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Effectiveness of Measuring Genetic Polymorphisms in Metabolizing Enzymes of Tacrolimus within One Medical Facility

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Objectives: Because genetic polymorphisms cause diverse activity in drug metabolizing enzymes, drug concentrations in the blood may be variable among patients. We analyzed the genotypes of CYP3A5 and MDR1, and investigated their relationship with whole blood drug concentrations.

Methods: Eight patients were administered an oral dose of tacrolimus for one week or longer prior to enrollment in this study. Whole blood concentrations for tacrolimus were measured 12 hours post oral administration, on the same day as genotyping, within our hospital using a fully automated gene analyzer. The procedures became so rapid that collection of blood samples could be completed within the same day (approximately one hour).

Results: The genotype frequency of CYP3A5 was *3/*3 in five patients, *1/*3 in two patients, and *1/*1 in one patient. All five patients with *3/*3 showed favorable increases in tacrolimus blood concentrations. In the two patients with *1/*3, an increase in tacrolimus blood concentration was not readily achieved in one patient, but increased favorably in the other patient. In the patient with *1/*1, tacrolimus was not detectable in the patient's blood. A favorable treatment effect was obtained by changing tacrolimus to cyclosporine. It is notable that genotypes in patients where tacrolimus was not detected in the blood were wild types: 2677G/G and 3435C/C in MDR1.

Conclusions: The measurement of genetic polymorphisms in metabolizing enzymes of tacrolimus, within one medical facility, is applicable for the selection of immunosuppressants and individual dosing for the treatment of autoimmune disease. (J Nippon Med Sch 2017; 84: 274–279)

Key words: tacrolimus, genetic polymorphism, CYP3A5, MDR1

Introduction

Calcineurin inhibitors, such as tacrolimus and cyclosporine, are specific inhibitors for calcineurin, which is a dephosphorylation enzyme of T cells. These inhibitors have been used as therapies for various diseases. Tacrolimus binds with the 12 kD FK506 binding protein inside leucocytes, and then shows calcineurin inhibiting activity. Its side effects may include renal and central nervous system toxicity. The target cells for its pharmaceutical effects are T cells, and its blood concentration corresponds favorably to drug efficacy models. However, the therapeutic window for tacrolimus blood concentrations is narrow, ranging from 5–20 ng/mL. There are also inter-individual, and intra-individual, variations in terms of its *in vivo* pharmacokinetics. Thus, adjustment of the dose based on blood concentration monitoring is essential[1]. Even with consistent dosing, tacrolimus blood concentrations may not increase in some patients. The reasons for this have been reported to be multifactorial and include age, race, time of food intake, liver function, complications of gastrointestinal disease, concomitant use of other medications (**Table 1**), and food intake such as grapefruits. Recently, the influence of genetic polymorphisms of metabolizing enzymes has also been suggested.

Tacrolimus is metabolized by cytochrome P450 3A4

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Table 1 Drugs which were reported to influence the metabolism of tacrolimus

antifungal drugs	itraconazole, fluconazole, ketoconazole, clotrimazole
antibacterial drugs	erythromycin, clarithromycin, troleandomycin
calcium antagonists	nifedipine, diltiazem, amlodipine, nicardipine, verapamil
digestive medicines	cimetidine, metoclopramide
anticonvulsant drugs	carbamazepine, phenobarbital, phenytoin
antituberculosis drugs	rifabutin, rifampicin
others	bromocriptine, danazol, proteolytic enzyme, cyclosporine

Table 2 Baseline clinical characteristics of patients

Patient	Disease	Sex	Age	Body weight (kg)	u-P (g/g・Cr)	u-OB	sCre (mg/dL)	CRP (mg/dL)
1	SLE	female	24	41.0	1.29	(–)	0.54	0.18
2	SLE	male	36	73.9	1.31	(2+)	0.70	0.52
3	SLE	male	57	70.0	0.88	(1+)	1.05	1.04
4	SLE	female	56	53.9	1.03	(3+)	1.26	0.04
5	SLE	male	53	73.0	0.42	(–)	0.87	1.83
6	RA	male	66	64.5	0.36	(–)	2.68	1.83
7	CADM*1	female	75	53.4	(–)	(–)	0.60	0.05
8	HSPN	male	15	81.0	0.45	(2+)	0.58	0.05

*1 CADM: Clinically amyopathic dermatomyositis

(CYP3A4) and 3A5 (CYP3A5) in the small intestine and liver. Metabolites and unaltered substances are excreted to bile via the drug transporter P-glycoprotein (multidrug resistance protein 1: MDR1). Recently, many genetic polymorphisms for CYP3A4, CYP3A5, and MDR1 have been reported[2–5]. However, to date, the expression level of CYP3A4 has not been established to quantitatively predict the inter-variation or intra-variation of the *in vivo* pharmacokinetics of tacrolimus. On the other hand, genetic polymorphisms in CYP3A5 and MDR1 have been recently reported to influence the tacrolimus blood concentrations primarily in patients with organ transplants[6,7].

