

Regular Article

Impact of ABCC2, ABCG2 and SLCO1B1 Polymorphisms on the Pharmacokinetics of Pitavastatin in Humans

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Summary: Pitavastatin, a 3-hydroxyl-3-methylglutaryl-coenzyme A reductase inhibitor is distributed to the liver, a target organ of action and excreted mainly into the bile. To investigate the impact of influx (OATP1B1) and efflux (MRP2, BCRP) transporter alleles on its disposition, the pharmacokinetic (PK) parameters were compared among the following groups: *SLCO1B1* (*15 carrier and non-carrier), *ABCC2* (G1249A, C3972T, C–24T, G1549A, and G1774T), and *ABCG2* (C421A) single nucleotide polymorphisms in 45 healthy Korean volunteers. Pitavastatin AUC_{last} was higher in individuals carrying the *SLCO1B1**15 allele than those not carrying it (144.1 ± 55.3 vs. 84.7 ± 25.7 h·ng/mL [mean ± SD], *p* = 0.002). The AUC_{last} varied significantly according to the *ABCC2* C–24T allele (103.4 ± 42.2, 80.2 ± 23.8, and 39.0 h·ng/mL in CC, CT and TT, respectively; *p* = 0.027). Other SNPs of *ABCC2* and *ABCG2* were not significant. The effect of these transporters and body weight on the AUC_{last} and C_{max} were tested, and only *SLCO1B1* and *ABCC2* C–24T genotypes were significant factors by analysis of covariance. These variants accounted for almost 50% of the variation in AUC_{last} and C_{max} of pitavastatin. Therefore, *ABCC2* C–24T was significantly associated with pitavastatin human PK when the known effect of *SLCO1B1**15 was also considered.

Keywords: pitavastatin; MRP2; BCRP; OATP1B1; pharmacokinetic

Introduction

Pitavastatin is a potent competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and was approved for the treatment of dyslipidemia in 2009 by the Food and Drug Administration.¹⁾ The drug is more than 99% protein bound in human plasma,²⁾ and hardly metabolized by CYP450 enzymes.³⁾ In experimental animals, hepatoselective distribution to the liver, a target organ of the action, was shown.⁴⁾ Several membrane transporters are known to be involved in the hepatic distribution and elimination of pitavastatin.^{5–9)} Organic anion transporting polypeptide 1B1 (OATP1B1), which is encoded by *SLCO1B1* and located on the basolateral membrane of hepatocytes, is responsible for hepatocellular uptake of several drugs from portal blood.¹⁰⁾ Reduced activity of this transporter is associated with a decrease in the LDL-lowering effect and increased incidence of myopathy, likely due to lower hepatocellular and higher plasma

concentration of pravastatin.¹¹⁾ Although pitavastatin is more lipophilic than pravastatin or rosuvastatin,³⁾ OATP1B1 could explain approximately 90% of the total hepatic uptake of pitavastatin.¹²⁾ The *SLCO1B1**15 alleles (A388G and T521C) were found to increase systemic exposure and peak plasma concentrations of pitavastatin in healthy Korean,¹³⁾ Japanese⁷⁾ and Chinese¹⁴⁾ individuals. Following administration of a single 32 mg ¹⁴C-labeled pitavastatin dose, a mean of 15% of the radioactivity was excreted in the urine and 79% of the dose was excreted in the feces within 7 days.^{1,15)} Therefore, biliary excretion seems to be the primary route of pitavastatin elimination.

