[12:00 - 12:30]

Pharmacogenomic Association Study between Genetic Polymorphic Variations of MDR-1and CYP3A5 and Efficacy and Adverse Drug Reactions of Calcinuerin Inhibitors

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Introduction

Tacrolimus, a major immunosuppressive agent for organ transplantation, is characterized by a wide inter-individual variability in their pharmacokinetics with a potential impact on their therapeutic efficacy and toxicity. Because of this inter-individual variability and a narrow therapeutic index, tacrolimus requires strict therapeutic monitoring. P-glycoprotein and the drug metabolizing enzymes have major pharmacokinetic effects of tacrolimus. Tacrolimus is a substrate for cytochrome P450 (CYP) 3A5 which is the most important contributors to tacrolimus metabolism, while the intestinal efflux-pump P-glycoprotein pump (Pgp) which is encoded by multidrug resistance 1 *MDR1* (ATP-binding cassette B1, ABCB1) modulates its bioavailability. P-gp which is distributed on the surface of enterocytes plays an important role in controlling the absorption of the drug within the gastrointestinal tract. Variability in tacrolimus absorption is influenced by P-gp activity which, in turn, is affected by single nucleotide polymorphisms (SNPs) within the (MDR-1. In 2000, Hoffmeyer et al.

performed a study that screened the SNPs of MDRI where they found more than 15 SNPs. They also found that, as the 3435 location in the exon 26 (C3435T) is a silent mutation that affects the expression of the *MDRI* protein in the duodenum. Further, it was also found through many studies that a total of 29 SNPs are located in the 28 *MDRI* genes. Although there are racial differences in *MDRI*'s SNP, it is most popularly expressed in exons 12 (C1236T), 21 (G2677T/A), and 26 (C3435T). It was found that the activity of the CYP3A5 enzyme is increased in patients with CYP3A5*1 allele, As the level of expression and heterogeneity of CYP3A5 enzyme vary by patient, the pharmacokinetics and adverse reaction to tacrolimus metabolized by CYP450 appears genetically different in each patient, making a serious impact on the survival rate of the patient and the transplanted organ.

The objective of this study was to analyze the effect of the donor's and recipient's CYP3A5 and *MDR1* polymorphisms on tacrolimus dose requirements, trough blood concentrations and adverse effects of tacrolimus in recipients of living-donor liver transplantation (LDLT).

Methods

Korean liver transplant recipients treated with tacrolimus and their corresponding donors were enrolled in this study. Polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) analysis method was used to determine the genotype of *MDR1* (exons 12, 21, 26) and CYP3A5 (CYP3A5*1, *3, *6) in liver transplant recipients and their corresponding donors (fig 1).

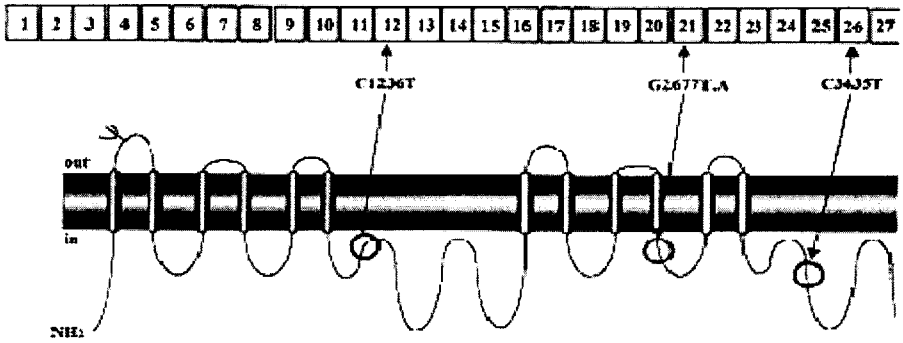

Figure 1. Sites of analysis for *MDR1* polymorphism

Primers and restriction enzymes for each genetic variants are as shown in table 1.

Table 1 Primers and restriction enzymes for each genetic variants

Gene	Primer(sense/antisense, 5'-3')	Restriction enzyme
CYP3A5*3	CTT TAA AGA GCT CTT TTG TCT CTC	*Dde I*
	CCA GGA AGC CAG ACT TTG AT	
MDR1 C3435T	TGC TGG TCC TGA AGT TGA TCT GTG AAC	*Mbo I*
	ACA TTA GGC AGT GAC TCG ATG AAG GCA	
MDR1 G2677A	TAC CCA TCA TTG CAA TAG CAG	*Rsa I*
	TTT AGT TTG ACT CAC CTT CCC	
MDR1 G2677T	TGC AGG CTA TAG GTT CCA GG	*Ban I*
	TTT AGT TTG ACT CAC CTT CCC G	
MDR1 C1236T	TCT TTG TCA CTT TAT CCA GC	*Eco0109 I*
	TCT CAC CAT CCC CTC TGT	

Tacrolimus whole-blood trough concentrations were measured by immunoassays on the IMx analyzers (Abbott Diagnostics Laboratories, Abbott-Park, IL).

