의학박사 학위논문

Impact of drug metabolism related-polymorphisms of donors and recipients on pharmacokinetics of slow-release tacrolimus in liver transplant patients

수혜자와 공여자의 약물대사와 관련된 염기 다형성이 간이식 환자에서 Tacrolimus 서방형 제제의 약동학에 미치는 영향

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서울대학교 대학원

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이 논문을 의학박사 학위논문으로 제출함

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Abstract

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Background: The effects of multidrug resistance-1 (MDR1), ABCC2, and P450 oxidoreductase (POR)*28 gene polymorphisms on tacrolimus metabolism following a switch to once-daily dosing have not been elucidated. We investigated the effects of recipient and donor CYP3A5, MDR1, ABCC2, and POR*28 polymorphisms on tacrolimus pharmacokinetics following a switch to once-daily tacrolimus dosing.

Methods: Eighty-seven liver transplant recipients who were switched from twice- to once-daily tacrolimus dosing following living-donor liver transplantation and 81 matched donors were genotyped for CYP3A5, MDR1 (1236C>T, 2677G>T/A, and 3435C>T), ABCC2 (-24C>T, 1249G>A, and 3972C>T), and POR*28. Tacrolimus dose-adjusted trough levels (C0/dose) before and after the switch were determined and calculated based on past medical records. Recipients were divided into two groups, one group constituted of 38 patients with a C0/dose decrease of less than 30% following conversion (group 1) and the other constituted of 49 patients with a C0/dose decrease of ≥30% (group 2).

Results: CYP3A5 *1/*3 and *3/*3 were more frequent in recipients in group 1 (60.5% vs. 36.8%), while CYP3A5 *1/*1 was more

frequent in group 2 (59.2% vs. 32.7%) (p = 0.016). The proportions of donor ABCC2 1249G>A genotypes AA and AG were higher in group 2 than in group 1 (20.4% vs. 5.3%; p = 0.042).

Conclusion: Recipient CYP3A5 polymorphism and donor ABCC2 1249G>A polymorphism affected tacrolimus pharmacokinetics following the switch to once-daily dosing. Dose adjustment to maintain therapeutic tacrolimus levels following the switch to once-daily dosing should be considered based on polymorphisms in both the recipient and donor.

Keyword : liver transplant recipient, tacrolimus, once-daily dosing, pharmacokinetics, dose-adjusted trough level, polymorphism
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Table of Contents

Chapter 1. Introduction

1.1. Study Background

Tacrolimus, a calcineurin inhibitor, is usually administered twice daily following solid organ transplantation, guided by therapeutic drug monitoring. Maintaining drug concentrations within the required range is essential as out-of-range drug concentrations can induce graft rejection or nephrotoxicity. The pharmacokinetics of tacrolimus depend mainly on the activities of cytochrome P450 (CYP) 3A5, a predominant intestinal metabolic enzyme, and P-glycoprotein (ABCB1), an efflux transporter located in enterocyte membranes.[1] As the pharmacokinetics of tacrolimus vary with race, many studies have been conducted on several genes encoding CYP3A5 and ABCB1.

CYP3A5 impact on drug pharmacokinetics has been extensively studied in heart, kidney, and liver transplantation patients.[2] The wild-type CYP3A5 polymorphism, CYP3A5*1, is reportedly related to reduced tacrolimus therapeutic levels in liver transplant patients.[3-7] In particular, CYP3A5*1/*1 or CYP3A5*1/*3 (CYP3A5 expressers), mostly in Asians, increases the activity of CYP3A compared to CYP3A5*3/*3 (CYP3A5 non-expresser), which is the majority of Caucasians. The concentration is reduced, which eventually leads to the need for dose adjustment of the drug.

The relationship between ABCB1, encoded by the multidrug resistance-1 (MDR1) gene, and tacrolimus pharmacokinetics has also been evaluated in kidney and liver transplant patients; however, the results have been controversial.[2, 3, 8-11] Moreover, another transporter, multidrug resistance-associated protein 2 (MRP2),

encoded by ABCC2, reportedly influences tacrolimus pharmacokinetics in kidney transplant patients[12–15]; however, no study has evaluated its effects on tacrolimus pharmacokinetics following liver transplantation. Furthermore, the effects of P450 oxidoreductase (POR), a CYP enzyme modulator encoded by the POR*28 gene, on tacrolimus pharmacokinetics have been evaluated mainly following kidney transplantation, and the results have been controversial.[14, 16–18]

Once–daily extended–release tacrolimus administration, introduced to promote patient compliance and increase graft survival, is reportedly safe and feasible, and its effects were comparable to those of conventional tacrolimus dosing in liver transplant patients.[19–21] Zhang et al. showed that stable tacrolimus concentration was maintained compared to conventional formulation even after 24 hours had elapsed after taking once–daily formulation in liver transplant patients.[22] In addition, Florman et al. reported that AUC_{0-24} was not significantly different from that of conventional tacrolimus even after switching to once–daily tacrolimus for most Caucasian patients who had undergone liver transplantation for more than 6 months in the United States.[23] However, tacrolimus levels are reported to decrease after the twice– to once–daily dosing switch; thus, restoring drug concentrations to the therapeutic range requires dose adjustment.[19, 24]

