Regular Article

Association between CYP3A5 Genotypes in Graft Liver and Increase in Tacrolimus Biotransformation from Steroid Treatment in Living-donor Liver Transplant Patients

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Summary: We retrospectively examined whether cytochrome P450 (CYP) 3A5 genotypes are associated with high-dose steroid pulse treatment-induced functional gain of tacrolimus biotransformation in living-donor liver transplant patients. Concentrations of tacrolimus and its 3 primary metabolites, 13-O-demethyl tacrolimus (M-I), 31-O-demethyl tacrolimus (M-II), and 15-O-demethyl tacrolimus (M-III), were measured in trough blood samples from 18 liver transplant patients, by liquid chromatography–tandem mass spectrometry/mass spectrometry (LC-MS/MS). In patients engrafted with a CYP3A5*1-carrying liver but not with a CYP3A5*3/3-carrying liver, the concentration/dose ratio of tacrolimus significantly fell after therapy, while ratios of M-I/tacrolimus, M-II/tacrolimus, and M-III/tacrolimus were significantly higher after therapy than before (p = 0.032, p = 0.023, and p = 0.0078, respectively). After steroid pulse therapy, the concentration of tacrolimus measured by immunoassay was significantly higher than that measured by LC-MS/MS in patients engrafted with a CYP3A5*1-carrying liver, but not those engrafted with a CYP3A5*3/3-carrying liver. This suggests that the increased ratio of tacrolimus metabolites/tacrolimus can be explained by induction of CYP3A5 via high-dose steroid pulse therapy. Further, the concentrations of tacrolimus measured by the immunoassays were overestimated, partly because of cross-reactivity of the monoclonal antibody they incorporated to detect tacrolimus, with the increased metabolites in patients with a CYP3A5*1-carrying graft liver.

Keywords: metabolite; tacrolimus; induction; overestimation; transplantation

Introduction

Tacrolimus is widely used as the primary immunosuppressant in patients undergoing solid organ transplantation. The therapeutic range of tacrolimus for liver transplantation is between 5 and 15 ng/mL, but the blood concentration measured during the development of acute cellular rejection has been shown to vary between patients.1) Patients diagnosed with acute cellular rejection receive high-dose steroid pulse therapy (usually for 3 days at 10 mg·kg−1·day−1) to suppress immune reactions.2) We previously reported that enterocyte expression of CYP3A4, but not of MDR1, was markedly enhanced by high-dose steroid pulse treatment in 3 small-bowel transplant recipients.3) However, it remains unknown whether high-dose steroid pulse treatment affects biotransformation of tacrolimus, because general and indirect analytical methods such as the microparticle enzyme-linked immunoassay (MEIA; IMx® system by Abbott, Tokyo, Japan) and the chemiluminescent immunoassay (CLIA; ARCHITECT® system by Abbott) cannot reliably distinguish between tacrolimus and its metabolites.4, 5)

Tacrolimus is metabolized mainly by cytochrome P450 (CYP) 3A4/5 to 3 primary metabolites, namely 13-O-demethyl tacrolimus (M-I), 31-O-demethyl tacrolimus (M-II), and 15-O-demethyl tacrolimus (M-III) (Fig. 1) in the small intestine and liver.6-1) M-I is the major metabolite in human, dog, and rat liver microsomes,9) and anti-tacrolimus monoclonal antibody exhibits negligible cross-reactivity. In contrast, the reactivity of M-II (109%)...
and M-III (90.5%) to a monoclonal antibody against tacrolimus was shown to be comparable to that of the unchanged form. Data from a mixed lymphocyte reaction system indicate that M-II exhibits pharmacological activity (IC_{50}, 0.23 nM) comparable to that of the unchanged form (IC_{50}, 0.15 nM), while M-III has negligible immunosuppressive activity (IC_{50}, >127 nM). Therefore, the presence of M-III in whole blood may lead to an overestimation of immunosuppressive activity when measured using indirect immunosassays.