Tacrolimus dosage and blood trough concentration were investigated at 1 week, 2 weeks, and 1 month after transplantation. Doses required to achieve the target blood concentrations, dose-adjusted trough concentrations (concentration/dose [C/D] ratios)

and the types and incidences of adverse reactions of tacrolimus were compared among patients according to allelic status of *MDR1* (exons 12, 21, 26) and CYP3A5 (CYP3A5*1, *3, *6). This study was performed in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Seoul National University Hospital. Written informed consent was obtained from all subjects.

Results

Basic characteristics of patients are shown in Table 2.

Table 2. Pattern of genotypes in recipients and donors of liver transplantation

Genotype			**Recipients**	**Donors**
CYP3A5		*1/*1	3(7.0%)	2(4.7%)
		*1/*3	15(34.9%)	13(30.2%)
		*3/*3	25(58.1%)	28(65.1%)
		total	43(100%)	43(100%)
ABCB1	C3435T	CC	14(32.6)	14(32.6)
		CT	22(51.2)	21(48.8)
		TT	7(16.3)	8(18.6)
		total	43(100%)	43(100%)
	G2677T/A	GG	5(11.6)	7(16.3)
		GA	6(14.0)	5(11.6)
		GT	11(25.6)	13(30.2)
		AT	9(20.9)	7(16.3)
		TT	9(20.9)	11(25.6)
		AA	3(7.0)	0(0)
		total	43(100%)	43(100%)
	C1236T	CC	7(16.3)	5(11.6)
		CT	21(48.8)	21(48.8)
		TT	15(34.9)	17(39.5)
		total	43(100%)	43(100%)

No difference was observed in the distribution of genes between recipients and donors. As 69.8% of cases are blood related familial living donor liver transplantation, the association between familial relationship and genotype was investigated. 58.1% of CYP, 46.5% of *MDR1* 3435, 27.9% of 2677, and 60.5% of 1236 were identical in both recipient and donor.

However, no gene had significantly different distribution according to blood relationship (Chi-square test, $p<.05$). Significant difference in L/D was found between the recipient's CYP3A5 expressor versus nonexpressor genotypes. Carriers of CYP3A5*3/*3 allele showed higher L/D overall, which led less dose requirement than CYP3A5*1 carriers for similar drug level (Table 3).

Table 3. Comparison of Tacrolimus L/D and Recipient's CYP3A5 Genotype

		***1/*1(n=3)**	***1/*3(n=15)**	***3/*3(n=25)**	**Total(n=43)**	***P***
Dose (mg)	1week	1.66(.76)	1.9(1.17)	1.9(1.17)	1.9(1.17)	.904
	2week	3.16(.14)	3.30(1.28)	1.67(.85)	2.34(1.26)	.000
	3week	3.41(.87)	2.75(1.35)	2.17(.97)	2.45(1.15)	.100
	4week	3.16(.57)	3.21(1.31)	2.12(1.01)	2.57(1.21)	.012
Concentration	1week	6.03(3.52)	7.15(4.87)	9.88(3.95)	8.66(4.43)	.093
	2week	10.56(3.23)	11.34(2.56)	11.34(2.83)	11.29(2.70)	.896
	3week	8.00(1.90)	9.06(3.21)	10.28(3.28)	9.70(3.21)	.331
	4week	7.16(1.46)	7.45(2.21)	10.60(3.38)	9.26(3.29)	.005
L/D (ng/mL/mg)	1week	3.46(1.31)	4.23(2.88)	6.50(3.15)	5.50(3.16)	.042
	2week	3.36(1.14)	3.95(1.86)	9.07(5.94)	6.89(5.31)	.004
	3week	2.53(1.13)	5.11(5.94)	6.02(4.09)	5.46(4.71)	.463
	4week	2.32(.68)	2.74(1.60)	6.16(3.72)	4.70(3.44)	.003

The *MDR1* C1236T, G2677T and C3435T genotypes as well as donor's CYP3A5 polymorphisms had no effect on L/D after transplantation. The relationship between the

genotypes of *MDR1* and CYP3A5 and the adverse reaction of tacrolimus will be presented at the meeting.

Conclusion

This study confirms that tacrolimus dosing in adult liver transplant patients is associated with recipient's not donor's CYP3A5 gene polymorphisms.