Polymorphisms of \textit{CYP3A5} Affect Serum Levels and Maintenance Doses of Tacrolimus in Myasthenia Gravis Patients

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Abstract

\textbf{Objectives:} To evaluate the influences of polymorphisms of cytochrome P450 (CYP) 3A5 (A6986G, CYP3A5*3) on serum levels of tacrolimus and cyclosporine (CyA) in patients with myasthenia gravis (MG).

\textbf{Methods:} This study included 74 MG patients treated with tacrolimus (n=65) or CyA (n=22). Genomic DNA was extracted and amplified with specific primers, and CYP3A5 alleles were confirmed by direct sequencing of PCR products on an automated AB13100 DNA sequencer. We measured trough blood level (C\textsubscript{0}) of tacrolimus and CyA. Clinical disabilities were evaluated with the MG-ADL scale.

\textbf{Results:} For tacrolimus C\textsubscript{0}, the CYP3A5*3/*3 genotype was associated with higher levels than the CYP3A5*1/*3 genotypes (7.1 ng/ml versus 2.9 ng/ml; P=0.0001) and CYP3A*1/*1 (7.1 ng/ml versus 1.3 ng/ml; P=0.0004). The improvement in the mean MG-ADL scores tended to be better in MG patients with the CYP3A5*3/*3 or CYP3A5*1/*3 than those with CYP3A5*1/*1. For the CyA concentrations, CYP3A5 genotypes did not have significant effects.

\textbf{Conclusion:} In MG patients, CYP3A5 polymorphism significantly affects serum levels of tacrolimus and thereby treatment effects, but not those of CyA. The maintenance dose of tacrolimus should be determined considering CYP3A5 polymorphism.

Keywords: Myasthenia gravis; Cytochrome P450; CYP3A; Calcineurin inhibitor; Tacrolimus; Cyclosporine

Abbreviations: CyA: Cyclosporine; MG: Myasthenia Gravis; AChR: Acetyl Choline Receptor

Introduction

Myasthenia gravis (MG) is an autoimmune disorder of neuromuscular transmission caused primarily by autoantibodies specific to the human nicotinic acetylcholine receptor (AChR). Characteristic symptoms include fatigability and weakness of the striated muscles [1,2]. Immunosuppressant therapy is commonly employed to improve MG symptoms and prevent the destruction of the neuromuscular junctions by AChR-antibodies. Currently, MG is treated with several immunomodulating or immunosuppressive agents, including tacrolimus and cyclosporine (CyA) that inhibit calcineurin.

Calcineurin inhibitors (CNIs) interfere with T-lymphocyte activation and transcription of inflammatory cytokines such as interleukin-2. A recent study indicates that while low-dose tacrolimus is effective for treating MG [3-6], CNIs have a narrow therapeutic index with variable pharmacokinetics and bioavailability after oral administration [7]. In recent years, the biological activity of the cytochrome P450 (CYP) enzyme system has been shown to play an important role in CNI efficacy [8,9]. Specifically, CYP3A is responsible for the phase I metabolism of more than 50% of drugs [9] including CNIs. As CYP3A5 is expressed in a limited number of individuals and is absent in 73% of Chinese and 70% of Caucasians. The expression of CYP3A5 has recently been correlated with a genetic polymorphism (CYP3A5*3) [10,11]. Moreover, at least one CYP3A5*1 allele is required for substantial expression of CYP3A5. Because CYP3A5 may comprise up to 50% of total CYP3A protein in individuals polymorphically expressing CYP3A5, it may play a major role in variations of CYP3A-mediated drug metabolism. Indeed, dose normalized blood concentrations of tacrolimus have been correlated with CYP3A5 genotypes in kidney, heart, lung, and liver transplanted patients [12-17]. In these studies, tacrolimus concentrations (trough levels) in kidney, heart, and lung transplanted patients after tacrolimus administration were lower in patients who expressed CYP3A5*1/*1 and CYP3A5*1/*3 alleles than in those who expressed CYP3A5*3/*3 allele [12-16]. However, the translational link between interindividual variability in CYP3A-mediated drug disposition and clinical improvement of MG patients with polymorphisms in CYP3A5 genes remains to be examined.