The purpose of the present study was to clarify whether high-dose steroid pulse treatment affects tacrolimus biotransformation in living-donor liver transplant (LDLT) patients. We analyzed blood concentrations of tacrolimus and its 3 primary metabolites, M-I, M-II, and M-III, by using the liquid chromatography–tandem mass spectrometry (LC-MS/MS) method, and examined the specificity of 3 indirect immunosassays for blood tacrolimus level in comparison with LC-MS/MS analysis.

**Materials and Methods**

**Materials:** Analytical-grade tacrolimus, 13-0-demethyl tacrolimus (M-I), 31-0-demethyl tacrolimus (M-II), and 15-0-demethyl tacrolimus (M-III) were kindly provided by Astellas Pharma Inc. (Tokyo, Japan). Sirolimus (rapamycin) was purchased from LC Laboratories (Woburn, MA) and used as an internal standard. All other chemicals were of the highest purity available.

**Patients:** Eighteen patients who developed acute cellular rejection within 30 days after they underwent an LDLT procedure at Kyoto University Hospital between November 2001 and August 2002 were enrolled after obtaining written informed consent from them. Among the 18 patients, there were no patients treated concomitantly with inducers or inhibitors of CYP3As or P-glycoprotein, and no patients showing abnormal levels of serum creatinine, urea nitrogen, serum albumin, total protein, blood flow, aspartate aminotransferase (AST), alanine aminotransferase (ALT), or total bilirubin. Data were collected retrospectively by a review of routine therapeutic drug monitoring (TDM) data before and after steroid pulse therapy. This study was conducted in accordance with the Declaration of Helsinki and its amendments, and was approved by the Kyoto University Graduate School and Faculty of the Medicine Ethics Committee.

**Tacrolimus dosing regimen and criteria for acute cellular rejection:** Blood group matching for the ABO system was identical in 9 patients, compatible in 6, and incompatible in 3. In the case of incompatible matching, pre-transplant blood exchange or plasmapheresis was done to reduce the antibody titer before LDLT. The basic immunosuppression regimen consisted of tacrolimus (Prograf®, Astellas Pharma Inc.) with low-dose steroids. Blood samples were collected once a day in the morning before the next administration of tacrolimus. Tacrolimus was administered orally at a dose of 0.075 mg/kg every 12 h after the evening of postoperative day 1. The target for post-transplantation whole-blood trough concentration of tacrolimus was set between 10 and 15 ng/mL during the first 2 weeks. Steroid treatment was initiated at graft reperfusion at a dose of 10 mg/kg, with a gradual reduction from 2 mg·kg⁻¹·day⁻¹ to 0.3 mg·kg⁻¹·day⁻¹ during the first 2 weeks after surgery. The tacrolimus dosage was adjusted on the basis of whole-blood trough concentrations measured approximately 12 h after the evening dosage every day, by using a semiautomated MEIA method (IMx® system; Abbott). A target whole-blood trough concentration of tacrolimus was set between 10 and 15 ng/mL during the first 2 weeks after surgery. The tacrolimus dosage was adjusted on the basis of whole-blood trough concentrations measured approximately 12 h after the evening dosage every day, by using a semiautomated MEIA method (IMx® system; Abbott).

Acute cellular rejection was defined by re-elevation of AST and ALT levels, and diagnosed by histological evaluation of liver biopsy specimens according to criteria based on the Banff schema. Patients diagnosed with acute cellular rejection received high-dose intravenous methylprednisolone or corticosterone at 10 mg·kg⁻¹·day⁻¹ for 3 days, followed by 5 mg·kg⁻¹·day⁻¹, which was then quickly tapered off over the following days in addition to an increased dose of tacrolimus.