Recently, Kim et al. reported a decrease in tacrolimus levels in recipients who were expressers of CYP3A5 (*1/*1 or *1/3) following the twice– to once–daily dosing switch after liver transplantation; however, this study was limited by a relatively small sample size and a short follow–up period following the

switch.[25] Previously, Miyata et al.[26] reported a decrease in tacrolimus levels in patients who had received donor livers with the CYP3A5 *1 allele following the switch to once-daily dosing. However, tacrolimus was administered intravenously rather than orally and the time to switch dosing was relatively short, i.e., 14 days after transplantation.

Although several studies have evaluated the pharmacokinetics of tacrolimus following twice-daily administration, research on its pharmacokinetics following once-daily administration is limited. Moreover, the most investigated genes affecting tacrolimus pharmacokinetics following all transplantation types are CYP3A5 and MDR1, and most studies on ABCC2 or POR*28 have focused mainly on kidney transplant patients.

1.2. Purpose of Research

We hypothesized that these genes might affect tacrolimus pharmacokinetics following once-daily dosing. This study, therefore, sought to determine whether CYP3A5, MDR1, ABCC2, and POR*28 polymorphisms in donors or recipients could affect tacrolimus dose-adjusted trough level in liver transplant patients following the switch to once-daily dosing.

Chapter 2. Methods

2.1. Study design

This single-center; retrospective study analyzed prospectively collected patient samples. DNA was extracted from patient blood samples or paraffin blocks stored in a cancer tissue bank. This study was approved by the Institutional Review Board of Seoul National University Hospital, Korea (1908-172-1059).

2.2. Patients & immunosuppressants

Patients who had been regularly followed up after having been transplanted living-donor livers between March 1999 and January 2018 were considered; the included patients had consistently been treated with tacrolimus (Tacrobell®, Chong Kun Dang Pharma, Seoul, Korea or Prograf®, Astellas Pharma, Tokyo, Japan) following liver transplantation by twice-daily dosing and then switched to once-daily dosing (Advagraf®, Astellas Pharma, Inc., Deerfield, IL, USA) at the same total daily dose. The inclusion criteria were patients who underwent liver transplantation at least one month before switching to once-daily dosing, who had steadily taken tacrolimus twice daily, who had stable renal (serum creatinine level lower than 2.0 mg/dL) and liver function (serum aspartate aminotransferase and alanine aminotransferase levels within normal range), and who had been followed up for more than 6 months after transplantation. The exclusion criteria were patients with low compliance with taking tacrolimus twice daily, impaired renal and liver function, at least one episode of rejection, and history of re-transplantation. A

triple regimen (tacrolimus, mycophenolate mofetil, and corticosteroids) was administered to the patients following liver transplantation. Corticosteroids were tapered and discontinued within 6 months. Donors for each recipient were also included in the study. Patients were enrolled in the study after voluntarily consenting for their tissues to be used as human research material. Patients who did not provide this consent were not enrolled.

2.3. Data collection

Blood (3 μL) was collected from recipients and donors enrolled in this study during outpatient visits within the study period; this was performed after they voluntarily consented for their tissues to be used as material for human research. In case of difficulty in obtaining recipient or donor blood samples, DNA was extracted from their paraffin blocks of liver tissues stored in a cancer tissue bank following surgery to confirm single-nucleotide polymorphisms (SNPs) and CYP3A5, MDR1, ABCC2, and POR*28 genotypes. Additionally, ABCC2 haplotypes were determined and analyzed as reported previously.[14] Haplotypes were classified into three groups, the high expression (H1/H2 and H2/H2) group, the wild/average expression (H1/H1, H2/H10, and H2/H12) group, and the low expression (H1/H9, H1/H10, H1/H12, H9/H12, and H12/H12) group. Each patient's medical records were reviewed for the determination of serum tacrolimus levels, as well as its administered dosage before and after the switch to once-daily dosing.

2.4. DNA sequencing and genotyping

Genomic DNA was extracted from whole blood samples and paraffin blocks using a DNA Extraction Kit (Intron Bio, Sungnam, Korea), according to the manufacturer's instructions. To describe the relationship between detailed genotypes and trough levels, CYP3A5 (6986G>A in intron 3), MDR1 (1236C>T in exon 12, 2677G>T/A in exon 21, and 3435C>T in exon 26), ABCC2 (−24C>T, 1249G>A, and 3972C>T), and POR*28 (1508C>T) expression levels were determined using a TaqMan assay kit (Thermo Fisher Scientific, Applied Biosystems, Waltham, MA, USA). Six SNPs in MDR1 and ABCC2 were genotyped by direct sequencing. For the TaqMan assay, the reaction was performed in a final volume of 10 μL constituted of 5 ng of genomic DNA, 1X TaqMan Genotyping Master Mix, and 20X TaqMan SNP Genotyping Assay solution. Polymerase chain reaction (PCR) was performed in thermal cyclers with Prism 7900HT devices (Applied Biosystems, Foster City, CA, USA) using the following conditions: 10 min at 95 ℃, then 40 cycles at 92 ℃ for 15 s, and 60 ℃ for 1 min. Alleles were assigned using the SDS version 2.1 software (Applied Biosystems). Direct sequencing was performed using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) for comparative sequencing according to the manufacturer's instructions. Sequence variants were verified using the SeqMan version 7.0 software (DNASTAR Inc., Madison, Wisconsin, USA).

