Review

Metabolism of Tacrolimus (FK506) and Recent Topics in Clinical Pharmacokinetics

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Summary: Tacrolimus (FK506), an immunosuppressive drug, is co-medicated with multiple drugs under clinical conditions. Tacrolimus is highly lipophilic and is excreted from the body after receiving extensive metabolism. Due to its narrow therapeutic window following organ transplantation, tacrolimus requires therapeutic drug monitoring by an enzyme immunoassay using the monoclonal antibody raised against tacrolimus. Therefore, metabolism studies including drug-drug interaction and metabolite identification studies are essential for the efficient development and clinically optimal usage of this drug. Tacrolimus was metabolized by the cytochrome P450 (CYP) 3A subfamily. Metabolic drug-drug interaction studies were conducted to provide information regarding the optimal usage of tacrolimus, and its metabolism was inhibited by known CYP3A inhibitors such as ketoconazole, cyclosporine A, and nifedipine. Recent reports on clinical pharmacokinetics indicate that dose levels of tacrolimus need to be adjusted in transplant patients with CYP3A5 polymorphism.

Key words: tacrolimus; FK506; oxidative metabolism; CYP3A4; CYP3A5; drug-drug interaction; immunoassay; immunosuppressant

Introduction

Tacrolimus (FK506) has been marketed as an immunosuppressive agent for preventing or treating graft rejection in organ transplantation patients, or for treating autoimmune diseases including myasthenia gravis, arthritis, and atopic dermatitis.1) Tacrolimus is available as an injection formulation, capsules, or an ointment for a continuous intravenous (i.v.) infusion, an oral dosing, or a topical administration, respectively. Tacrolimus is a 23-membered macrolide antibiotic (molecular weight of non hydrate: 806) produced by Streptomyces tsukubaensis (Fig. 1), and is highly lipophilic and insoluble in water.2) These physicochemical properties of tacrolimus cause a large variation of oral absorption and receiving extensive metabolism for the clearance from the body.

Tacrolimus was initially developed as a drug for the prevention and/or treatment of graft rejection in organ transplantation patients. Before and after organ transplantation, patients are co-medicated with multiple drugs to prevent rejection of the transplanted organ and to maintain them in a good condition. Due to its narrow therapeutic window, tacrolimus requires therapeutic drug monitoring (TDM) by an enzyme immunoassay (EIA) using the monoclonal antibody raised against tacrolimus. Evaluation of immunocrossreactivity of tacrolimus metabolites against the antibody is very important to the proper estimation of blood levels determined by this nonspecific EIA. These medical situations necessitate metabolite identification and special considerations of drug-drug interactions between tacrolimus and co-medicated drugs during the drug development stage. After administration, tacrolimus, either injected or absorbed into the body, is excreted from the body after receiving extensive metabolism. Characterization of tacrolimus metabolism is essential for prompt and efficient development of tacrolimus and its proper usage under clinical conditions.

Although the clinical pharmacokinetics of tacrolimus have been reviewed repeatedly,3–5) there is no review focusing on the metabolism of tacrolimus. This review deals primarily with metabolism and metabolism-related issues, and includes some recent topics relating to
Metabolism of Tacrolimus

Tacrolimus was excreted from the body after receiving extensive metabolism. After i.v. injection of $^{14}$C-tacrolimus to rats, radioactivity was mainly excreted in the feces (95% of dose) and in the bile (82% of dose) in intact rats and in bile-duct cannulated rats, respectively. Only a very minor portion (less than 0.4% of dose) of unchanged tacrolimus was detected in the urine, bile and feces after i.v. and oral administration to rats. Radioactivity in the samples was analyzed by high performance liquid chromatography (HPLC). The elution profile of radioactivity in the urine, feces and bile was very complex and no major radioactive peaks were observed (Fig. 2). The mono-demethylated metabolite at the 13-methoxy group (M-I) was detected in these matrices but its proportion was very small. After the administration of $^{14}$C-tacrolimus to the rat, M-I was also detected in the blood but its concentration was low (less than 10% of total radioactivity). These results indicate the difficulty in isolation and identification of metabolites from animal matrices after tacrolimus administration, and primary and secondary metabolites were identified in the in vitro system consisting of liver microsomes and NADPH under aerobic conditions.