**Measurement of tacrolimus metabolites:** After measuring concentrations of tacrolimus, M-I, M-II, and M-III by LC-MS/MS using surplus blood samples from all patients after routine monitoring, we retrospectively analyzed data for patients with acute cellular rejection. Briefly, all whole blood samples (150 µL) were transferred to glass tubes and spiked with 25 µL of sirolimus (100 ng/mL), which served as the internal standard. Then, 600 µL of water and 2 mL of extraction solution (methyl-i-butyl ether/cyclohexane, 1:3 v/v) were added to the glass tubes. Each tube was capped securely, mixed on a horizontal shaker for 30 min, and centrifuged at 3,000 rpm for 10 min. The organic layer was transferred to a new tube and evaporated using an Automatic Environmental Speed Vac® System (Thermo Fisher Scientific Inc., Waltham, MA). Each sample was reconstituted with 150 µL of the mobile phase and vortexed for 1 min. A 20-µL aliquot of each sample was injected into the LC-MS/MS system. Briefly, the system comprised 2 pumps, an analytical column (Inertsil-ODS3, 150 × 2.1 mm i.d.; GL Sciences, Inc., Tokyo, Japan), and an MS/MS detector (API4000 System, Applied Biosystems by Life Technologies, CA). The mobile phase consisted of a multiple gradient of solvent A (methanol/1 mM ammonium acetate) and solvent B (1 mM ammonium acetate). The flow rate was set at 250
Increased Tacrolimus Metabolism by CYP3A5 Rather than CYP3A4

µL/min, and the eluent was introduced directly into the electrospray ion source of the mass spectrometer. Selected reaction monitoring transitions in the positive ion mode were m/z 821 → m/z 768 for tacrolimus, m/z 807 → m/z 722 for M-I, m/z 807 → m/z 754 for M-II, m/z 807 → m/z 754 for M-III, and m/z 931 → m/z 864 for sirolimus. Tacrolimus and its metabolites were detected as ammonium adducts (m+ NH₄). Peak areas were linear from 0.5 to 60 ng/mL for tacrolimus and its metabolites.¹⁵

For examination of the characteristics of assay methods for measuring blood tacrolimus, two immunoassays, the CLIA, and the antibody-conjugated magnetic immunoassay (ACMIA; Dimension® system by Siemens, Tokyo, Japan) were compared to the LC-MS/MS method (n = 5 replicates), with 10 ng/mL of each metabolite spiked into whole blood samples with or without a target background concentration of 10 ng/mL tacrolimus. The Architect and Dimension assay acceptance criteria were followed per the manufacturer’s instructions.

**Identification of CYP3A5 genetic variants:** Genomic DNA was extracted from the peripheral blood of living donors with a Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison, WI). The liver is the main eliminatory organ for tacrolimus, and steroid pulses are given intravenously; therefore, the graft liver is considered more likely to induce CYP3A5 expression than the intestine. Because the CYP3A4*1B, CYP3A5*6, and CYP3A5*7 alleles have not been detected in Japanese or other Asian ethnic groups,¹⁶-¹⁹ we focused on the more frequent CYP3A5*3 allele to examine interindividual variation of tacrolimus pharmacokinetics in patients after liver transplantation. Accordingly, the patients were divided into 2 groups based on graft liver CYP3A5 genotype; group *1, CYP3A5*1/*1 or CYP3A5*1/*3, and group *3, CYP3A5*3/*3. The CYP3A5*3 polymorphic variant was detected by PCR-restriction fragment length polymorphism (RFLP).¹⁶,²⁰,²¹

**Data collection and analysis:** We defined at least 3 days prior to high-dose steroid pulse therapy as the event-free condition, which ranged from postoperative day 0 to 18. Then, we retrospectively collected data at least 3 days prior to and during high-dose steroid pulse therapy. Comparisons of findings before and after steroid pulse therapy were not performed in 1 case (patient age, <15 years, *1 group) in which data during the event-free period were unavailable. However, his blood tacrolimus concentration data were included in the analysis of comparison between MEIA and LC-MS/MS. The metabolite/tacrolimus ratio between the 2 groups was compared using the Mann–Whitney U-test. We used the Wilcoxon signed-rank test to compare the median concentration ratios of metabolite/tacrolimus before and after steroid pulse therapy. Data are expressed as the median and range, or as mean ± SD, depending on the data type. For all analyses, a two-tailed p value of <0.05 was considered statistically significant. All statistical analyses were conducted using GraphPad PRISM, version 4 (GraphPad Software, San Diego, CA).