2.4. Outcomes

The primary endpoint of this study was the determination of the

effects of CYP3A5, MDR1, ABCC2, and POR*28 polymorphisms in recipients and donors on blood tacrolimus trough levels in recipients following the switch to once-daily dosing. The secondary endpoint was the comparison of tacrolimus level changes between CYP3A5 expressers and non-expressers, in association with polymorphisms in MDR1, ABCC2, and POR*28 that significantly affect tacrolimus levels.

2.5. Statistical analysis

In similar previously published multicenter studies, the rate of reduction in tacrolimus concentration following the switch to once-daily dosing was reported to be 28.8 ± 23.5% in the CYP3A5 expression group and 14.2 ± 35.2% in the CYP3A5 non-expression group.[25] Assuming a two-sided type I error of 5% and a power of 80%, 40 patients were drawn from each group. Therefore, at least 80 recipients and 80 donors were required for this study.

The paired t-test and the chi-square test were used to determine factors affecting tacrolimus trough levels following the switch to once-daily dosing; these included patient characteristics and the investigated genes. To compensate for missing data, logistic regression was used to ascertain factors found to significantly affect tacrolimus trough levels through the t- and chi-square tests. The Mann-Whitney U test and Kruskal-Wallis test were used for further analysis. Furthermore, ABCC2 haplotypes were predictably determined and analyzed based on previous study findings.[13, 14, 27] Statistical analyses were performed using SPSS Version 28.0

(SPSS Inc., Chicago, IL, USA).

Chapter 3. Results

3.1. Patient characteristics

Based on patient selection criteria, 91 recipients and donors were found eligible. After patient registration, 87 recipients and 81 donors were enrolled and studied. Hepatocellular carcinoma was the most common indication for liver transplantation in the two groups followed by hepatitis B virus-related liver cirrhosis (Table 1). Median time for switching to once-daily dosing was 30.0 month and median ratio of decrease in tacrolimus dose-adjusted trough levels (C0/dose) was 31.8%. Polymorphic genotypes presented with missing data. After switching to once-daily dosing, there were no events of acute cellular rejection (ACR). However, six patients had abnormal liver function test (LFT) which was mostly recovered by dose adjustment. There were no bacterial or viral infections in patients following switching. The patients were divided into two groups based on the decrease in C0/dose as reported previously[24] i.e., patients with less than a 30% decrease in C0/dose and patients with a ≥30% decrease in C0/dose following the switch to once-daily dosing (Table 2). There were no significant differences in age, sex, or bodyweight between groups. The median time between transplantation and the switch to once-daily dosing was significantly shorter in group 2 (37.0 months [range, 4-163] vs. 25.0 months [range, 1-161]; $p = 0.025$).

3.2. Polymorphisms affecting tacrolimus C0/dose

Recipient CYP3A5 *1/*3 and *3/*3 were more frequent in group 1 than in group 2, while CYP3A5 *1/*1 was more frequent in group 2

than in group 1 (p = 0.016) (Table2). However, there were no significant differences in the frequency of donor CYP3A5 variants between the two groups. As concerns donor ABCC2 1249G>A, the proportions of genotypes AA and AG were higher in group 2 than in group 1 (p = 0.042). The donor high-activity ABCC2 group was significantly more frequent in group 2 than in group 1, while the low-activity group was more frequent in group 1 than in group 2 (0% vs. 14.3%; 36.8% vs. 20.4%; p = 0.013). However, there were no significant differences in the proportion of ABCC2 haplotypes between recipients. Shorter switching time, recipient CYP3A5 *1/*1, and donor ABCC2 1249G>A AA and AG genotypes were found to be factors favoring the decrease in tacrolimus C0/dose as determined through the logistic regression analysis following missing data exclusion to reduce bias (Table 3). After adjusting switching time due to significant differences between the two groups (Table 2 and 3), no factors were found to be associated with the decrease in tacrolimus C0/dose. As shown in Table 2, a combination of recipient CYP3A5 expression and donor ABCC2 1249G>A genotypes AA and AG significantly induced a decrease in tacrolimus C0/dose as compared to a combination of recipient CYP3A5 non-expression and donor ABCC2 1249G>A genotype GG (p = 0.021) (Table 4 and Figure 1).

Chapter 4. Discussion

In this study, recipient CYP3A5 polymorphism and donor ABCC2 1249G>A were found to play an important role in tacrolimus pharmacokinetics following the switch to once-daily dosing. To the best of our knowledge, ours is the first study to demonstrate the effects of donor and recipient MDR1, ABCC2, POR*28, CYP3A5 polymorphisms on tacrolimus levels in the liver transplant patients following the switch to once-daily dosing.