The aim of a pharmacogenetics is to optimize drug therapy through characterization of the patients’ genotype and to ensure maximum efficacy with minimal adverse effects [18-20]. This illustrates the concept of personalized medicine, in which drugs and drug combinations are optimized for an individual’s unique genetic makeup [21,22]. The wider use of pharmacogenetic testing is currently viewed as an important tool to improve safety and efficacy [18-21]. This study is the first to show the utility of genotyping in individualizing therapy for MG patients.

Patients and Methods

Patients

All MG patients visiting the outpatient clinic of Chiba University Hospital, Chiba, Japan, who were treated with CNIs at least 4 months after transplantation were included in this study. Genomic DNA was extracted from peripheral blood and amplified with specific primers, and CYP3A5 alleles were confirmed by direct sequencing of PCR products on an automated AB13100 DNA sequencer. We measured trough blood level (C\textsubscript{0}) of tacrolimus and CyA. Clinical disabilities were evaluated with the MG-ADL scale.
before the start of the study, were eligible for this study. Seventy-four Japanese MG patients receiving either tacrolimus (n=65) or CyA (n=22) were recruited, and they gave informed consent. Thirteen patients had received treatment with tacrolimus and CyA previously. During routine visits, blood samples were collected for analysis of tacrolimus and CyA concentrations and clinical parameters at 4 months after CNI treatment. Tacrolimus (3 mg/day) was administrated orally in a single dose every evening. CyA (3 mg/kg of the body weight) was administrated daily in 2 equal oral doses. All patients treated with CyA used the micro emulsion formulation. Patients with liver dysfunction or severe gastrointestinal disorders that could interfere with their ability to absorb oral medications and those receiving other drugs that may interfere with immunosuppressant drug disposition were excluded. Patients taking medications that are known to interact with calcineurin inhibitors, such as calcium channel blockers (diltiazem, nicardipine, and verapamil), antiepileptics (phenytoin and carbamazepine), antmycotics (fluconazole and ketoconazole), and macrolide antibiotics (erythromycin and clarithromycin) were not eligible for entry into the study.

Clinical studies

We recorded the daily dose of CNIs and prednisolone (PSL) and measured steady-state blood drug concentrations at trough level (C₀) 4 months after administration of CNIs. All patients were clinically and biochemically monitored once a month for at least 4 months. Patients were assessed using the MG-activities of daily living (MG-ADL) scale [23]. This semi-quantitative scale has previously been validated in assessing MG [23] and was used in relation to steady-state drug concentrations (C₀).

Genotyping

Genomic DNA was isolated from 5 fingernails of MG patients using the QIAamp Blood Kit (Qiagen, Hilden, Germany). Genetic polymorphisms of CYP3A4 (A-392G) and CYP3A5 (A6986G) were identified using specific primers. A fragment containing A6986G polymorphism was amplified in 10x PCR buffer containing 2 mM dNTP, 0.1 mM primers (forward: 5'-taccaagttcgatgtcacc-3' and reverse: 5'-gaactgttctgatcacgtc-3'). The polymerase (Applied Biosystems, Foster City, CA, ABI) and incubated at 95°C for 10 min, followed by 40 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 30 s, and finally 72°C for 7 min. After purification using cational intestinal phosphate (CIP; Promega, Madison, WI), 1.0 µl of purified PCR product was mixed with 2.5 µl of SNaPshot Ready Reaction Mix (ABI) and 20 pmol/µl of SNaPshot primer (5'-aagccttggccttccat-3'). The cycling program consisted of 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 30 s. Post-extension reactions were purified with CIP, incubated at 37°C for 45 min, and then at 75°C for 10 min. Final reaction samples (1.0 µl) containing extension products were added to 9 µl of Hi-Di formamide (Applied Biosystems). The mixture was incubated at 95°C for 5 min, incubated on ice for 5 min, and then analyzed by electrophoresis in the ABI Prism 3730 DNA analyzer. Results were analyzed using GeneScan Analysis 3.1 (Applied Biosystems). Since previous reports suggest that CYP3A5*3 is the major defective allele and that other functional exonic single-nucleotide polymorphisms (SNPs) are rare in Japanese subjects [10], we detected this allele by identifying CYP3A5 A6986G polymorphism. Patients were divided into 2 groups according to CYP3A5 genotype as follows: CYP3A5*1/*1 or CYP3A5*1/*3 (expressers) and CYP3A5*3/*3 (non-expressers).