**Results**

**Patient characteristics:** The median patient age was 13.1 years (range, 0.5–62 years) and female patients comprised 55.6% of the study population (Table 1). Before acute cellular rejection was diagnosed, the mean duration of event-free conditions was 4.8 ± 1.5 days. The mean duration of treatment with high-dose steroid pulse therapy for acute cellular rejection was 5.2 ± 1.1 days. The median amount of total glucocorticoids administered was 120.8 mg (40–750 mg), in accordance with the standard administration of 10 mg·kg⁻¹·day⁻¹.

**Tacrolimus concentration determined by immunoassay:** Figure 2 shows the concentration/dose (C/D) ratio of tacrolimus under event-free conditions using the data obtained by MEIA. The median C/D ratio of tacrolimus in the *1 group was 227.6 (ng/mL)/(mg·kg⁻¹·day⁻¹) (range, 79.7–439.7 [ng/mL]/[mg·kg⁻¹·day⁻¹]), which was significantly higher than that of the *3 group, which was 82.8 (ng/mL)/(mg·kg⁻¹·day⁻¹) (range, 61.1–271.2 [ng/mL]/[mg·kg⁻¹·day⁻¹]) (p = 0.020) (Fig. 2A). We evaluated the influence of graft-to-recipient body weight ratio (GRWR) on the C/D ratio of tacrolimus. Under event-free conditions, the C/D ratio of tacrolimus in the low-GRWR group (defined as <1.5%, mainly comprised adult patients [age, >15 years]) was higher than that of the high-GRWR group (defined as >1.5%, mainly comprised children [age, <15 years]) (Fig. 2B). This difference was because of the larger proportion of adult patients in the *1 group (6 of 10 patients) than in the *3 group (2 of 8 patients), and the GRWR in adult patients (*1 group, 1.09 ± 0.14; *3 group, 0.95) was higher than that in pediatric patients (*1 group, 1.45 ± 0.43; *3 group, 2.71 ± 0.90) (Fig. 2A). This is consistent with the higher C/D ratio values of tacrolimus and lower GRWR in the *1 group (Fig. 2B). This is also consistent with previous reports that the C/D ratio of tacrolimus in patients with a low GRWR was higher than those with a high GRWR, when the graft liver CYP3A5 genotype was the same.²⁰ Although the C/D ratio of tacrolimus in group *1 was reduced by 27.1% after steroid pulse treatment compared to that under the event-free conditions (p = 0.0046, n = 10), in group *3 there was no significant difference between the ratio under the event-free conditions and the ratio after steroid pulse treatment (p = 0.42, n = 8) (Figs. 2C, 2D, and 2E).

**Influence of steroid pulse treatment on blood concentration ratios of metabolite/tacrolimus:** Next, we examined the influence of high-dose steroid pulse treatment on blood concentration ratios of metabolite/tacrolimus in LDLT patients (Fig. 3). In patients with a CYP3A4*1A-carrying graft liver, M-I/tacrolimus, M-II/tacrolimus, and M-III/tacrolimus ratios were significantly higher after high-dose steroid pulse treatment than before, regard-

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**Table 1. Patient and donor characteristics**

<table>
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<tr>
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<th>Patient</th>
<th>Donor</th>
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<tr>
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</tr>
<tr>
<td>Age, years</td>
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<tr>
<td></td>
<td>0.5–62</td>
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<td>Body weight, kg</td>
<td>Range</td>
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<td>6.7–76.4</td>
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<td>Primary biliary cirrhosis</td>
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<tr>
<td></td>
<td>Byler disease</td>
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<tr>
<td></td>
<td>Fulminant hepatic failure</td>
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</table>
less of the age of the patients ($p = 0.032, p = 0.023$, and $p = 0.0078$, respectively) (Figs. 3A, 3B, and 3C). In contrast, high-dose steroid pulse therapy did not increase the ratio of metabolites/tacrolimus in patients with a CYP3A5*1/*1 or *1/*3-carrying graft liver.