In addition to recipient CYP3A5 polymorphism, the presence of the CYP3A5*1 allele in donors was also been reported to affect tacrolimus levels following liver transplantation.[3, 4, 9, 11, 28, 29] Moreover, tacrolimus C0/dose was found to be significantly decreased in patients who had received donor livers with the CYP3A5*1 allele following the administration tacrolimus once daily.[26] In this study, recipient CYP3A5*1/*1 induced a decrease in tacrolimus C0/dose, as reported in previous studies. However, unlike in previous studies, donor CYP3A5*1 was found not to affect tacrolimus C0/dose in this study, even through sub-analysis. Uesugi et al. reported that intestinal and hepatic CYP3A5 play an important role in tacrolimus pharmacokinetics following liver transplantation.[7] Based on the findings of this study and those of previous studies, recipient CYP3A5 can particularly be important as the first pharmacokinetic mediator in the intestines.

ABCC2 polymorphism has been reported to affect tacrolimus pharmacokinetics in kidney transplant patients.[12, 13] Genotypes AA and AG of ABCC2 1249G>A and genotype CC of ABCC2 3972C>T were found to be related to a decrease in tacrolimus C0/dose; in addition, the ABCC2 high activity group also showed a reduction in the dose-normalized concentration of tacrolimus.[13]

The findings of this study were consistent with these previous findings, as ABCC2 1249G>A and its high activity group induced a significant decrease in tacrolimus C0/dose. In contrast, ABCC2 polymorphism was found not to be associated with changes in tacrolimus levels in other studies.[14, 15] Vanhove et al. also reported that no relationship existed between ABCC2 diplotypes and tacrolimus C0/dose; however, for CYP3A5 non-expressers, tacrolimus C0/dose was lower in the ABCC2 low activity group than in the average and high activity groups.[30] MRP2, an ATP-binding cassette transporter, is located in hepatocyte membranes, gallbladder epithelial cells, renal tubular cells, and enterocytes; it is mainly expressed in hepatocyte apical canalicular membranes, where it contributes to the detoxification and biliary excretion of xenobiotics.[31] It can be inferred that ABCC2 1249G>A was more active in hepatocytes, as in this study, tacrolimus levels decreased only in donors, but not in recipients. This study is the first to demonstrate the effects of ABCC2 polymorphism on the pharmacokinetics of tacrolimus administered once daily in liver transplant patients.

Few studies have reported controversial findings on MDR1 polymorphisms in liver transplant patients. MDR1 1236C>T and MDR1 2677G>T/A were found to affect tacrolimus pharmacokinetics, but MDR1 3435C>T was not in another study[8], MDR1 3435C>T was found to be significantly associated with tacrolimus pharmacokinetics.[11] However, Bruendía et al. reported that MDR1 1236C>T and 2677G>T/A did not affect the pharmacokinetics of tacrolimus administered once or twice daily in liver transplant patients.[3] The findings of other studies have also shown that tacrolimus C0/dose is not affected by MDR1 2677G>T/A

and 3435C>T.[9, 10] Furthermore, MDR1 3435C>T was shown not to affect tacrolimus levels, even with the once-daily extended-release regimen[26]; this is consistent with the findings of our study. In this study, none of the three donor or recipient MDR1 SNPs was found to have any effects on tacrolimus C0/dose.

POR*28 polymorphism has been reported to be related to reduced tacrolimus levels in renal transplant patients.[14, 16, 17] However, there was a discrepancy in the findings reported by these three studies. In one of the studies, the dose-normalized tacrolimus trough level was reported to decrease in CYP3A5 non-expressers but not in CYP3A5 expressers.[14] In the other studies, in CYP3A5 expressors, the tacrolimus trough level was significantly lower in patients with the POR*28 CT and TT genotypes than in those with the CC genotype, and dose adjustment was necessary; however, there were no differences between the two CYP3A5 non-expresser patient groups.[16, 17] These findings indicated that POR*28 did not exert any effect on its own, but elicited its effect when combined with CYP3A5. However, in this study, POR*28 polymorphism did not affect tacrolimus C0/dose. Although our study did not show any significant results, it is the first study to attempt elucidating the relationship between POR*28 polymorphism and tacrolimus pharmacokinetics in liver transplant patients.

With once-daily extended-release tacrolimus, from the beginning of administration, reaching the target concentration was more difficult and delayed than with twice-daily tacrolimus.[21] Tacrolimus dose and trough levels were higher in patients who were switched to once-daily dosing within 1 year of transplantation than in those who were switched more than 1 year after transplantation; in addition, dose adjustment was more necessary in

the group that was switched early because of the need for higher therapeutic levels within the period following transplantation.[32] Therefore, administering twice-daily at first and then switching to once-daily dosing later is presumed to be better. Conversion time may be possible when the liver function is stable. However, according to our previous study[24], there is a possibility for ACR or LFT abnormality to occur in inverse proportion to time, so 1:1.5 conversion or close monitoring is required for early conversion. Furthermore, ACR and LFT abnormalities occurred more frequently in patients with decreased trough levels, but ACR also occurred in the patient group with maintained or increased trough levels[24]. In this study, switching time was found to be shorter in patients with a ≥30% decrease in tacrolimus C0/dose following the switch.

