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Benefits of minimizing immunosuppressive dosage according to cytochrome P450 3A5 genotype in liver transplant patients: findings from a single-center study

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ABSTRACT. We evaluated the clinical efficacy of tailoring tacrolimus dosage to cytochrome P450 (CYP) 3A5 genotype in liver transplant patients. One hundred patients who received tacrolimus-based therapy were included in the retrospective study in which the relationship between the tacrolimus blood trough concentration/dosage ratio and the CYP3A5 genotype of both donors and recipients was determined. Subsequently, 106 patients were continuously enrolled in a prospective study and followed-up for 6 months; the relationship between tacrolimus dosage and CYP3A5 genotype was also determined. Rates of acute rejection, hepatotoxicity, renal toxicity, neurotoxicity, hypertension, and hyperglycemia were compared between the groups. During the 6 months following liver transplantation, the mean tacrolimus concentration/dosage ratio among patients who did not have the CYP3A5*1 genotype and who received a transplant from a

donor with the same genotype (24/100, 24% of patients) was higher than that among patients who did have the CYP3A5*1 genotype and/or had a donor with the same genotype (76/100, 76% of patients). In the second part of the study, the tacrolimus dosage was tailored to CYP3A5 genotype and 24 patients (22.64%) received a lower dose. There was an obvious decrease in acute rejection, hepatotoxicity, renal toxicity, neurotoxicity, hypertension, hyperglycemia, and *Pneumocystis carinii* infection among the latter group. A lower tacrolimus dose was suitable for about 25% of the liver transplant patients, as these patients did not have the CYP3A5*1 genotype and received a transplant from a donor with the same genotype. Tailoring the tacrolimus dosage according to the CYP3A5 genotype could reduce rejection and adverse effects.

Key words: Cytochrome P450 3A5; Liver transplantation; Tacrolimus; Transplant rejection; Renal toxicity

INTRODUCTION

Research in cases of renal, lung, and heart transplantation has confirmed that there is a strong correlation between cytochrome *P450 (CYP) 3A5* gene polymorphism and patient-specific differences in responses to tacrolimus (Zheng et al., 2003, 2004; Macphee et al., 2005; Li et al., 2007; Zuo et al., 2013). However, the situation is more complex in cases of liver transplantation. The CYP3A5 enzyme is distributed in both the liver and the small intestine, and the genetic backgrounds of both the donor and the recipient affect tacrolimus metabolism. It is not clear which has the greatest influence. Therefore, we performed a retrospective analysis to explore the major factors affecting the tacrolimus blood trough concentration. Subsequently, we conducted a prospective study in which we tailored the tacrolimus dosage to the patient's CYP3A5 genotype and observed the clinical effects.

MATERIAL AND METHODS

Retrospective study

The retrospective study included 100 adult Chinese orthotopic liver transplantation patients who were treated with tacrolimus at our center between January 2010 and December 2011. Tacrolimus was administered orally twice daily (at 6:00 and 18:00 h). The average initial dose was 4-6 mg/day, but this was adjusted based on the physician's judgment, clinical syndromes, trough concentration, renal function, and immune function. In addition, we determined the *CYP3A5* gene polymorphism status of both donors and recipients and calculated the blood trough concentration/dosage (C/D) ratio of tacrolimus for the 6 months after transplantation. Subsequently, we analyzed the relationship between the C/D ratio of tacrolimus and the CYP3A5 genotypes of donors and recipients. Concentration values influenced by liver dysfunction, intestine dysfunction, fluconazole therapy, amlodipine, nicardipine, or a lack of regular outpatient visits were excluded.