Ethics

The study was performed in accordance with the Declaration of Helsinki and its amendments. The protocol was approved by the Ethics Committee of the Chiba University and written informed consent was obtained from all subjects.

Results

Clinical characteristics of the study population (n=74) are shown in Table 1. The majority of patients were homozygous for CYP3A5*3 variant allele (n=44; 59.5%) and were expected to lack CYP3A5 activity. On the other hand, 22 patients (29.7%) carried 1 CYP3A5*1/*3 allele and 8 patients (10.8%) carried CYP3A5*1/*1 allele. The estimated allele frequencies of CYP3A5*1 and CYP3A5*3 alleles were 25.7% and 74.3%, respectively. There were no CYP3A4 mutations among the patients in this study. Among 74 MG patients assessed for CYP3A5 genotype, 65 were treated with tacrolimus and 22 with CyA. Thirteen patients received treatment with tacrolimus and CyA previously. Among patients treated with tacrolimus, CYP3A5 wild-type genotype (*1/*1) was observed in 7 patients (10.8%), whereas 20 patients (30.8%) were heterozygous and 38 (58.4%) homozygous for CYP3A5*3 allele. A significant difference in tacrolimus C₀ and improvement of clinical symptoms was found between the 2 groups at 4 months. Figure 1 shows the differences in trough blood concentrations (C₀) of tacrolimus between CYP3A5 genotypes 4 months after tacrolimus treatment. The mean tacrolimus C₀ for MG patients (n=38) carrying CYP3A5*3/*3 allele was significantly higher than those (n=20) carrying CYP3A5*1/*1 allele (7.05 ng/mL versus 2.87 ng/mL, p=0.0005, Mann-Whitney’s U test) and those with CYP3A5*1/*1 (n=7; 7.05 ng/mL versus 1.30 ng/mL, p=0.014). In contrast, CYP3A5 polymorphisms did not affect blood CyA concentrations at C₀.

The improvements of mean MG-ADL scores (from baseline to 4 months) after tacrolimus administration in MG patients carrying either CYP3A5*3/*3 (5.4 to 3.4) or CYP3A5*1/*3 (6.2 to 4.3) alleles tended to be better than for those with CYP3A5*1/*1 allele (4.0 to 3.7) in Figure 2. The average dose of PSL (alternate days) for MG patients carrying either CYP3A5*3/*3 (32.1 mg to 25.2 mg; p=0.008, Wilcoxon signed-rank test) or CYP3A5*1/*3 (24.6 mg to 15.6 mg; p=0.010) alleles was significantly reduced at 4 months, but not for CYP3A5*1/*1 carriers (8.8 mg to 8.3 mg). Side effects of tacrolimus, including deterioration of diabetes mellitus, elevation of serum creatinine, hyperlipidemia, and finger tremors, were noted in 5 of 38 MG patients carrying CYP3A5*3/*3 (7.6%), 3 of 20 patients carrying CYP3A5*1/*3 (15.0%), and 2 of 7 patients carrying CYP3A5*1/*1 (28.6%) polymorphisms. The side effects of tacrolimus in patients carrying CYP3A5*1/*1 allele tended to be more prevalent than in patients carrying other alleles. On the other hand, there were no significant differences between