From our previous study, we understood that some patients showed a significant reduction in the trough level after conversion.[24] In this study, we proved that both specific donor and recipient SNPs affect this reduction. Therefore, it might be necessary to check polymorphism first before switching to once-daily tacrolimus dosage. However, we believe that routine check of SNP is not necessary, and we suggest that considering the prevalence of specific types of SNP in the region and recipient conditions (such as postoperative day after transplantation and combined hepatocellular carcinoma or not), the conversion ratio strategy needs to be tailored.

CYP3A5*1 was reported to affect graft loss of patients.[33] In this study, there were no significant differences in graft loss or patient survival between the patient groups (Table 4). However, the decrease rate of C0/dose was large, so increase in dosage is required.

Due to the cost of DNA sequencing to reveal SNPs in blood or tissue samples, it is not clinically useful to test the major genes identified in this study before switching in all patients. However, in our study, by presenting SNPs in specific genes of donors and recipients with a decrease of 30% or more in C0/dose of tacrolimus and by suggesting their frequencies in general donors and recipients, it is necessary to increase the total dose during switching or to measure the trough level after switching. Thus, it is thought that there is clinical significance in reducing serious complications after switching.

This study has several limitations that must be considered. First, the sample size was small owing to missing data on the genotypes of each polymorphism. DNA sequencing was performed using the stored paraffin blocks of patients who had undergone transplantation long ago, with difficulty in blood sample collection; thus, the results obtained were not as accurate as they should have been due to the long storage period and possible damage, which resulted in a small sample number. To prevent specimen damage, blood sample collection should be performed during the hospitalization period immediately before and after transplantation. Second, the measurement period for tacrolimus levels following the switch to once-daily dosing was not the same for every patient. This period ranged from 5-102 days due to different outpatient visiting schedules. It is necessary to measure tacrolimus levels within a fixed period following the switch to once-daily dosing. Third, unlike most previous studies, the time period between transplantation and the switch to once-daily dosing in this study was different for each patient due to the retrospective nature of the study. In the future, the same switching period should be

established for all patients.

In conclusion, recipient CYP3A5*1 allele and donor ABCC2 1249G>A AA and AG genotypes induced a reduction in tacrolimus C0/dose following the switch to once-daily extended-release tacrolimus administration. Dose adjustment to maintain tacrolimus therapeutic levels following the switch to once-daily dosing should be considered based on polymorphisms in both the recipient and donor.

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Table 1. Demographics of patients

Characteristics			Patients (n=87)
Age (Years, mean ± SD)			48.2 ± 16.2
Male sex			65 (74.7%)
Bodyweight (kg, mean ± SD)			62.0 ± 11.5
Indications for transplantation Hepatocellular carcinoma Hepatitis B virus-related liver cirrhosis Alcoholic liver cirrhosis Biliary atresia Hepatoblastoma Wilson's disease			 47 (54.0%) 14 (16.1%) 6 (6.9%) 6 (6.9%) 2 (2.3%) 2 (2.3%)
Tacrolimus trough level (ng/mL, mean ± SD)		Before	4.5 ± 1.7
		After	3.8 ± 1.9
C0/Dose (mean ± SD)		Before	2.2 ± 1.4
		After	1.6 ± 0.9
Δ C0/Dose (%, median, range)			−31.8 (−100 − 1067)
Switching time (Months, median, range)			30.0 (1 − 163)
Recipient	CYP3A5		*1/*1: 43 (49.4%) *1/*3, *3/*3: 39 (44.8%) Missing: 5
	MDR1	1236C>T	TT: 24 (27.6%) CC, CT: 62 (71.3%) Missing: 1
		2677G>T/A	AA, AT, TT: 28 (32.2%) CC, AC, CT: 55 (63.2%) Missing: 4
		3435C>T	CT, TT: 43 (49.4%) CC: 43 (49.4%) Missing: 1
	ABCC2	−24C>T	CT, TT: 38 (43.7%) CC: 46 (52.9%) Missing: 3
		1249G>A	AA, AG: 15 (17.2%) GG: 71 (81.6%) Missing: 1
		3972C>T	CT, TT: 38 (43.7%) CC: 45 (51.7%) Missing: 4