Comparison of measurement by immunoassay and LC-MS/MS: To investigate whether tacrolimus concentration after steroid pulse treatment measured by MEIA was overestimated, we compared the measurements determined by the MEIA and LC-MS/MS methods (Fig. 4). Under event-free conditions, there was no significant difference between the tacrolimus concentrations determined by MEIA and those determined by LC-MS/MS, regardless of the CYP3A4 genotype of the graft liver (Figs. 4A and 4C). After steroid pulse treatment, however, tacrolimus concentration determined by MEIA significantly exceeded the other measurement in patients with a CYP3A4*1-carrying graft liver ($p = 0.0078$) (Fig. 4B). In contrast, there was no significant difference between the tacrolimus concentrations measured by MEIA and those measured by LC-MS/MS in patients with a CYP3A4*3/*3-carrying graft liver (Fig. 4D).

Characteristics of assay methods for tacrolimus: Because the MEIA assay system for tacrolimus was discontinued at the end of 2009, the cross reactivity of the anti-tacrolimus antibody was examined with CLIA, ACMIA, and LC-MS/MS systems with or
without spiking the samples with M-I, M-II, or M-III metabolite. As shown in Table 2, the data obtained for tacrolimus alone, which contained the unchanged form only, was similar, whether derived from the CLIA method (9.2 ± 0.3 ng/mL; Architect® system by Abbott) or the LC-MS/MS method (9.7 ± 0.7 ng/mL) (mean ± SD of 5 measurements). Because the antibody against tacrolimus used in the CLIA method cross-reacts with M-II and M-III, the measurement of a spiked sample containing 10 ng/mL of each metabolite, M-I, M-II, and M-III, was 22.1 ± 1.1 ng/mL (mean ± SD of 5 measurements) using this method. However, measurements derived from the same sample were 9.6 ± 0.9 ng/mL as determined by the ACMIA method, and 10.7 ± 0.8 ng/mL for CLIA and 10.1 ± 1.1 ng/mL for ACMIA (SD of 5 measurements).

**Table 2. Cross-reactivities of immunoassays (CLIA and ACMIA) among unchanged tacrolimus, M-I, M-II, and M-III, compared to data obtained by LC-MS/MS**

<table>
<thead>
<tr>
<th>Number</th>
<th>Prepared spiked samples</th>
<th>CLIA</th>
<th>ACMIA</th>
<th>LC-MS/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tacrolimus M-I (ng/mL)</td>
<td>M-II</td>
<td>M-III</td>
<td>Tacrolimus (ng/mL)</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
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<td>4</td>
<td>0</td>
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<td>0</td>
<td>10</td>
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<td>10</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
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</table>

Measurements obtained by CLIA and ACMIA were compared to those obtained using the LC-MS/MS method (5 replicates), with 10 ng/mL of each metabolite spiked into whole blood samples with or without a target background concentration of 10 ng/mL of tacrolimus. The Architect® and Dimension® assay acceptance criteria were followed per the manufacturer’s instructions. Data are expressed as mean ± SD of 5 measurements. N.D., not detected (or under the lower detection limits; 0.5 ng/mL for CLIA and LC-MS/MS, and 1.5 ng/mL for ACMIA).

**Discussion**

In clinical settings, immunoassays such as MEIA and CLIA are routinely used to examine tacrolimus blood concentrations, but tacrolimus metabolites with similar structures can cross-react with the primary antibody used to identify the parent drug, thus interfering with parent drug measurement.2–8 In the literature, compared to LC-MS/MS, immunoassays have been reported to overestimate the concentration of tacrolimus.22 To date, no findings describing the effects of high-dose steroid pulse therapy on the overestimation of tacrolimus by immunoassays have been reported. In this study, several interesting observations were made. Although there was no significant potential bias in the immunoassay under event-free conditions, high-dose steroid pulse therapy affected tacrolimus biotransformation, which led to a significant positive bias in the results of the immunoassays in LDLT patients. This overestimation was observed in patients with a CYP3A5*1/*1 or *1/*3-carrying graft liver, but not in those with a CYP3A5*3/*3-carrying graft liver. On the basis of these findings, we expect that donor CYP3A5 genotype will be a useful tool for predicting overestimation of tacrolimus concentration after high-dose steroid pulse therapy.

