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Impact of CYP3A5 genetic polymorphism on cross-reactivity in tacrolimus chemiluminescent immunoassay in kidney transplant recipients

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ABSTRACT

Background: Tacrolimus immunoassays possess cross-reactivity with metabolites in the blood. The aim of this study was to evaluate the cross-reactivity in tacrolimus chemiluminescent immunoassay (CLIA) in kidney transplant recipients and to characterize the cross-reactivity according to CYP3A5 genetic polymorphism.
Methods: The subjects were 50 kidney transplant recipients receiving low-dose tacrolimus. Blood levels of tacrolimus at 12 h (C_{12}) measured by CLIA were compared with that by LC–MS/MS using Bland–Altman analysis. The influence of CYP3A5 genotypes on the cross-reactivity in tacrolimus CLIA was evaluated by interaction plots.
Results: No significant difference was observed in tacrolimus C_{12} between the *CYP3A5*1/*3* and *CYP3A5*3/*3* genotypes. The dose-normalized C_{12} of tacrolimus was significantly higher in the *CYP3A5*3/*3* genotype than in the *CYP3A5*1/*3* genotype. The C_{12} ratio of 13-*O*-demethylate to tacrolimus was significantly lower in the *CYP3A5*3/*3* genotype than in the *CYP3A5*1/*3* genotype. Tacrolimus C_{12} measured by CLIA was 35% higher than that by LC–MS/MS. A higher cross-reactivity was observed in the patients with a tacrolimus C_{12} of less than 3 μg/l and *CYP3A5*1/*3* genotype.
Conclusion: This study confirmed the cross-reactivity in CLIA in kidney transplant recipients receiving low-dose tacrolimus. High metabolic capacity associated with the *CYP3A5*1* genotype affected the cross-reactivity in patients with low tacrolimus levels.

1. Introduction

Tacrolimus, a calcineurin inhibitor, is more effective than cyclosporine at improving allograft survival and preventing acute rejection at 1 year [1]. However, at currently recommended doses, tacrolimus shares several adverse reactions with cyclosporine, including nephrotoxicity, neurotoxicity, and infectious complications [2,3]. Recently, the Efficacy Limiting Toxicity Elimination (ELITE)-Symphony study demonstrated that low-dose tacrolimus is superior to other immunosuppressive regimens with respect to renal function, allograft survival, and acute rejection rates in kidney transplant recipients [4]. The 2007 European Consensus Conference on Tacrolimus Optimization recommends that the lower limit of quantification of the monitoring method for tacrolimus should be less than 1 μg/l, especially in patients with blood tacrolimus levels of 3 μg/l or less [5].

Tacrolimus is extensively metabolized in the liver by the cytochrome P450 (CYP) 3A subfamily, with little excretion of the unchanged drug in the urine, bile, or feces [6]. The primary metabolites have been identified as 13-*O*-, 31-*O*-, and 15-*O*-demethylates in human liver microsomes [7]. These metabolites account for most of the metabolic clearance of tacrolimus. Among the metabolites, 13-*O*-demethylate was detectable in the blood of liver transplant recipients [6]. CYP3A5 is the most important genetic contributor to interindividual differences in CYP3A-dependent drug clearance [8]. The *CYP3A5*3/*3* genotype requires less tacrolimus to reach target blood levels compared with *CYP3A5*1* allele carriers [9–11]. The homozygotes for *CYP3A5*3* alleles result in the almost complete absence of the CYP3A5 protein, and at least one **1* allele contributes to the CYP3A5 expression.

Routine therapeutic drug monitoring of tacrolimus is an integral part of the clinical management because of intra- and inter-patient pharmacokinetic variability and a narrow target range [12]. Recently, several immunoassays for measuring tacrolimus in whole blood have been introduced in clinical practice. Tacrolimus chemiluminescent immunoassay (CLIA) meets the 1 μg/l or less recommendation [5]. Tacrolimus CLIA is superior to microparticle enzyme immunoassay in measuring lower levels of tacrolimus [13] and does not suffer from the interferences seen with antibody-conjugated magnetic immunoassay

Abbreviations: CYP, cytochrome P450; CLIA, chemiluminescent immunoassay; C_{12}, blood level at 12 h; LC–MS/MS, liquid chromatography–tandem mass spectrometry.