		Haplotype [a]	High: 9 (10.3%) Wild/average: 40 (46.0%) Low: 33 (37.9%) Missing: 5
	POR*28		CC: 26 (29.9%) CT, TT: 60 (69.0%) Missing: 1
Donor	CYP3A5		*1/*1: 33 (37.9%) *1/*3, *3/*3: 28 (43.7%) Missing: 26
	MDR1	1236C>T	TT: 30 (34.5%) CC, CT: 49 (56.3%) Missing: 8
		2677G>T/A	AA, AT, TT: 26 (29.9%) CC, AC, CT: 39 (44.8%) Missing: 22
		3435C>T	CT, TT: 35 (40.2%) CC: 35 (40.2%) Missing: 17
	ABCC2	−24C>T	CT, TT: 30 (34.5%) CC: 46 (52.9%) Missing: 11
		1249G>A	AA, AG: 12 (13.8%) GG: 62 (71.3%) Missing: 13
		3972C>T	CT, TT: 25 (28.7%) CC: 48 (55.2%) Missing: 14
		Haplotype [a]	High: 7 (8.0%) Wild/average: 38 (43.7%) Low: 24 (27.6%) Missing: 18
	POR*28		CC: 26 (29.9%) CT, TT: 50 (57.5%) Missing: 11
Adverse events after switching Liver function test abnormality Acute cellular rejection			6 (6.9%) 0 (0%)

[a] Haplotypes were predictably determined and analyzed based on the findings of previous studies.[13, 14, 27]

Table 2. Differences in patient characteristics between groups 1 and 2

			Group 1 (n = 38)	Group 2 (n = 49)	p-
Age (Years, mean ± SD)			46.8 ± 19.2	50.0 ± 12.8	0.277
Male sex			28 (73.7%)	37 (75.5%)	0.846
Bodyweight (kg, mean ± SD)			62.7 ± 11.6	61.5 ± 11.6	0.633
Indications for transplantation Hepatocellular carcinoma Hepatitis B virus-related liver cirrhosis Alcoholic liver cirrhosis Biliary atresia Hepatoblastoma Wilson's disease Miscellaneous			 20 (52.6%) 6 (15.8%) 2 (5.3%) 5 (13.2%) 1 (2.6%) 0 (0.0%) 4 (10.5%)	 27 (55.1%) 8 (16.3%) 4 (8.2%) 1 (2.0%) 1 (2.0%) 2 (4.1%) 6 (12.2%)	0.366
Tacrolimus trough level (ng/mL, mean ± SD)		Before	3.8 ± 1.5	5.0 ± 1.6	<0.001
		After switching	4.6 ± 2.3	3.3 ± 1.3	0.005
C0/Dose (mean ± SD)		Before	1.3 ± 0.6	2.8 ± 1.4	<0.001
		After switching	1.6 ± 1.0	1.5 ± 0.8	0.594
ΔC0/Dose (%, median, range)			-3.5 (-29 - 1067)	-39.3 (-100 - -30)	0.001
Switching time (Months, median, range)			37.0 (4 - 163)	25.0 (1 - 161)	0.025
Recipient	CYP3A5		*1/*1: 14 (36.8%) *1/*3, *3/*3: 23 (60.5%) Missing: 1	*1/*1: 29 (59.2%) *1/*3, *3/*3: 16 (32.7%) Missing: 4	0.016
	MDR1	1236C>T	TT: 11 (28.9%) CC, CT: 26 (68.4%) Missing: 1	TT: 13 (26.5%) CC, CT: 36 (73.5%)	0.743
		2677G>T/A	AA, AT, TT: 14 (36.8%) CC, AC, CT: 23 (60.5%) Missing: 1	AA, AT, TT: 14 (28.6%) CC, AC, CT: 32 (65.3%) Missing: 3	0.478
		3435C>T	CT, TT: 21 (55.3%) CC: 16 (42.1%) Missing: 1	CT, TT: 22 (44.9%) CC: 27 (55.1%)	0.276
	ABCC2	-24C>T	CT, TT: 17 (44.7%) CC: 21 (55.3%)	CT, TT: 21 (42.9%) CC: 25 (51.0%) Missing: 3	0.933
		1249G>A	AA, AG: 5 (13.2%) GG: 33 (86.8%)	AA, AG: 10 (20.4%) GG: 38 (77.6%) Missing: 1	0.352
		3972C>T	CT, TT: 18 (47.4%) CC: 20 (52.6%)	CT, TT: 20 (40.8%) CC: 25 (51.0%) Missing: 4	0.790