The direct association between acute cellular rejection and patient mortality rate is weak,23 but it can be a trigger for other severe complications such as infections, drug-induced renal injury, neurotoxicity, malignancy, and recurrence of hepatitis with viral amplification in patients receiving antirejection treatment.24–26 Therefore, it is important to control the concentration of tacrolimus, especially after acute cellular rejection. In our study, steroid pulse therapy significantly decreased the tacrolimus C/D ratio in the *1 group, but not in the *3 group. In addition, we found significantly increased metabolites of tacrolimus in the *1 group, but not the *3 group, suggesting that tacrolimus could be increasingly metabolized via hepatic CYP3A5. Because the reduction in the blood concentration of tacrolimus after steroid-pulse therapy is a consequence of the induction of CYP3A in the liver,27 it is possible that steroid-pulse therapy could induce hepatic CYP3A5 rather than CYP3A4 in LDLT patients, and that whether this induction occurs or not depends on the hepatic CYP3A5 genotype.

The increased metabolism caused an overestimation of tacrolimus levels by immunoassay in the *1 group, leading to reduced doses of tacrolimus in an effort to avoid further complications. Because the tacrolimus antibody is the same in both the MEIA (IMx®) and CLIA (Architect®) systems, similar phenomena would be likely to be observed in patients whose tacrolimus blood concentrations are monitored by the Architect® system (Table 2). However, data yielded by the ACMIA system revealed that the antibody in that system is more specific for the unchanged form of tacrolimus than the antibody used in the MEIA and CLIA systems. This finding is consistent with a report that tacrolimus concentration measured by ACMIA was lower than that obtained by MEIA, especially in patients with lower hematocrit values.28,29 The hematocrit values of liver transplant patients are often low, and this is usually controlled by transfusion with red blood cells. Since we usually measure the blood level of tacrolimus with the CLIA (Architect®) system in practice, we did not optimize our ACMIA (Dimension®) system; relatively low levels were observed in our ACMIA system. Taken together, the blood concentration of tacrolimus measured with the CLIA method was shown to include measurements of M-II and M-III caused by the cross-reactivity of the primary antibody against tacrolimus.

Among the 3 primary metabolites, the reactivity of the antitacrolimus antibody against M-II is similar to that against tacrolimus, and its reactivity against M-III is approximately 40% of that to tacrolimus; however, the antibody exhibits almost no reactivity to M-I.11 Therefore, the presence of M-II and M-III could cause an overestimation of the tacrolimus concentration in immunoassays such as MEIA and CLIA. Indeed, these metabolites represent only a small fraction of the total concentration, but the therapeutic index of tacrolimus for liver transplantation is narrow. On the other hand, the underestimation of blood concentration of pharmacologically active tacrolimus measured by ACMIA may be increased in the patients grafted with CYP3A4*1 carrying liver.

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under the condition of steroid pulse therapy. Therefore, the therapeutic window of tacrolimus measured by MEIA and CLIA should be largely different with that by ACMIA. Moreover, the target concentration of tacrolimus measured by ACMIA method would show large variation between the patients grafted CYP3A5*1 carrying liver and CYP3A5*3/*3 carrying liver, especially after the steroid pulse therapy. Taken together, both the overestimation of unchanged form tacrolimus concentration by the MEIA and CLIA methods and the underestimation of pharmacologically active tacrolimus concentration by the ACMIA method are important clinical problems to be overcome for standardization of tacrolimus therapy in the setting of the therapeutic window.