In this study, we analyzed genetic polymorphisms in CYP3A5 and MDR1 genes in patients with autoimmune disease, and investigated their relationship with tacrolimus blood concentrations.

Materials and Methods

Eight Asian patients (five men and three women, aged 48 ±21 years) with systemic lupus erythematosus (SLE, n=5), rheumatoid arthritis (RA, n=1), dermatomyositis (DM, n=1) and purpura nephritis (HSPN, n=1) were recruited for this study. Diagnosis of SLE was made based on the SLICC 2012 classification criteria, RA was based on the 2010 ACR-EULAR classification criteria, DM was based on the diagnostic criteria of Bohan & Peter, and HSPN was based on the Chapel Hill Consensus Conference 2012. **Table 2** shows their demographics. All patients had no liver dysfunction or gastrointestinal disease. Tacrolimus was prescribed at the discretion of the attending physician, and doses varied among the patients. No patients received drugs or foods that had been reported to influence the metabolism of tacrolimus. The study was approved by the Institutional Review Board of Nippon Medical School Hospital and informed consent was obtained from all the patients prior to enrollment.

Tacrolimus was administered orally after supper once a day, for at least one week, to ensure a constant blood concentration. Whole blood concentration was then measured 12 hours after administration. Whole blood samples were also collected from all patients for measurement of genotypes. Measurements of genotypes in CYP3A5 and MDR1, and measurements of tacrolimus blood concentrations, were conducted on the same day. The genotypes in CYP3A5 and MDR1 were analyzed using a fully automated gene analyzer, i-densy IS-5320 (Arkray, Kyoto, Japan) and direct PCR sequencing.

The gene locus of CYP3A5 is on the short arm 22 of the 7th chromosome (7q22) and the important SNP (CYP3A5 6986A > G; rs776746 in dbSNP (Single Nucleotide Polymorphism Database)) is at the intron 3. Allele A is a wild type. Polymorphism is represented by a variant from A to G. The CYP3A5 6986A allele is expresses the

Table 3 The blood concentration of tacrolimus and genetic polymorphisms of CYP3A5 and MDR1

Patient	Dose of tacrolimus	Blood concentrations of tacrolimus	*CYP3A5* rs776746 (A6986 G)	*MDR1*-rs2032582 (G2677T/A)	*MDR1*-rs1045642 (C3435T)
1	4 mg	<2.4 ng/mL	A/A (*1/*1)	G/G	C/C
2	3 mg	10.2 ng/mL	G/G (*3/*3)	G/G	C/C
3	3 mg	10.3 ng/mL	A/G (*1/*3)	G/T	C/T
4	3 mg	2.6 ng/mL	A/G (*1/*3)	T/A	C/T
5	3 mg	6 ng/mL	G/G (*3/*3)	T/A	C/T
6	3 mg	5.2 ng/mL	G/G (*3/*3)	G/G	C/C
7	3 mg	10 ng/mL	G/G (*3/*3)	G/A	C/C
8	2.5 mg	10.6 ng/mL	G/G (*3/*3)	A/T	C/T

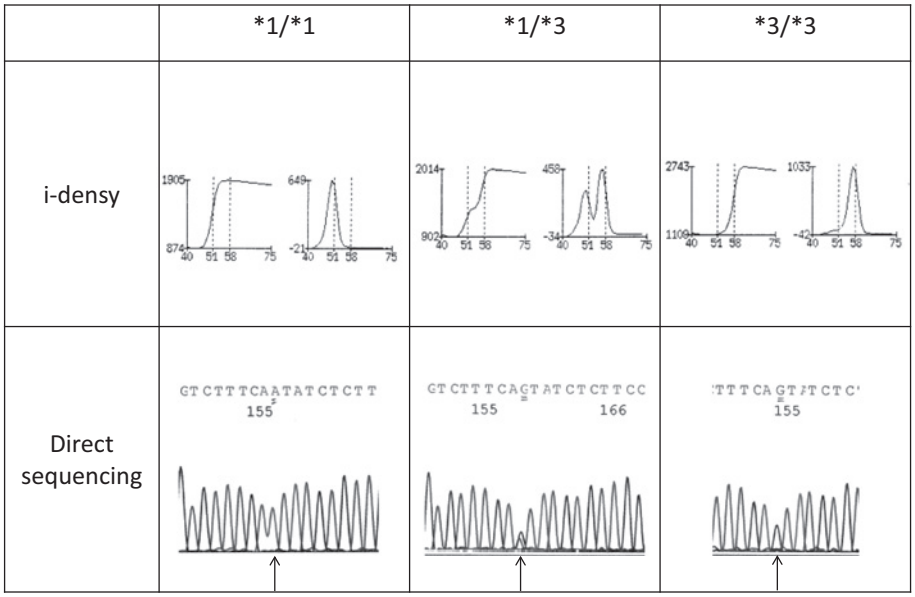

Fig. 1 The analysis results of genotypes *1/*1, *1/*3, *3/*3 in CYP3A5 using a fully automated gene analyzer, i-densy IS-5320, and a direct PCR sequencing method.