		Haplotype [a]	High: 4 (10.5%) Wild/average: 17 (44.7%) Low: 17 (44.7%)	High: 5 (10.2%) Wild/average: 23 (46.9%) Low: 16 (32.7%) Missing: 5	0.739
	POR*28		CC: 11 (28.9%) CT, TT: 27 (71.1%)	CC: 15 (30.6%) CT, TT: 33 (67.3%) Missing: 1	0.817
Donor	CYP3A5		*1/*1: 12 (31.6%) *1/*3, *3/*3: 14 (36.8%) Missing: 12	*1/*1: 21 (42.9%) *1/*3, *3/*3: 14 (28.6%) Missing: 14	0.283
	MDR1	1236C>T	TT: 14 (36.8%) CC, CT: 22 (57.9%) Missing: 2	TT: 16 (32.7%) CC, CT: 27 (55.1%) Missing: 6	0.878
		2677G>T/A	AA, AT, TT: 11 (28.9%) CC, AC, CT: 17 (44.7%) Missing: 10	AA, AT, TT: 15 (30.6%) CC, AC, CT: 22 (44.9%) Missing: 12	0.919
		3435C>T	CT, TT: 17 (44.7%) CC: 13 (34.2%) Missing: 8	CT, TT: 18 (55.3%) CC: 22 (44.9%) Missing: 9	0.334
	ABCC2	−24C>T	CT, TT: 14 (36.8%) CC: 19 (50.0%) Missing: 5	CT, TT: 16 (32.7%) CC: 27 (55.1%) Missing: 6	0.645
		1249G>A	AA, AG: 2 (5.3%) GG: 30 (78.9%) Missing: 6	AA, AG: 10 (20.4%) GG: 32 (65.3%) Missing: 7	0.042
		3972C>T	CT, TT: 14 (36.8%) CC: 21 (55.3%) Missing: 3	CT, TT: 11 (22.4%) CC: 27 (55.1%) Missing: 11	0.320
		Haplotype [a]	High: 0 (0%) Wild/average: 18 (47.4%) Low: 14 (36.8%) Missing: 6	High: 7 (14.3%) Wild/average: 20 (40.8%) Low: 10 (20.4%) Missing: 12	0.013
	POR*28		CC: 10 (26.3%) CT, TT: 24 (63.2%) Missing: 4	CC: 16 (32.7%) CT, TT: 26 (53.1%) Missing: 7	0.428

Group 1: C0/dose reduction rate of <30%; group 2: C0/dose reduction rate of ≥ 30%

[a] Haplotypes were predictably determined and analyzed based on the findings of previous studies.[13, 14, 27]

Table 3. Analyses of risk factors for a decrease in tacrolimus C0/dose following the switch to once-daily dosing, with missing value cases (n = 70) excluded

		Group 1 (n = 31)	Group 2 (n = 39)	Multivariate analysis		
				OR	95% CI	p-value
Switching time (Months, median, range)		37.0 (4 - 163)	25.0 (9 - 161)	0.980	0.965 - 0.996	0.013
Recipient	CYP3A5	*1/*1: 13 (41.9%) *1/*3, *3/*3: 18 (58.1%)	*1/*1: 25 (64.1%) *1/*3, *3/*3: 14 (35.9%)	0.350	0.118 - 1.037	0.058
Donor	ABCC2 1249G>A	AA, AG: 2 (6.5%) GG: 29 (93.5%)	AA, AG: 10 (25.6%) GG: 29 (74.4%)	0.182	0.031 - 1.062	0.058

Group 1: C0/dose reduction rate of <30%; group 2: C0/dose reduction rate of ≥ 30%

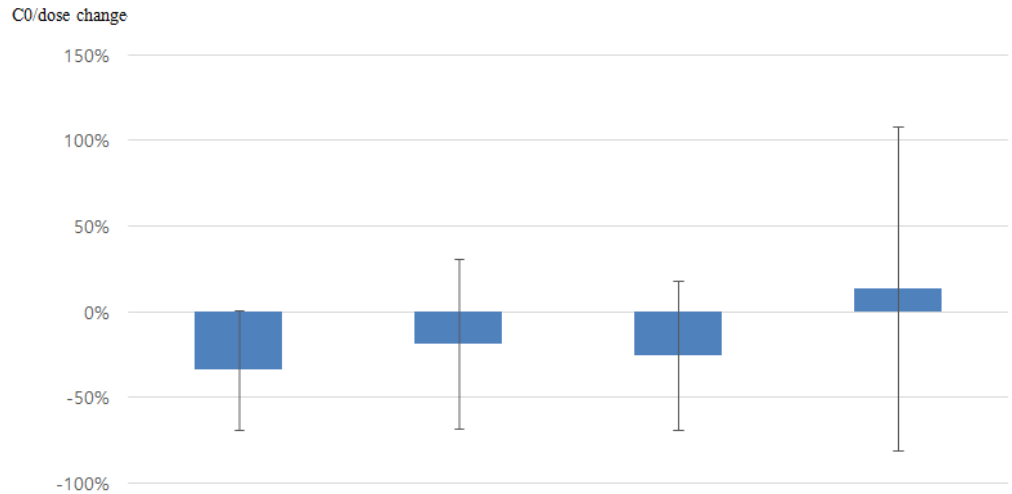
Table 4. Characteristic of patients on combination of recipient CYP3A5 and donor ABCC2 1249G>A, with missing value cases (n = 70) excluded