Methylprednisolone or corticosteroids increase tacrolimus clearance, probably by induction of CYP3A enzymes.30) The expression of CYP3A4 is regulated by the pregnane X receptor (PXR, also known as the steroid and xenobiotic receptor, or SXR), which binds to many steroids, their metabolites, and xenobiotics.31 CYP3A5 mRNA is also elevated by treatment with PXR ligand, and reduced by transfection with small interference (si) RNA specific for CYP3A5.34,35) Burk et al.36) showed that both PXR and constitutively activated receptor (CAR) activate transcription of CYP3A5 in the human liver and intestine. Interestingly, PXR-mediated induction of CYP3A5 was observed in liver samples carrying the CYP3A5*1 allele,36) suggesting that the PXR-mediated expression of CYP3A5 induced by steroids might be related to its genotype.

In the present study, we focused on the CYP3A45 genotype in graft liver. This is because steroid pulse treatment is administered intravenously, and thus the steroid-induced expressional change of CYP3A4/5 primarily occurs in the liver rather than the small intestine. Furthermore, we previously reported that induction of CYP3A4 in graft intestine because steroid pulse therapy occurred without pharmacokinetic changes in orally administered tacrolimus, suggesting a lesser significance of intestinal CYP3A4 compared to hepatic CYP3A4 on tacrolimus pharmacokinetics.3) Therefore, the expressional change of hepatic CYP3A5 could have a greater effect on tacrolimus pharmacokinetics compared to CYP3A4, especially after steroid pulse therapy. In the present study, we found that LDLT patients were characterized by 2 groups: one group in which patients showed a reduced C/D ratio of tacrolimus after steroid-pulse therapy, and the other in which patients showed no significant change in this ratio after the therapy (Figs. 2D and 2E). Although steroid pulse therapy did not significantly affect the C/D ratio of tacrolimus in all patients (Fig. 2C), stratified by graft liver CYP3A45 genotype, there was significant decrease in the C/D ratio of tacrolimus in patients with a CYP3A5*1/*1 or *1/*5-carrying liver, but not those with a CYP3A5*3/*3-carrying liver. These results suggest that steroid-pulse therapy could induce the enzymatic function of CYP3A5 rather than CYP3A4. In line with this finding, the metabolite/tacrolimus ratio after high-dose steroid pulse therapy in patients with a CYP3A5*1/*1 or *1/*3-carrying graft liver was significantly higher than that before therapy, suggesting that M-I, M-II, and M-III are largely generated by CYP3A5. In patients with a CYP3A5*3/*3-carrying graft liver (CYP3A5 non-expressers), there was no significant difference in the metabolite/tacrolimus ratio before and after therapy, suggesting that high-dose steroid pulse treatment had almost no influence on increased metabolism via CYP3A4. Thus, the increased ratio of metabolites/tacrolimus can be explained by the induction of CYP3A5 function. This is the first report suggesting an association between the influence of CYP3A activity on tacrolimus metabolism and CYP3A45 genotypes after liver transplantation.

Our study must be interpreted within the context of its potential limitations. First, our sample size was too small to create a general linear model with co-variate analysis to assess the potential influence of confounding factors, and therefore, further work with a larger sample size is needed. Second, we could not estimate the tacrolimus plasma concentration curves under various conditions because it is clinically difficult to obtain multiple blood samples from pediatric patients undergoing steroid pulse treatment after acute cellular rejection. Lastly, we did not assess steroid pharmacokinetic measurements in individual subjects, because these can vary substantially in liver transplant patients.

In conclusion, we observed an increased ratio of metabolites/tacrolimus that can be explained by the induction of CYP3A5 via high-dose steroid pulse therapy. Further, the concentrations of tacrolimus as determined by the CLIA and MEIA immunoassays were underestimated, partly because of cross-reactivity of the monoclonal anti-tacrolimus antibody utilized by these methods with the increased tacrolimus metabolites in patients with a CYP3A5*1-carrying graft liver. Therefore, graft liver CYP3A5 genotyping might be useful for predicting potential bias in tacrolimus concentrations determined by the CLIA and MEIA methods after high-dose steroid pulse therapy in liver transplant patients.

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References
Increased Tacrolimus Metabolism by CYP3A5 Rather than CYP3A4