Various types of extrusion transporters may play a role in the biliary secretion of pitavastatin. Among the ATP-binding cassette (ABC) superfamily members, breast cancer resistance protein (BCRP; *ABCG2*), which is expressed on the apical membrane of hepatocytes,¹⁶⁾ has been reported to be involved in pitavastatin elimination in *Bcrp1*(–/–) mice.⁵⁾ In addition, multidrug resist-

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ance-related protein 2 (MRP2) encoded by *ABCC2* was linked to the hepatic clearance of several statins.^{5,17,18} MRP2 transports glutathione, glucuronide, and sulfate conjugates of endogenous and exogenous compounds into bile, feces, and urine,¹⁹ and its absence causes Dubin-Johnson syndrome, resulting in symptomatic conjugated hyperbilirubinemia. In heterozygotes for *ABCC2* C1446G ($n = 3$), systemic exposure and peak plasma concentration (C_{\max}) of pravastatin were lower than in wild-type homozygotes (CC, $n = 35$) as a consequence of increased MRP2 mRNA expression.¹⁸ However, the variation of this allele is synonymous and has been only reported in one small Caucasian population. *ABCC2* G1249A was not associated with altered pharmacokinetic (PK) profiles of pravastatin in European- or African-Americans,¹⁷ and 1774delG was a significant predisposing factor for cholestatic toxic liver injury in Korean patients.²⁰ *ABCC2* C-24T was reported to reduce the clearance of methotrexate,²¹ mycophenolic acid,^{22,23} and irinotecan.^{24,25} However, the relationship between *ABCC2* polymorphisms and the PK of statins is unknown except for pravastatin.¹⁸ The impact of *ABCC2* on the biliary excretion of pitavastatin was reported in rat models, but results were inconsistent. In MRP2-deficient (TR-) UNC (University of North Carolina) rats, the biliary excretion of pitavastatin was decreased,²⁶ but not in Eisai hyperbilirubinemic rats.⁵

This study aimed to investigate whether genetic polymorphisms of the *ABCC2* could influence human pharmacokinetics of pitavastatin. Given the dominating effect of *SLCO1B1**15 and known *ABCG2* effects, these genetic polymorphisms should be considered together to explore the net effect of *ABCC2* polymorphisms. We investigated the functional impact of polymorphisms of influx (OATP1B1) and efflux (MRP2, BCRP) transporters on the disposition of pitavastatin in healthy Korean individuals. This allowed us to evaluate the extent to which inter-individual PK variability can be explained by genetic polymorphisms of these three transporters.

Materials and Methods

Study participants: Forty-five healthy Korean men participated in this study after providing additional written consent for genotyping. They were enrolled from among 50 healthy male volunteers who had participated in a bioequivalence study of a 2 mg dose of pitavastatin. Genotypes were assessed retrospectively and were related to the PK results, and only data from the reference formulation were used in the current analysis. All participants were in good health, as ascertained by their medical history, physical examination, 12-lead electrocardiogram, and routine laboratory tests (hematology, blood chemistry, and urine analysis).

Clinical study: We obtained the PK data from the previous single dose study of pitavastatin 2 mg. The study protocol was approved by the Institutional Review Board of Severance Hospital in the Yonsei University Health System, Seoul, Korea. After an overnight fast, all participants were given a 2 mg tablet of pitavastatin calcium (Livalo®; ChoongwaePharma Corporation, Seoul, Korea) with 240 mL water at approximately 8 AM. Participants were kept in a fasting state until 4 h after drug administration, except water 1 h after dosing. Peripheral venous blood was collected in sodium heparin tubes before dosing and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after dosing. All blood samples were centrifuged immediately and stored at -70°C until analysis. Blood sampling for genotyping was also done before

drug administration, and genotyping was done after the end of the study. Participants were not permitted to take any medications; consume alcohol, beverages containing caffeine, or grapefruit products; or smoke during this study.