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in some patients [14]. CLIA is useful for the measurement of tacrolimus in organ transplant patients treated with ordinary dosages of tacrolimus. However, tacrolimus CLIA tends to overestimate actual blood levels due to cross-reaction with metabolites [15].

To date, a growing number of reports have demonstrated that CYP3A5 genetic polymorphism is associated with the interindividual pharmacokinetic variation of tacrolimus in organ transplant recipients [9–11], however, its influence on the cross-reactivity of tacrolimus CLIA remains to be clarified in clinical settings. The aim of this study was to evaluate the cross-reactivity in tacrolimus CLIA in kidney transplant recipients receiving low-dose tacrolimus. In addition, we characterized the cross-reactivity in tacrolimus CLIA according to the CYP3A5 genotype and the blood levels of tacrolimus.

2. Materials and methods

2.1. Ethics

The study was performed in accordance with the Declaration of Helsinki and its amendments, and the protocol was approved by the Ethics Committee of Hamamatsu University Hospital. The patients were briefed about the scientific aim of the study and each patient provided written informed consent.

2.2. Subjects

Fifty kidney transplant recipients receiving tacrolimus (Prograf® capsule, Astellas Pharma, Tokyo, Japan) twice daily at Hamamatsu University Hospital were enrolled. All patients were treated with an immunosuppressive regimen consisting of prednisolone and antimetabolite for more than 6 months. Tacrolimus administration was started on the day of transplantation and then adjusted to blood levels at 12 h (C_{12}) of 10–15 μg/l during the first month, 7–10 μg/l during the second month, and thereafter 5–7 μg/l until the sixth month. The target C_{12} of tacrolimus in the study population was less than 5 μg/l. Exclusion criteria were as follows: (1) patients on hemodialysis or peritoneal dialysis; (2) patients who were being co-treated with triazole antifungal agents or rifampin; and (3) patients with poor compliance with respect to their medications. Blood specimens were drawn into tubes containing ethylenediaminetetraacetic acid disodium salt at 12 h after the evening dosing.

2.3. Determination of blood levels of tacrolimus and its demethylates using LC–MS/MS

Tacrolimus and 13-*O*- and 31-*O*-demethylates were kindly donated by Astellas Pharma. All other reagents were of analytical grade and commercially available. Blood levels of tacrolimus and 13-*O*- and 31-*O*-demethylates were determined by isocratic LC–MS/MS [15]. The calibration ranges were 0.5–20 μg/l for tacrolimus and 0.1–5 μg/l for 13-*O*- and 31-*O*-demethylates in human blood. The intra- and interassay accuracies were 94–109% and 99–108% for tacrolimus, 98–109% and 99–107% for 13-*O*-demethylate, and 97–107% and 95–108% for 31-*O*-demethylate, respectively. The intra- and interassay imprecisions were 7–12% and 7–9% for tacrolimus, 5–13% and 3–10% for 13-*O*-demethylate, and 2–10% and 3–9% for 31-*O*-demethylate, respectively. The lower limits of quantification were 0.5 μg/l for tacrolimus and 0.1 μg/l for 13-*O*- and 31-*O*-demethylates.

2.4. Determination of blood tacrolimus levels using CLIA

Tacrolimus levels in whole blood specimens were measured using CLIA according to the manufacturer's protocol (ARCHITECT Tacrolimus, Abbott Japan, Tokyo) using an automated immunoassay analyzer (ARCHITECT i1000 SR®, Abbott Japan). The intra- and interassay accuracies were 106–108% and 103–109% for tacrolimus, respectively. The intra- and interassay imprecisions were 3–12% and 4–11% for tacrolimus, respectively. The lower limit of quantification was 0.5 μg/l.