CYP3A5*1 protein and the 6986G allele is expresses the CYP3A5*3 protein. The CYP3A5*3/*3 genotype is a CYP3A5 enzyme protein deficient type. In genetic polymorphism of MDR1, variants in 2677G > T/A (rs 2032582 in dbSNP) of exon 21 (rs 1045642 in dbSNP) and 3435C > T of exon 26 have been primarily reported[8,9].

Results

Table 3 shows the blood concentrations of tacrolimus and genotypes of CYP3A5 and MDR1 in the eight patients. Frequencies of genotypes of CYP3A5 were *1/*1 in one, *1/*3 in two, and *3/*3 in five patients using both a fully automated gene analyzer, i-densy IS-5320, and a direct PCR sequencing method. **Fig. 1** shows the genotyping patterns of CYP3A5. In the CYP3A5*1/*1, homogeneous genotype, there was no increase in tacrolimus blood concentrations over the course of tacrolimus administration (Patient 1). In the CYP3A5*1/*3 genotype, a favorable increase in blood concentrations occurred in one patient and no increase was seen in the other patient. In the CYP3A5*3/*3 genotype, there were favorable increases in blood concentrations in all five patients. The influence of genetic polymorphisms of MDR1 on tacrolimus blood concentrations could not be clearly established. However, it is notable that the wild types of 2677G/G and 3435C/C were present in Patient 1.

Case presentation: Patient 1 is a 22-year-old woman with SLE, who complained primarily of pyrexia and systemic arthralgia. Her SLEDAI (Disease Activity Index) was 29 and she was diagnosed with complications of lupus nephritis Class III (A/C), based on the ISN/RPS classification. Following steroid pulse therapy, multitarget therapy with oral administration of steroids, tacrolimus, and mizoribine was conducted. Blood concentrations of tacrolimus remained lower than the sensitivity threshold even with the maximum dose of 4 mg/

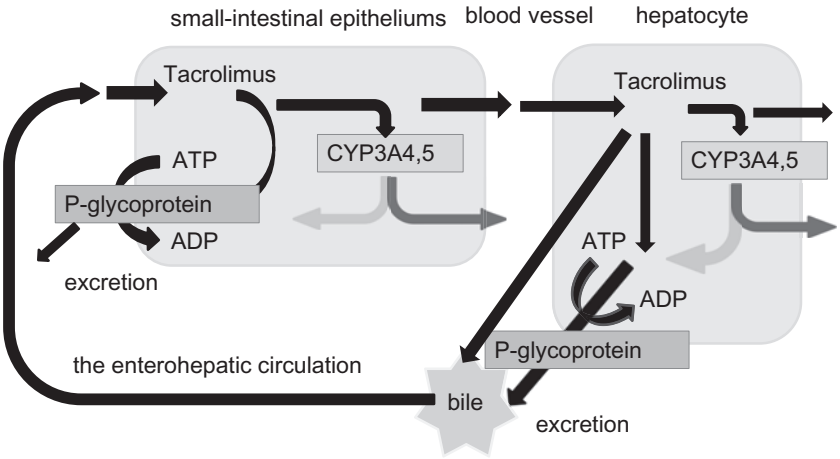

Fig. 2 Pharmacokinetics of tacrolimus

day and the treatment effect was not achieved. An intravenous infusion of tacrolimus of 1 mg/day was attempted. However, her blood concentration was detected at 4 ng/mL. Following the intravenous administration, the patient was switched back to an oral administration, and their blood concentration again dropped below the sensitivity threshold. The treatment drug was then changed to cyclosporine. Symptoms and laboratory values subsequently improved when the blood concentrations of cyclosporine reached the treatment concentration.

Discussion

Tacrolimus, a calcineurin inhibitor, has demonstrated a strong immunosuppressant effect by inhibiting production of interleukin 2 from sensitized T cells, as well as inhibiting activation and proliferation of T cells. The drug has been used for autoimmune diseases such as RA, lupus nephritis, and interstitial pneumonia with dermatomyositis, as well as for organ transplantations. The bioavailability of tacrolimus has been reported to have a large inter-individual variation, ranging from 4–89%[10]. Thus, it is essential to frequently monitor blood concentrations and establish an optimal dose.