Prospective study

In 2012, we continuously enrolled 106 adult orthotopic liver transplantation patients in a prospective study. Their CYP3A5 genotypes were determined at the time of transplantation and tacrolimus-based immunosuppressive therapy was tailored to each patient's genetic profile. Among CYP3A5*1*1/*1*3 or donor CYP3A5*1*1/*1*3 patients, the initial dose of tacrolimus was 4 mg/day, which was soon increased to 6 mg/day. Among donors and recipients who both had the CYP3A5*3*3 genotype, the initial dose of tacrolimus was 2 mg/day and this was increased to 3-4 mg/day. We took great care when increasing the tacrolimus dosage in CYP3A5*3*3 patients who also had a CYP3A5*3*3 donor. The incidence of acute rejection, hepatotoxicity, renal toxicity, neurotoxicity, hypertension, and hyperglycemia was compared among the 100 patients treated in 2010 and 2011, and the 106 patients treated in 2012. The study was approved by the hospital Ethics Committee [approval number: 2013 (8)].

Diagnoses of acute rejection and hepatotoxicity were dependent on clinical symptoms, liver function, and liver biopsy findings. The following definitions were used for each condition:

Liver toxicity: glutamic-pyruvic transaminase levels exceed the upper limit and/or bilirubin levels exceed 50% of the upper limit of normal.

Diagnosis of renal toxicity: preoperative serum creatinine >167 μM in patients with normal renal function or show a >30% increase postoperatively in patients with abnormal renal function before surgery (after the exclusion of other factors).

Diagnosis of neurotoxicity: hand tremor, numb lips, and numbness in limbs.

Hypertension: systolic pressure >140 mmHg (1 mmHg = 0.133 kPa) or diastolic pressure >90 mmHg.

Hyperglycemia: blood glucose >7.0 mM for three consecutive days.

Genotype analysis

Peripheral blood samples or liver specimens were obtained from recipients and donors and DNA was extracted. The single nucleotide polymorphism A6986G in CYP3A5 intron 3 was identified by polymerase chain reaction-restriction fragment length polymorphism.

The tacrolimus concentration (ng/mL) was identified by microparticle enzyme immunoassay using an IMx analyzer (Abbott Japan, Tokyo, Japan). In total, 1854 blood samples were collected and the daily dosage of tacrolimus was recorded at the detection spot.

Statistical analysis

All concentration values are reported as means ± SD. The distribution of genotypes was analyzed using the chi-squared test. A non-parametric test was used to compare differences in C/D ratios of tacrolimus among groups. All statistical analyses were performed using the SPSS v.17.0 software. A P value <0.05 was considered to be statistically significant.

RESULTS

Patients

In total, 206 liver transplantation patients were included in both studies; there were no

instances of living donor transplantation. The demographic characteristics of the 206 patients are shown in Table 1. The mean age of the liver transplant recipients was 49.06 ± 8.64 years.

Table 1. Characteristics of the study population (N = 206).

Demographics	Patients treated in 2010-2011 (N = 100)	Patients treated in 2012 (N = 106)
Gender (male/female)	78/22	81/25
Age (years)	49.58 ± 9.48	48.578 ± 7.77
Primary disease		
Cirrhosis	44	55
Cancer	38	39
Primary biliary cirrhosis	5	6
Alcoholic cirrhosis	2	4
Other (drug-induced liver injury)	1	2

Genotype data

The CYP3A5 genotype frequencies in donors and recipients are summarized in Table 2. There was no statistically significant difference between recipients and donors in terms of the proportion of patients with each genotype ($P > 0.05$). In approximately 25% of the liver transplant patients, neither the donor nor the recipient had the CYP3A5*1 genotype.

Table 2. Pharmacogenomic data for the study population (N = 206).

Genotype frequency	Patients treated in 2010-2011 (N = 100)	Patients treated in 2012 (N = 106)
Donor *1/*1 and *1/*3 + recipient *1/*1 and *1/*3	31 (31%)	20 (18.87%)
Donor *1/*1 and *1/*3 + recipient *3/*3	21 (21%)	26 (24.53%)
Donor *3/*3 + recipient *1/*1 and *1/*3	24 (24%)	35 (33.02%)
Donor *3/*3 + recipient *3/*3	24 (24%)	25 (23.58%)

Influence of pharmacogenomic data on tacrolimus C/D ratio

To investigate the effects of *CYP3A5* gene polymorphisms in donors and recipients on tacrolimus C/D ratio, we divided the patients who were treated in 2010-2011 (N = 100) into four groups according to patient and donor genotype and then compared the tacrolimus C/D ratios among the groups. Because patient-specific with one CYP3A5*1 allele express CYP3A5 (Kuehl et al., 2001), the CYP3A5*1/*1 and CYP3A5*1/*3 genotypes were combined and defined as "expressed"; the CYP3A5*3/*3 genotype was defined as "not expressed". Table 3 shows the tacrolimus C/D ratios for the four groups. Age, gender, and weight were not significantly different between the patients included in the retrospective and prospective studies.