<table>
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Table 1: Clinical characteristics of myasthenia gravis (MG) patients.
Among the CYP3A subfamily, CYP3A4 and CYP3A5 are metabolized by CYP3A enzymes to form active and inactive metabolites, and their polymorphisms affect the clearance and bioavailability of cyclosporine (CyA) and tacrolimus (FK506). These immunosuppressive therapies for MG patients are known to have a high variance in pharmacokinetics between individuals, with CyA concentrations ranging from 1.30 ng/mL to 2.87 ng/mL, and tacrolimus concentrations from 6.2 to 4.3 ng/mL. The CYP3A5 polymorphism, in particular, has been shown to affect CyA and tacrolimus concentrations. In our study, the CYP3A5*1/*1 genotype was observed in 73% of Chinese, 85% to 95% of Caucasian, and 73% of Japanese patients, indicating a significant genetic variation in the distribution of this allele.

The mean tacrolimus C0 for MG patients carrying CYP3A5*1/*1 allele carriers in effective CyA dose (mg/kg) or CyA C0 (ng/mL) at 4 months was 7.05 ng/mL versus 2.87 ng/mL, which was significantly higher than that for patients carrying CYP3A5*1/*3 allele (7.05 ng/mL versus 1.30 ng/mL, \( p = 0.014 \)). In contrast, the Cyclosporine concentration (C0) for MG patients carrying CYP3A5*1/*1 allele (6.2 to 4.3) alleles tended to be 7.05 ng/mL versus 5.4 to 3.4, or CYP3A5*1/*3 allele (6.2 to 4.3) alleles tended to be better than for those with CYP3A5*1/*1 allele (4.0 to 3.7).

### Discussion

The calcineurin inhibitors tacrolimus and CyA are new immunosuppressive therapies for MG patients. However, their bioavailability when administered orally is variable. Tacrolimus is metabolized by CYP3A enzymes to form active and inactive metabolites [24-27]. Among the CYP3A subfamily, CYP3A4 and CYP3A5 are the most abundant and important. Hence single nucleotide polymorphisms in CYP3A5*1/*3 correlate with CYP3A5 expression and function [10]. In vitro, CYP3A5 enzyme is twice as effective at clearing tacrolimus as CYP3A4 enzyme [28] and transplant recipients carrying CYP3A5*1 allele(s) have higher clearance, lower concentrations, and delayed time to therapeutic concentration [29-35].

In this study, we examined whether CYP3A5 SNPs contribute to the total metabolic clearance of tacrolimus and CyA. We observed a clear association between CYP3A5*1/*3 genotype and a lower tacrolimus concentration compared with CYP3A5*1/*3 MG patients at 4 months after administration. This observation is in agreement with data showing that CYP3A5*3 allele results in the loss of hepatic CYP3A5 activity [10,11], and indicates that patients with CYP3A5*1/*3 genotype require more tacrolimus to achieve the same target blood concentrations than patients with CYP3A5*3/*3 genotype. The frequency of CYP3A5 polymorphisms is significantly variable between ethnic groups. For example, African-Americans have a 27% to 55% frequency of CYP3A5*3 allele compared with 85% to 95% among Caucasian subjects [10,11]. CYP3A5*3 allele appears to be fairly common with an estimated allele frequency of 73% in the Chinese population [11]. In our 74 Japanese MG patients, the estimated allele frequency of CYP3A5*3 allele (74.3%) is similar to that reported in the Chinese population. Thus, genotyping of MG patients for CYP3A5 variants could be used to predict dose requirements and to avoid the adverse effects of overdosing. Moreover, identification of CYP3A5 variants among patients with other autoimmune disorders and organ transplants might be useful to predict CNI dose requirements.

### Conclusion

In conclusion, we found a significant association between CYP3A5 polymorphism and tacrolimus C0 and clinical efficacy in MG patients. Patients carrying CYP3A5*1 allele required significantly more tacrolimus to reach target concentrations compared with CYP3A5*3 homozygotes, leading to poorer clinical outcomes. Thus, they may have a higher probability of under immunosuppression and poor clinical improvement. This information might be used to prospectively individualize immunosuppressive therapy.

### References
