P-Glycoprotein-Dependent Disposition Kinetics of Tacrolimus: Studies in mdr1a Knockout Mice

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Purpose. This study was performed to evaluate the involvement of P-glycoprotein in disposition kinetics of tacrolimus (FK506), a substrate of P-glycoprotein, in the body.

Methods. The blood and tissue concentrations of FK506 after i.v. or p.o. administration (2 mg/kg) to normal and mdr1a knockout mice were measured by competitive enzyme immunoassay.

Results. The blood concentrations in knockout mice were significantly higher than those in normal mice. The value of the total clearance (CLtot) for knockout mice (19.3 mL/min/kg) was about 1/3 of that for normal mice (55.8 mL/min/kg) (P < 0.001), although there was no significant difference in the distribution volume at the steady-state (Vdss) (about 4.6 L/kg) between both types of mice. FK506 rapidly penetrated the blood-brain barrier and the brain concentration reached a maximum, which was about 10 times higher in knockout mice than in normal mice, 1 hr after administration. The brain concentration in normal mice thereafter decreased slowly, whereas in knockout mice, an extremely high concentration was maintained for 24 hr.

Conclusions. The pharmacokinetic behavior of FK506 in the tissue distribution is related with the function of P-glycoprotein encoded by the mdr1a gene. The brain distribution of FK506 is dominantly by the P-glycoprotein-mediated drug efflux and presumably also by the binding to FK-binding proteins (immunophils) in the brain.

KEY WORDS: tacrolimus; disposition kinetics; P-glycoprotein; mdr1a knockout mice; brain distribution.

INTRODUCTION

P-glycoprotein is encoded by the multidrug-resistance (mdr) gene and is expressed, not only in multidrug-resistant cancer cells, but also in various normal tissues such as the adrenal, kidney, liver, small intestine, colon, and capillary endothelium in the brain (1–3). Three mdr genes have been reported in rodents, while two have been identified in humans. In the mouse, mdr1a and mdr1b have been shown to confer multidrug resistance in cancer cells, whereas the mdr2 gene does not (4). Therefore, although P-glycoprotein is considered to play an important role in the tissue-distribution, clearance, and gastrointestinal absorption in animals, the contributions of individual gene products to the drug pharmacokinetics have not yet been clarified. Recently, Borst et al. (5) established mdr1a gene-deficient mice. Subsequently, Schinkel et al. (6, 7) reported the distribution of several P-glycoprotein substrates, such as ivermectin, vinblastine, digoxin, cyclosporin A, and dexamethasone, to the brain is significantly increased in the mdr1a knockout mice, when compared with normal mice. This evidence clearly indicates the P-glycoprotein encoded by mdr1a gene operates as a part of the blood-brain barrier in mice (8).

Tacrolimus (FK506) is a potent immunosuppressant having a macrolide lactone structure. It was isolated from Streptomyces tsukubaensis and is commonly used to prevent the rejection of organ transplants. FK506 has some adverse effects such as renal and hepatic toxicities (9), and there is also evidence of central nervous and heart toxicity (10). On the other hand, FK506 has recently been reported to have a neuroprotective effect in focal cerebral ischemia by inhibiting calcineurin in the brain (11, 12). It has already been shown that FK506 is a substrate of P-glycoprotein, by means of in vitro studies using vincristine resistant mouse leukemia P388 cells (13) and LLC-PK1 cells transfected with human MDR1 cDNA (14). However, the involvement of P-glycoprotein in the in vivo tissue-distribution, absorption, and elimination of FK506 has not yet been clarified.

In this study, we examined the involvement of P-glycoprotein in the pharmacokinetics of FK506 in the brain, using mdr1a knockout mice.

MATERIALS AND METHODS

Materials

FK506, mouse anti-FK506 monoclonal antibody (FKmAb) and FK506-conjugated peroxidase (FK-POD) were gifts from Fujisawa Pharmaceutical Co. (Osaka, Japan). Mouse anti IgG polyclonal antibody (goat) was purchased from Incstar Co. (Minn, USA). All other chemicals were of reagent grade and were used without further purification.

Animal Experiments

Experiments were performed on male mdr1a (−/−) knockout mice (body weight 22–28 g, Taconic Farms Inc., NY, USA). We used male C57BL/6 mice (body weight 21–26 g, SLC, Hamamatsu, Japan) as the control mice because genetically compatible wild type (+/+ ) mice, F2 and F3 generations of 129/Ola × FVB mice (6), were not available in Japan. We confirmed in a preliminary study that male C57BL/6 mice showed similar disposition kinetics of several drugs, including tacrolimus, to the wild type (+/+) mice (data not shown).