As shown in Table 3, during the 6-month postoperative follow-up period, patients with the CYP3A5*3/*3 genotype whose donors had the same genotype had higher tacrolimus C/D ratios than patients in the other three groups. In addition, the tacrolimus C/D ratio of patients who had the same CYP3A5*1 genotype as their donor was lower than that in the other three groups, but the difference was not significant. To further investigate this hypothesis, we expanded our dataset by analyzing all 206 patients enrolled during 2010-2012, and the outcome was the same (Figure 1).

Table 3. Concentration/dosage ratios (ng/mL)/(mg·kg^{-1}·day^{-1}) of tacrolimus 6 months after transplantation among patients treated in 2010-2011 and grouped according to donor and recipient cytochrome P450 3A5 genotype (N = 100).

Donor Recipient	*1/*1 and *1/*3 *1/*1 and *1/*3	*1/*1 and *1/*3 *3/*3	*3/*3 *1/*1 and *1/*3	*3/*3 *3/*3
Number	31	21	24	24
Week 1	44.06 ± 49.50	55.86 ± 53.95	49.82 ± 41.41	137.55 ± 123.96*
Week 2	41.10 ± 48.12	59.94 ± 61.20	53.36 ± 48.40	121.84 ± 109.26*
Week 3	35.46 ± 31.50	56.51 ± 52.50	50.65 ± 48.40	134.28 ± 129.28*
Week 4	48.55 ± 46.10	61.86 ± 56.87	57.20 ± 57.15	125.54 ± 121.73*
Month 2	60.38 ± 61.05	79.00 ± 75.60	79.02 ± 79.13	155.83 ± 156.96*
Month 3	67.09 ± 66.20	74.76 ± 66.50	61.11 ± 58.50	133.98 ± 136.00*
Month 4	51.44 ± 50.15	60.09 ± 58.12	62.83 ± 61.85	144.79 ± 134.75*
Month 5	57.27 ± 59.40	65.38 ± 63.55	64.43 ± 63.90	133.41 ± 122.80*
Month 6	72.89 ± 72.33	82.30 ± 78.53	89.80 ± 83.82	201.66 ± 197.06*

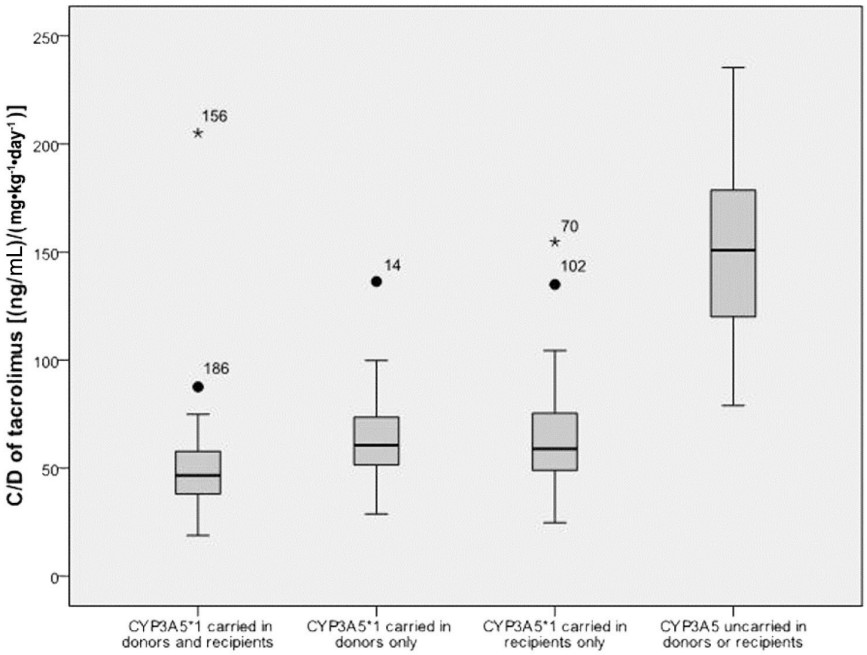

Figure 1. Tacrolimus concentration/dosage ratio among the four groups classified according to combination donor and recipient cytochrome P4503A5 genotypes (N = 206).