Recipient CYP3A5	*1/*1	*1/*1	*1/*3, *3/*3	*1/*3, *3/*3	p-value
Donor ABCC2 1249G>A	AA, AG	GG	AA, AG	GG	
No. of patients	9 (12.9%)	29 (41.4%)	3 (4.3%)	29 (41.4%)	
Before switching					
Age (Years)	53.4 ± 10.6	42.4 ± 17.7	52.3 ± 8.5	51.8 ± 14.1	0.096*
Male sex	9 (100%)	18 (62.1%)	3 (100%)	20 (69%)	-
Switching time (Months)	51.8 ± 46.8	46.6 ± 44.9	33.0 ± 5.2	37.6 ± 36.2	0.765*
C0/Dose	3.4 ± 2.0	2.5 ± 1.3	2.5 ± 0.9	1.4 ± 0.6	<0.001*
ΔC0/Dose (%)	−33.8 ± 35.0	−18.5 ± 49.5	−25.4 ± 43.7	13.7 ± 94.8	0.073*
After Switching					
C0/Dose	1.9 ± 0.9	1.7 ± 0.8	1.6 ± 0.2	1.4 ± 1.0	0.049*
LFT abnormality	0 (0%)	2 (6.9%)	0 (0%)	2 (6.9%)	-
Nephrotoxicity	0 (0%)	2 (6.9%)	0 (0%)	2 (6.9%)	-
eGFR (mL/min/1.73m^2)	93.4 ± 12.1	89.2 ± 21.4	86.4 ± 5.4	75.0 ± 22.6	0.034*
Graft failure	0 (0%)	0 (0%)	0 (0%)	0 (0%)	-
Patient survival (Months)	89.1 ± 31.4	105.1 ± 22.6	57.0 ± 3.6	89.6 ± 28.4	-

eGFR: estimated glomerular filtration rate, LFT: liver function test
* Kruskal-Wallis test

Figure 1. Combined effects of recipient CYP3A5 and donor ABCC2 1249G>A on tacrolimus C0/dose changes (%, mean ± SD), with missing value cases (n = 70) excluded.

The combination of recipient CYP3A5 expression and donor ABCC2 1249G>A genotypes AA and AG induced a significant decrease in tacrolimus C0/dose as compared to the combination of recipient CYP3A5 non-expression and donor ABCC2 1249G>A genotype GG.



Recipient CYP3A5	*1/*1	*1/*1	*1/*3, *3/*3	*1/*3, *3/*3
Donor ABCC2 1249G>A	AA, AG	GG	AA, AG	GG
No. of patients	9 (12.9%)	29 (41.4%)	3 (4.3%)	29 (41.4%)
Mean ± SD	-33.8 ± 35.0	-18.5 ± 49.5	-25.4 ± 43.7	13.7 ± 94.8

p = 0.173*, **p = 0.538***, **p = 0.349***

p = 0.864*

p = 0.084*

p = 0.021*

* Mann-Whitney test

국문 초록

수혜자와 공여자의 약물대사와 관련된 염기 다형성이 간이식 환자에서 Tacrolimus 서방형 제제의 약동학에 미치는 영향

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서론: MDR1, ABCC2, POR*28 유전자 다형성이 간이식 환자에서 하루 2회 복용하는 기존의 tacrolimus 제제에서 1회 복용하는 서방형 제제로 변경하였을 때 약물 대사에 미치는 영향은 현재까지 밝혀지지 않았다. 간이식 수혜자와 기증자의 CYP3A5, MDR1, ABCC2, POR*28 다형성이 tacrolimus 서방형 제제로 전환한 후에 tacrolimus 약동학에 미치는 영향을 조사하였다.

방법: 생체 간이식 후 기존의 tacrolimus 제제에서 서방형 제제로 전환한 87명의 간이식 수혜자들과 이에 대한 81명의 기증자들의 CYP3A5, MDR1(1236C>T, 2677G>T/A, 3435C>T), ABCC2(−24C>T, 1249G>A, 3972C>T), POR*28에 대한 유전자형을 분석하였다. 서방형 제제로 전환 전후의 tacrolimus 용량 대비 혈중 농도 (C0/dose)는 환자의 의무기록을 참조하여 계산 후에 결정되었다. 수혜자들은 두 그룹으로 나누어졌는데, 한 그룹은 전환 후 C0/dose의 감소가 30% 미만이거나 증가한 38명의 환자로 구성되었고 (그룹 1), 다른 그룹은 C0/dose의 감소가 30% 이상인 49명의 환자로 구성되었다 (그룹 2).

결과: 수혜자의 CYP3A5 *1/*3 및 *3/*3은 그룹 1에서 많았던 반면에 (60.5% vs. 36.8%), 수혜자의 CYP3A5 *1/*1은 그룹 2에서 더 많았다.

(59.2% vs. 32.7%) (p = 0.016). 공여자의 ABCC2 1249G>A 유전자형 AA와 AG의 비율은 그룹 1보다 그룹 2에서 더 높았다 (20.4% vs. 5.3%; p = 0.042).

결론: 수혜자의 CYP3A5 다형성과 기증자의 ABCC2 1249G>A 다형성은 간이식 환자에서 tacrolimus를 서방형 제제로 전환한 후 약물 약동학에 영향을 주었다. 서방형 제제로 전환한 후 최소한의 tacrolimus의 혈중 농도를 유지하기 위해서 투여 용량의 조정은 수혜자와 기증자의 다형성에 근거하여 고려되어야 한다.

주요어 : 간이식 수혜자, tacrolimus, 서방형 제제, 약동학, 혈중 농도, 염기 다형성

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