FK506 (2 mg/kg) was injected via the jugular vein in a volume of 50 μl or was orally administered in a volume of 200 μl. Blood samples were collected from the intraorbital venous plexus using a heparinized capillary tube under light ether anesthesia, at designated time intervals. To determine the apparent tissue-to-blood concentration ratio (Kb.app), the mice

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were euthanized 5 hr after a single intravenous (i.v.) injection of FK506. The tissues were quickly excised, rinsed well with ice-cold saline, blotted dry, and weighed. The samples were homogenized in ice-cold saline (10%, w/v). For the biliary recovery of FK506, the bile duct was cannulated with polyethylene tubing (type sp-8 O. D. 0.5 mm, Natsume, Tokyo, Japan) under light ether anesthesia. Cannulated mice were kept in a supine position on restraining plates. Blood, tissue, and bile samples were kept at -30°C until assay.

Assay for FK506

According to the method of Kobayashi et al. (15), FK506 was measured by competitive enzyme immunoassay with FKmAb and FK-POD. Briefly, FK506 in whole blood and bile was extracted with methanol. FK506 in tissue samples was extracted with n-hexane containing 2.5% isoamyl alcohol. The extraction solvent was evaporated and the residue was dissolved in FK-POD solution. The solution was added to a microtiter plate well, previously coated with FKmAb, to determine competitive binding of FK506 and FK-POD with FKmAb. POD activity was measured using o-phenylenediamine and hydrogen peroxide as substrates. The reaction was stopped by addition of H₂SO₄, and the optical density was measured by a microplate reader (CS-9300PC, Shimadzu, Kyoto, Japan). FK506 content was determined by comparison with a standard curve.

Data Analysis

The steady-state distribution volume (Vdss) and the total body clearance (Cltot) were estimated by means of model-independent moment analysis as described by Yamaoka et al. (16). The data were analyzed by using Student’s t test for comparing the unpaired means of two sets of data. The number of determinations (N) is noted in each table and figure. A P value of 0.05 or less was used as the criterion of a significant difference between sets of data.

### RESULTS

#### Time Course of Blood Concentration of FK506

The time courses of blood concentration of FK506 after i.v. administration of FK506 (2 mg/kg) to normal and knockout mice are shown in Fig. 1. They are biphasic, with half-times for the distribution and elimination phases of 10.5 and 111 min in normal, and 19 and 209 min in knockout mice, respectively. The blood concentrations in knockout mice were significantly higher than those in normal mice.

As shown in Table 1, the value of the area under the blood concentration-time curve (AUC(0→∞)) of FK506 from time zero to infinity for knockout mice was significantly larger than that for normal mice (P < 0.001). The value of the total clearance (Cltot) for knockout mice was about 1/3 of that for normal mice (P < 0.001), though there was no significant difference in the distribution volume at the steady-state (Vdss) between them.

### Table 1. Pharmacokinetic Parameters of FK506 in Normal and Knockout Mice After i.v. Administration (2 mg/kg)

<table>
<thead>
<tr>
<th></th>
<th>Normal mice</th>
<th>Knockout mice</th>
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<tbody>
<tr>
<td></td>
<td>AUC (ng · min/mL)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>35900 ± 3900</td>
<td>104000 ± 13800*</td>
</tr>
<tr>
<td></td>
<td>MRT (min)</td>
<td>92 ± 22</td>
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<tr>
<td></td>
<td>Vdss (L/kg)</td>
<td>5.14 ± 1.59</td>
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<tr>
<td></td>
<td>Cltot (mL/min/kg)</td>
<td>55.8 ± 6.2</td>
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<tr>
<td></td>
<td>A1 (ng/mL)</td>
<td>937 ± 92</td>
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<tr>
<td></td>
<td>λ1 (min⁻¹)</td>
<td>0.0662 ± 0.006</td>
</tr>
<tr>
<td></td>
<td>A2 (ng/mL)</td>
<td>120 ± 14</td>
</tr>
<tr>
<td></td>
<td>λ2 (min⁻¹)</td>
<td>0.00062 ± 0.00057</td>
</tr>
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</table>

* Determined by model-independent moment analysis.  
 Determined by applying the MULTI program to the biexponential equation:

$$C_t = A_1 \cdot \exp^{-λ1t} + A_2 \cdot \exp^{-λ2t}$$

Each value represents the mean ± SD of four to eight mice.

* Significantly different from normal mice at P < 0.01 and P < 0.001.

### Fig. 2. Tissue and blood concentrations of FK506 at 5 hr after i.v. administration (2 mg/kg) to normal (●) and knockout (●) mice. Each column with bar represents the mean ± SE of four or five mice. **, *** Significantly different from normal mice at P < 0.01 and P < 0.001, respectively.