds examined influence the pathways of absorption. Contribution of absorption through the transappendageal route is relatively large in ionized or hydrophilic compounds. Partitioning to the intracellular lipid of stratum corneum was observed in compounds with a partition coefficient of more than 1000 in n-octanol/water system, so these compounds are absorbed through the transepidermal route. The partition coefficient of FK506 was more than 1000 and this result suggested that FK506 would be absorbed through the transepidermal route.

In the transepidermal pathway, the stratum corneum functions as a barrier of drug absorption and the absorption is enhanced under the condition of damaged skin. The absorption of FK506 from the skin increased markedly after applying FK506 ointment to the damaged skin of rats. Bronaugh reported that percutaneous absorption of compounds was increased in damaged skins such as UV irradiated, abraded or tape-stripped skins, and the highest absorption was observed in the tape-stripped skin owing to the removal of the stratum corneum. FK506 percutaneous absorption was also increased in damaged skin, and the degree of FK506 absorption would be maximum because the ointment was
applied to the tape-stripped skin. We have reported similar results in which blood concentrations and excretion of radioactivity increased drastically after a single dermal application of 14C-labeled FK506 ointment under the same dressing condition. These results indicate that the stratum corneum of the rat skin functions as a barrier to the absorption of FK506 in the ointment and FK506 is absorbed through the transepidermal route in the rat.

The blood concentrations of FK506 after application of the ointment to the intact skin of rats increased proportionally to the applied area of the ointment. The concentrations were almost constant even when the amount of ointment was increased on the same area under the same condition. The results indicate that the percutaneous absorption of FK506 from the ointment through the rat skin was dependent on the size of the area applied, and not the amount or thickness of the ointment under the same skin condition.

Table III Effects of application amount on pharmacokinetics of FK506 after single dermal application of 0.3% FK506 ointment to rats

<table>
<thead>
<tr>
<th>Amount of FK506 applied (mg/rat)</th>
<th>Pharmacokinetic parameters</th>
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<tbody>
<tr>
<td></td>
<td>$C_{\text{max}}$ (ng/mL)</td>
</tr>
<tr>
<td>10</td>
<td>0.84±0.37</td>
</tr>
<tr>
<td>30</td>
<td>0.76±0.35</td>
</tr>
<tr>
<td>100</td>
<td>0.91±0.35</td>
</tr>
<tr>
<td>300</td>
<td>0.47±0.16</td>
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The application area of 0.3% FK506 ointment was 10 cm$^2$/rat under occlusive condition. Values represent mean ± S.E. of 5 to 7 animals.

Fig. 3. Relationship between application area and pharmacokinetic parameters of FK506 after a single dermal application of FK506 ointment to the rat.

Amount of 0.3% FK506 ointment applied was 100 mg/rat under occlusive condition of intact skin. Each point represent mean (± S.E.) of 5 to 7 rats.

Table III Effects of application amount on pharmacokinetics of FK506 after single dermal application of 0.3% FK506 ointment to rats

References