2.5. Genotyping of *CYP3A5*3*

Genomic DNA was extracted from peripheral whole blood from each patient using a DNA Extractor WB Kit (Wako Pure Chemicals, Osaka, Japan) and stored at −20 °C until analysis. The primer sequences and conditions of the polymerase chain reaction-fragment length polymorphism for analyses of CYP3A5 A6986G (**3*) used were as described previously, with some modifications [16].

2.6. Statistical analysis

All statistical analyses were performed using SPSS 15.0J (SPSS Japan Inc., Tokyo). The blood 31-*O*-demethylate levels could not be statistically analyzed, because they were less than 0.1 μg/l. The blood levels of tacrolimus and 13-*O*-demethylate were evaluated using the following parameters: the C_{12} of tacrolimus and 13-*O*-demethylate, their normalized values for the daily dose, and the C_{12} ratio of 13-*O*-demethylate to tacrolimus. The differences in these parameters of tacrolimus and 13-*O*-demethylate between the *CYP3A5*1/*3* and *CYP3A5*3/*3* genotypes were analyzed using the Mann–Whitney *U* test, because only 2 patients with the *CYP3A5*1/*1* genotype were observed in this study. The cross-reactivity of tacrolimus CLIA was evaluated using Bland–Altman analysis and interaction plots [17]. The mean bias, calculated as the difference of (CLIA − LC–MS/MS)/(mean of tacrolimus C_{12}), was evaluated using two factors: tacrolimus C_{12} (with or without less than 3 μg/l) and CYP3A5 genotypes (**1/*3* or **3/*3*). A $P<0.05$ was considered to indicate statistical significance.

3. Results

3.1. Patient demographic characteristics

Table 1 shows the patient demographic characteristics in this study population. No patient had severe hepatic and/or renal function impairment. No significant differences in patient demographic characteristics were observed, with the exception of alanine aminotransferase. The numbers of patients with *CYP3A5*1/*1*, *CYP3A5*1/*3*, and *CYP3A5*3/*3* were 2 (4%), 16 (32%), and 32 (64%), respectively. The allele frequency of *CYP3A5*3* was 80.0%.

3.2. Cross-reactivity in tacrolimus CLIA

Fig. 1 shows that tacrolimus C_{12} determined by CLIA was significantly correlated with that by LC–MS/MS ($y = 1.0411x + 1.1725$, x = tacrolimus

Table 1
Patient demographic characteristics.

CYP3A5 genotypes	**1/*1*	**1/*3*	**3/*3*	*P*
Sex, male/female	2/0	12/4	14/18	0.08
Donor type, living/cadaveric	1/1	10/6	17/15	0.90
Age (years)	36, 34	45 (37–57)	50 (37–57)	0.92
Body weight (kg)	51, 51	54 (48–60)	52 (45–59)	0.61
Serum creatinine (mg/dl)	1.5, 1.6	1.5 (1.3–2.3)	1.3 (1.0–1.7)	0.07
Blood urea nitrogen (mg/dl)	16, 17	23 (17–30)	18 (17–30)	0.76
Total bilirubin (mg/dl)	1.1, 0.8	0.6 (0.6–1.0)	0.8 (0.7–1.1)	0.15
Aspartate aminotransferase (IU/l)	14, 18	15 (14–17)	16 (13–21)	0.26
Alanine aminotransferase (IU/l)	10, 10	11 (8.0–13)	14 (10–18)	0.04
Serum albumin (g/dl)	4.0, 4.6	4.4 (4.0–4.5)	4.3 (4.2–4.4)	0.51
Daily dose of prednisolone (mg)	5.0, 6.0	5.5 (5.0–7.9)	5.0 (5.0–6.0)	0.11

Data are expressed as median and interquartile range, which is in parentheses. Patient data with the *CYP3A5*1/*1* genotype were shown as each actual value. The differences in patient demographic characteristics between the *CYP3A5*1/*3* and *CYP3A5*3/*3* groups were tested using Fisher's exact test or the Mann–Whitney *U* test. The statistical results are shown as a *P* value.