Effect of tailoring tacrolimus dosage according to CYP3A5 genotype

We divided the 206 patients into two groups. Group 1 (N = 100) comprised patients who were treated in 2010-2011 and whose tacrolimus dosage was adjusted in accordance with the judgment of the physician. Group 2 (N = 106) comprised the patients treated in 2012; their tacrolimus dosage was adjusted according to their CYP3A5 genotype. The average daily dose of tacrolimus and the rates of acute rejection and other complications were then compared between the two groups (Tables 4 and 5).

Table 4. Average dosage of tacrolimus between the two groups of patients during 6-month follow-up after transplantation.

Group	Average dosage (mg/day)
Patients treated in 2010-2011 (N = 100)	42.94 ± 6.29
Patients treated in 2012 (N = 106)	
Donor recipient *1/*1 and *1/*3 (N = 82)	45.76 ± 1.42
Donor *3/*3 + recipient *3/*3 (N = 24)	31.5 ± 2.32

Table 5. Rates of acute rejection and of complications in the two patient groups during the 6-month follow-up after transplantation.

	Patients treated in 2010-2011 (N = 100)	Patients treated in 2012 (N = 106)
Acute rejection	19/100 (19%)	12/106 (11.32%)
Hepatotoxicity	9/100 (9%)	6/106 (5.66%)
Renal toxicity	25/100 (25%)	13/106 (12.26%)
Neurotoxicity	11/100 (11%)	6/106 (5.66%)
Hypertension	24/100 (24%)	8/106 (7.55%)
Hyperglycemia	24/100 (24%)	18/106 (16.98%)
Pneumocystis carinii pneumonia infection	4/100 (4%)	1/106 (1.89%)

DISCUSSION

Tacrolimus is a widely used immunosuppressant; it has a narrow therapeutic index and response varies greatly among patients, which makes setting a suitable dosage for transplant patients difficult. Apart from monitoring blood concentration, T lymphocyte function, liver function fluctuations, liver biopsy, and pharmacokinetic genetics can be used to adjust the dosage. Tacrolimus is metabolized by both CYP3A4 and CYP3A5, but CYP3A5 plays a more important role. *CYP3A5* gene polymorphism influences the C/D ratio of tacrolimus (López-Montenegro Soria et al., 2010); an A to G sequence variant at the 6986 nucleotide in *CYP3A5* gene intron 3 causes loss of functional CYP3A5. Studies of lung, heart, and renal transplantation have confirmed that CYP3A5*1 (6986A) plays an important role in tacrolimus metabolism (Chen et al., 2009).

However, in liver transplantation, the contributions of the donor liver and recipient gut complicate tacrolimus metabolism. The relationship between CYP3A5 polymorphism and variations in patient-specific responses to tacrolimus has rarely been studied in liver transplant patients, and studies that have been performed have reported contradictory findings.

One study of 53 liver transplantation patients by Yu et al. (2006) suggested that the donor CYP3A5*3 genotype made a greater contribution to the large inter-patient-specific variation in tacrolimus dose requirement than gut CYP3A5. Goto et al. (2004) indicated that the pharmacokinetics of tacrolimus are influenced by the graft CYP3A5 genotype. Yet another study revealed that patients with a liver graft with the CYP3A5*1 allele had a lower C/D ratio of tacrolimus than patients with a liver graft with the CYP3A5*3/*3 genotype (Rahsaz et al., 2012). Other studies have highlighted the contribution of the recipient's CYP3A5 genotype to tacrolimus metabolism. Shi et al. (2013) studied tacrolimus pharmacokinetics and concluded that patient-specific differences in tacrolimus response could probably be explained by different intestinal CYP3A5 genotypes. A significant difference in tacrolimus daily dose and C/D ratios has also been reported between recipients with the CYP3A5 6986GG allele and

those with the CYP3A5 6986AG/AA allele within 6 months of transplantation (Provenzani et al., 2011). A previous study also found that the genetic polymorphism of intestinal CYP3A5 in liver transplant patients was an important factor affecting tacrolimus blood concentration (Uesugi et al., 2006).