Tacrolimus was biotransformed to oxidized metabolites by liver microsomes. Three mono-demethylated metabolites, three di-demethylated metabolites, one mono-hydroxylated metabolite and one metabolite modified by multiple reactions were identified as shown in Fig. 1. M-I, M-II and M-III were the O-demethylated metabolites at the 13-, 31- and 15-methoxy group of tacrolimus, respectively, and M-IV was the mono-hydroxylated metabolite at the 12-position. The di-demethylated metabolites at the 15- and 31-, 13- and 31-, and 13- and 15-methoxy groups of tacrolimus, were respectively designated as M-V, M-VI and M-VII. M-
Fig. 2. Elution profile of radioactivity excreted in the urine, feces, and bile after i.v. and oral administration of 14C-tacrolimus (FK506) to rats. Dose was 1.0 mg/kg for both administrations. Each panel represents elution profile of radioactivity excreted to the urine (A) and feces (B) after oral dosing, and to the bile (C) after i.v. injection to rats. Radioactivity in the samples was analyzed by HPLC.

VIII was the metabolite produced after O-demethylation at the 31-methoxy group and formation of a fused 10-membered ring structure through the 19- to 22-carbon of the macrolide ring after oxidation of the 19-methyl group, and of the 36- and 37-vinyl group of tacrolimus.

**Metabolic pathway**

M-I formation (13-O-demethylation) was the major metabolic pathway of the first step of tacrolimus metabolism by liver microsomes fortified with NADPH under aerobic conditions (Fig. 3). Two other demethylation pathways at the 15- and 31-methoxy groups and a hydroxylation pathway at the 12-position were minor under the same conditions. Further demethylation pathways were detected, and M-VII (13- and 15-didemethylated metabolite) formation was the major metabolic pathway by human liver microsomes fortified with NADPH under aerobic conditions. Judging from the in vivo and in vitro results, tacrolimus received sequential metabolism through M-I, M-VII and further hydrophilic metabolites (Fig. 4).

**Immunocrossreactivity and pharmacological activity of identified metabolites**

Tacrolimus levels were determined by an EIA using monoclonal antibody against tacrolimus and the cross-reactivity of 8 identified metabolites was determined against the antibody. As shown in Table 1, reactivity to the antibody was almost equipotent between tacrolimus and the three metabolites, M-II, M-III, and M-V. M-I, M-IV, M-VI, M-VII, and M-VIII exhibited negligible or very weak cross-reactivity.

The pharmacological activity of tacrolimus and its metabolites were estimated using a mixed lymphocyte reaction system (Table 1). Among the metabolites, only M-II exhibited equipotent pharmacological activity to tacrolimus while the other metabolites exhibited negligible or very weak activity.

**Identification of metabolizing enzymes**

Tacrolimus was metabolized by animal and human liver microsomes in the system fortified with NADPH under aerobic conditions. The metabolism of tacrolimus was induced by treatment of phenobarbital and dexamethasone in the rat. The M-I formation from tacrolimus correlated with the 2β- and 6β-hydroxylation of testosterone by rat liver microsomes and with the 6β-hydroxylation by human liver microsomes. Tacrolimus was metabolized by the CYP3A subfamily including rat CYP3A2, human CYP3A4 and human CYP3A5. As shown in Table 2, the metabolism of tacrolimus and its major metabolite, M-I, were catalyzed by CYP3A4 and CYP3A5, and other human CYPs including the 1A, 2A, 2C, 2D and 2E subfamilies did not metabolize tacrolimus and M-I. CYP3A4 and CYP3A5 catalyzed the metabolism of tacrolimus (M-I formation and total metabolism) almost equally. M-I metabolism (M-VII formation and total metabolism) by CYP3A4 was higher than that by CYP3A5.

**Metabolic drug-drug interactions**

The effects of various drugs on the metabolism of tacrolimus by human liver microsomes were studied in the presence of an NADPH-generating system under aerobic conditions (Table 3). Drugs metabolized by CYP3A4/5 inhibited tacrolimus metabolism, with
Fig. 3. Time course of tacrolimus metabolism by pooled human liver microsomes. Elution pattern of authentic standards of tacrolimus and its metabolites is shown in panel A. Elution profile of radioactivity is depicted after incubation of $^{14}$C-tacrolimus with boiled liver microsomes for 30 min (B), and with intact microsomes for 2 min (C), 5 min (D), 10 min (E), 20 min (F), and 30 min (G).

Metabolism of Tacrolimus

Ketoconazole being the most potent. As reported previously, ketoconazole, cyclosporine A, diltiazem, erythromycin, and fluconazole were reported as the drugs that elicit clinically relevant drug interactions with tacrolimus. These results indicate the potential for metabolic interactions between tacrolimus and co-medicated drugs metabolized by CYP3A4/5.