Orally administered tacrolimus is endocytosed rapidly to small-intestinal epithelia from the gastrointestinal lumen owing to its lipophilicity. A portion of it is metabolized by CYP3A4 and CYP3A5 and is subsequently inactivated. The endocytosed tacrolimus is excreted back to the luminal side of the epithelium by MDR1, which is a foreign-matter removal transporter existing in the small-intestinal epithelium. Furthermore, tacrolimus that enters systemically is metabolized by CYP3A4 and CYP3A5 in the liver, and the metabolites are excreted into the bile via MDR1 at the biliary lateral membrane (**Fig. 2**)[11]. Thus, it is inferred that the genetic polymorphisms involving CYP3A4 and CYP3A5, as well as MDR1, influence the pharmacokinetics of tacrolimus.

In genetic polymorphisms of CYP3A5, CYP3A5*3 has a high frequency in Japanese populations. It is reported that the frequency of the allele in Japanese populations is about 56–64% in CYP3A5*3/*3 (non-expresser)[12]. CYP3A5*3/*3 does not express CYP3A5 proteins and has low metabolic activity, resulting in easily maintained blood concentrations of tacrolimus. On the other hand, CYP3A5*1 has a high enzymatic activity for metabolizing tacrolimus (expresser). Therefore, a larger dose is required to maintain the blood concentration. Furthermore, it has been shown that the enzymatic activity of CYP3A5*1/*1 as a homo-type is higher than that of *1/*3 as a hetero-type[13]. In this study, in one patient with CYP3A5*1/*1, blood concentrations of tacrolimus could not be detected even after oral administration at the maximum dose of 4 mg/day. In the two patients with CYP3A5*1/*3, an increase in tacrolimus blood concentrations was not readily achieved in one patient, but increased favorably in the other patient. We showed the concomitant medications in all patients in **Table 4**. Since all patients were taking many concomitant medications, influences of unknown mutual drug interactions could not be excluded. This may be one reason why blood concentrations of tacrolimus are slightly different even in the same genetic polymorphism, as in patients 2 and 6.

As for MDR1, although genetic polymorphisms for 2677G and 3435C have been studied frequently, there have been mixed reports on the presence or absence of their influence on drug efficacy[14,15]. Their frequencies, in Japanese populations, are reported to range from 15–20% for 2677G/G, and from 25–30% for 3435C/C, respec-

Table 4 Concomitant medications in all patients

Patient	Concomitant medications
1	prednisolone, mizoribine, rabeprazole sodium, ST-T combined drug, ethyl icosapentate, naproxen, warfarin
2	prednisolone, enalapril , carvedilol, lansoprazole, ST-T combined drug, febuxostat, atorvastatin, vildagliptin, alendronate, dilazep
3	prednisolone, losartan, lansoprazole, warfarin, ST-T combined drug, febuxostat, pregabalin, etizolam, zolpidem, loxoprofen, Vitamin B12, D
4	prednisolone, lansoprazole, ST-T combined drug, rosuvastatin calcium, furosemide, bifidobacterium
5	prednisolone, lansoprazole, ST-T combined drug, mosapride, bifidobacterium
6	salazosulfapyridine, actarit, cilnidipine, allopurinol, telmisartan
7	prednisolone, mizoribine, warfarin, alendronate, teprenone
8	prednisolone, esomeprazole, ST-T combined drug, Vitamin D, mizoribine, dipyridamole, diaminodiphenyl sulfone

tively[16]. The results in this study were slightly higher, with 37.5% in 2677G/G, and 50% in 3435C/C, respectively. Due to the small number of patient, no apparent relationship between these genetic polymorphisms and blood concentrations of tacrolimus could be established. Nevertheless, it is notable that in one patient whose blood concentration did not increase after administration of tacrolimus, both 2677G/G and 3435C/C were wild types with sufficient excreting functions. A larger cohort of patients is needed to further investigate this relationship in future studies.

In this study, analyses of genotypes in CYP3A5 and MDR1 were conducted within our hospital using a fully automated gene analyzer. Introduction of a fully automated gene analyzer enabled us to unify technological aspects and ensure consistent quality for gene examinations. Analysis software enabled us to evaluate genotypes automatically and avoid human errors. The procedures became so rapid that collection of blood samples and selection of drugs suitable for individuals could be completed within the same day (approximately one hour) and within one medical facility. Based on the results of genetic polymorphisms, patients who did not show an increase in their blood concentration of tacrolimus were switched from tacrolimus to cyclosporine. Blood concentrations of cyclosporine rapidly reached treatment concentrations and treatment effects were thus obtained. Consequently, rapid measurement results are likely to result in many benefits, including reduction of side effects and drug expenses.

Conclusions

Measurement of genetic polymorphisms in metabolizing enzymes of tacrolimus, within one medical facility, is applicable for the selection of immunosuppressants and individual dosing for the treatment of autoimmune disease.

Conflict of Interest: The authors have no financial conflicts of interest to declare with regard to the publication of this article.

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