In our study, we divided the patients into four groups according to donor and recipient genotype, and we compared the tacrolimus C/D ratio among these groups to investigate the relationship between CYP3A5 polymorphisms and tacrolimus metabolism. Concentration values influenced by fluconazole and nicardipine (Hooper et al., 2012) were excluded. We found that the tacrolimus C/D ratios of patients with the CYP3A5*3/*3 genotype who received a transplant from a donor who had the same genotype were greater than those of the other three groups, while no significant difference was found among the other three groups themselves. This finding confirms that both the hepatic and intestinal CYP3A5*1 alleles significantly influence the tacrolimus dose and C/D ratio. In other words, only patients who had the CYP3A5*3*3 genotype and whose donor had the same genotype had higher tacrolimus C/D ratios within the 6 months after the operation and required a lower tacrolimus dose to achieve immune tolerance. This finding is partly supported by other research (Elmachad et al., 2012; García-Roca et al., 2012), and also enabled us to tailor the immunosuppressant dose according to CYP3A5 genotype. Based on the findings from the retrospective study, we tailored the tacrolimus dosage in the prospective study to match the CYP3A5 genotypes of the donor and recipient. Among patients with the CYP3A5*3*3 genotype whose donor had the same genotype, the tacrolimus dosage was decreased to 2 mg/day initially and then slowly increased to 3-4 mg/day; these doses are lower than those prescribed for patients and/or donors with the CYP3A5*1 genotype.

After 6 months of follow-up, the total dosage of tacrolimus in patients with the CYP3A5*3*3 genotype who had a donor with that genotype was lower than that among patients in the other groups; no difference in the C/D ratio of tacrolimus was detected. Safety comparisons of the 106 patients in the prospective study and the 100 patients in the retrospective study indicated that the rates of acute rejection, hepatotoxicity, renal toxicity, neurotoxicity, hypertension, hyperglycemia, and *P. carinii* pneumonia infection were all lower among patients in the prospective study. It is not clear whether this protocol is suitable for every patient owing to the short follow-up period, but we inferred that approximately 25% of patients had the same CYP3A5*3/*3 genotype as their donor, required a reduced dosage of tacrolimus, and benefitted from this during the 6 months after transplantation.

However, the rates of acute rejection and adverse effects were not greatly improved, especially the rates of hyperglycemia and hypertension. In addition, we discovered that some patients were unsuited to tacrolimus therapy; their clinical symptoms included intractable elevated bilirubin levels or refractory hyperglycemia. It is worth mentioning that all of these individuals were CYP3A5*1*1/*1*3 patients or CYP3A5*1*1/*1*3 donors, and that immunosuppressant therapy was switched from tacrolimus to cyclosporine A for some of them.

Tacrolimus is not suitable for patients whose donors have identical CYP3A5*1*1/*1*3 genotypes because other enzymes also contribute to the metabolism of tacrolimus, including MDR1 and CYP3A4. For these patients, switching the immunosuppressant is more effective. Further research is required to improve the protocol for tailoring immunosuppressant therapy to CYP3A5 genotype.

In conclusion, our findings suggest that both donor and recipient CYP3A5 polymorphisms affect the C/D ratio of tacrolimus. Approximately 25% of patients had the

same CYP3A5*3*3 genotype as their donors and benefitted from a reduction in the tacrolimus dose. The reduced incidence of side effects proved that it is reasonable to tailor the tacrolimus dose to match the genotypes of donors and recipients, but further research is required to assess the long-term benefits.

Conflicts of interest

The authors declare no conflict of interest.

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