Rifampicin decreased the blood levels of tacrolimus in kidney and liver transplant patients. Rifampicin treatment caused a decrease of tacrolimus blood levels in healthy volunteers when compared to pretreatment levels. Co-administration of rifampicin significantly increased tacrolimus clearance (36.0 vs. 52.8 mL h$^{-1}$ kg$^{-1}$) and decreased tacrolimus bioavailability (14.4 vs.
Assay

Due to very low blood concentrations of tacrolimus, mouse monoclonal antibody against tacrolimus was raised and a highly sensitive EIA was developed.\textsuperscript{17)\textdagger} Tacrolimus has a narrow therapeutic index, and its blood concentration should be maintained at an optimal range in transplanted patients for the following reasons.\textsuperscript{18)\textdagger} If the concentration is lower than the range, the transplanted organ will be rejected due to the insufficient immunosuppression by tacrolimus. If the concentration is higher than the range, adverse effects will be observed due to the excess concentration of tacrolimus.

### Table 1. Immunocrossreactivity and pharmacological activity of tacrolimus and its metabolites

<table>
<thead>
<tr>
<th>Tacrolimus and metabolites</th>
<th>Immunocrossreactivity (IC50, ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrolimus</td>
<td>100</td>
</tr>
<tr>
<td>M–I</td>
<td>nil</td>
</tr>
<tr>
<td>M–II</td>
<td>109.0</td>
</tr>
<tr>
<td>M–III</td>
<td>90.5</td>
</tr>
<tr>
<td>M–IV</td>
<td>8.8</td>
</tr>
<tr>
<td>M–V</td>
<td>92.2</td>
</tr>
<tr>
<td>M–VI</td>
<td>nil</td>
</tr>
<tr>
<td>M–VII</td>
<td>nil</td>
</tr>
<tr>
<td>M–VIII</td>
<td>nil</td>
</tr>
</tbody>
</table>

### Table 2. Metabolism of tacrolimus and M–I by expressed human CYPs

<table>
<thead>
<tr>
<th>CYP isoform</th>
<th>Tacrolimus metabolism (nmol/nmol CYP)</th>
<th>M–I metabolism (nmol/nmol CYP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M–I formation</td>
<td>Total metabolism</td>
</tr>
<tr>
<td>1A2</td>
<td>&lt;10</td>
<td>&lt;25</td>
</tr>
<tr>
<td>2A6</td>
<td>&lt;10</td>
<td>&lt;25</td>
</tr>
<tr>
<td>2B6</td>
<td>&lt;10</td>
<td>&lt;25</td>
</tr>
<tr>
<td>2C8</td>
<td>&lt;10</td>
<td>&lt;25</td>
</tr>
<tr>
<td>2C9</td>
<td>&lt;10</td>
<td>&lt;25</td>
</tr>
<tr>
<td>2D6</td>
<td>&lt;10</td>
<td>&lt;25</td>
</tr>
<tr>
<td>2E1</td>
<td>&lt;10</td>
<td>&lt;25</td>
</tr>
<tr>
<td>3A4</td>
<td>214</td>
<td>324</td>
</tr>
<tr>
<td>3A5</td>
<td>190</td>
<td>212</td>
</tr>
</tbody>
</table>

Concentrations of tacrolimus and M–I were both 10 μM. Incubation time was 30 min.

### Table 3. Effect of various drugs on tacrolimus metabolism by human liver microsomes

<table>
<thead>
<tr>
<th>Inhibition (%)</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;90</td>
<td>Ketoconazole\textsuperscript{*}</td>
</tr>
<tr>
<td>70–61</td>
<td>Cyclosporin A, nifedipine</td>
</tr>
<tr>
<td>50–41</td>
<td>Diltiazem, ethinyl estradiol, nivadipine</td>
</tr>
<tr>
<td>40–31</td>
<td>Astemizole, erythromycin, prednisolone, terfenadine</td>
</tr>
<tr>
<td>30–21</td>
<td>Fluconazole, rifampicin</td>
</tr>
<tr>
<td>20–10</td>
<td>Amphotericin B, cefixime, ciprofloxacin, licomycin, norethindrone, ofloxacin, omeprazole, quinidine, sulindac</td>
</tr>
<tr>
<td>&lt;10</td>
<td>Acyclivir, azulene, cefalexine, cefotaxime, chlorpheniramine, cimetidine, clemastine, cyroheptadine, diclofenac, enoxacin, etizolam, fosfomycin, gentamycin\textsuperscript{**}, homochlorcyclizine, hydroxyzine, indomethacin, kanamycin, ketotifen, loosprofen, minocycline, phenobarbital, phenylbutazon, promethazine</td>
</tr>
</tbody>
</table>

Underlined drugs: clinically relevant drug interactions with tacrolimus were reported.\textsuperscript{45} Concentrations of tacrolimus and drugs were 10 and 100 μM, respectively.

\textsuperscript{*}: Concentration of ketoconazole was 10 μM.
\textsuperscript{**}: Concentration of gentamycin was 60 μg/mL.
Fig. 5. Correlation between tacrolimus levels measured by LC/MS and EIAs in the whole blood from kidney transplant patients. Each panel represents the correlation between (A) LC/MS and ELISA, and (B) LC/MS and IMx.

As described above, the immunocross-reactivity of 8 in vitro metabolites of tacrolimus was estimated (Table I). Although the major metabolite, M-I, did not exhibit cross-reactivity against the monoclonal antibody used in the assay of tacrolimus levels, the reactivity of M-II, M-III and M-V was almost equipotent with tacrolimus. The whole blood levels of tacrolimus were measured by nonspecific EIAs including an enzyme-linked immunosorbent assay (ELISA) and IMx, and compared with those determined by specific HPLC with mass spectrometric detection (LC/MS). ELISA was used in the tacrolimus research and development, and IMx was used in TDM of tacrolimus. As shown in Fig. 5, the values measured by ELISA and IMx in the whole blood from kidney transplant patients dosed with tacrolimus were correlated well with those determined by specific LC/MS method. The slopes of regression lines between ELISA and LC/MS and between IMx and LC/MS were respectively close to unity and the intercepts of the lines nearly passed through the origin of the coordinate. These results indicate that whole blood levels of tacrolimus measured by EIAs reflect those of the unchanged tacrolimus in kidney transplant patients.

Recent topics in clinical pharmacokinetics

Tacrolimus has a narrow therapeutic range, and its blood concentrations should be maintained at an optimal range in transplanted patients to prevent the rejection of transplanted organs due to insufficient concentrations of tacrolimus or the toxic events due to its excess concentrations.

Tacrolimus is a substrate of CYP3A4, CYP3A5 and P-glycoprotein (PGP, MDR1). Recently, a lot of attention has been focused on the relationship between clinical pharmacokinetics of tacrolimus and CYP3A5 genetic polymorphisms. Dose normalized blood concentrations of tacrolimus were correlated with the expression of the CYP3A5 genotype in kidney, heart, lung and liver transplanted patients. The concentrations (trough levels) in kidney, heart and lung transplanted patients after tacrolimus administration were lower in patients who expressed with CYP3A5*1/*1 and CYP3A5*1/*3 than in those with CYP3A5*3/*3. In the case of living-donor liver transplantation (LDLT), the expression levels of recipient PGP were a good predictor of dose requirement during the first week following transplantation, and subsequently, those of a homozygous CYP3A5*1 genotype from the transplanted liver required a higher tacrolimus dose than those of a homozygous or heterozygous genotype for CYP3A5*3. After oral dosing of 1 mg tacrolimus to healthy subjects, area under the concentration-time curve (AUC0-infinity) values were significantly higher in CYP3A5*3/*3 subjects (323.8 ng h mL−1) than in CYP3A5*1/*1 and CYP3A5*1/*3 subjects (131.5 ng h mL−1). Maximal blood concentration (Cmax) values were also higher in the former group (24.4 ng mL−1) than in the latter group (11.8 ng mL−1), but the time required to reach Cmax (tmax) and half-life (t1/2) values were not significantly different between the two groups. There was no significant difference in tacrolimus pharmacokinetics (AUC0-infinity, Cmax, tmax and t1/2) between the subjects with different MDR1 polymorphisms. These results indicate that CYP3A5 plays an important role in the metabolic clearance of tacrolimus in man, and the determination of the CYP3A5 genotypes of the recipients in organ transplantation and the donors in LDLT provides very useful information for selecting the optimal dosage of tacrolimus.

Conclusions

For the lipophilic drugs with high metabolic clearance and a narrow therapeutic window, metabolism studies such as the identification of metabolites and metabolizing enzymes, and drug-drug interactions, are essential to provide information for the optimal regimen of the drugs. In this review, the importance of metabolism stu-
Acknowledgments: This review was written as a memo-
devlopment stage and in clinical use.

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