Genotyping:

ABCC2 and *SLCO1B1*

DNA was extracted from peripheral blood samples using the QIAmp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Genotyping for SNPs in *ABCC2* (rs1885301 [G1549A], rs2273697 [G1249A], rs3740066 [C3972T], rs717620 [C-24T], and DL1000980 [G1774T]) and *SLCO1B1* (rs2306283 [A388G] and rs4149056 [T521C]) was performed by the single base primer extension assay using the ABI PRISM SNaPshot Multiplex kit (Applied Biosystems, Foster City, CA) according to the manufacturer's recommendations. Briefly, the genomic DNA flanking the SNP of interest was amplified by PCR with forward and reverse primers and standard PCR reagents in a 10- μL reaction containing 10 ng genomic DNA, 0.5 pM each oligonucleotide primer, 1 μL 10 \times PCR buffer, 250 μM dNTPs (2.5 mM each), and 0.25 units i-StarTaq DNA polymerase (5 unit/ μL ; iNtRON Biotechnology, Sungnam, Kyungki-Do, Korea). The PCR reactions were performed as follows: one cycle of 10 min at 95 $^{\circ}\text{C}$, 35 cycles of 95 $^{\circ}\text{C}$ for 30 s, Tm for 1 min, and 72 $^{\circ}\text{C}$ for 1 min, followed by one cycle of 72 $^{\circ}\text{C}$ for 10 min (Tm: 50 $^{\circ}\text{C}$ for T521C, 55 $^{\circ}\text{C}$ for G1249A and C3972T, and 60 $^{\circ}\text{C}$ for other SNPs). After amplification, the PCR products were treated with 1 unit each of shrimp alkaline phosphatase (USB Corporation, Cleveland, OH) and exonuclease I (USB Corporation) at 37 $^{\circ}\text{C}$ for 75 min and 72 $^{\circ}\text{C}$ for 15 min to purify the amplified products. One microliter of the purified amplification products was added to a SNaPshot Multiplex Ready reaction mixture containing 0.15 pmol genotyping primer for the primer extension reaction. The primer extension reaction was performed using 25 cycles of 96 $^{\circ}\text{C}$ for 10 s, 50 $^{\circ}\text{C}$ for 5 s, and 60 $^{\circ}\text{C}$ for 30 s. The reaction products were treated with 1 unit of shrimp alkaline phosphatase at 37 $^{\circ}\text{C}$ for 1 h and 72 $^{\circ}\text{C}$ for 15 min to remove excess fluorescent dye terminators. One microliter of the final reaction samples containing the extension products was added to 9 μL Hi-Di formamide (Applied Biosystems). The mixture was incubated at 95 $^{\circ}\text{C}$ for 5 min, followed by 5 min on ice, and analyzed by electrophoresis in an ABI Prism 3730xl DNA analyzer. Analysis was performed using Genemapper software (version 4.0; Applied Biosystems).

ABCG2

The genotype of the *ABCG2* polymorphism rs2231142 (BCRP, C421A) was screened using the TaqMan fluorogenic 5' nuclease assay (ABI). The final volume of the PCR reaction mixture was 10 μL , including 10 ng genomic DNA, 5 μL TaqMan Universal PCR Master Mix, and 0.5 μL 20 \times Assay Mix (Assay ID C_15854163_70). Thermal cycling conditions were as follows: 50 $^{\circ}\text{C}$ for 2 min to activate uracil N-glycosylase and to prevent carry-over contamination, 95 $^{\circ}\text{C}$ for 10 min to activate DNA polymerase, followed by 50 cycles of 95 $^{\circ}\text{C}$ for 15 s, and 60 $^{\circ}\text{C}$ for 1 min. All PCR reactions were performed in a Dual 384-Well GeneAmp PCR System 9700 (ABI) in 384-well plates, and the endpoint fluorescent readings were performed on an ABI PRISM 7300 HT Sequence Detection System (Applied Biosystems). Duplicate samples and negative controls were included to ensure the accuracy of genotyping.

Drug concentration analysis and PK: Plasma concentrations of pitavastatin were determined with a validated high-performance

Table 1. Pharmacokinetic parameters of pitavastatin according to *ABCC2*, *SLCO1B1*, or *ABCG2* genotypes

Genotype (n)		C _{max} (ng/mL)	AUC _{last} (h·ng/mL)	AUC _{inf} (h·ng/mL)	t _{max} (h)	t _{1/2} (h)	CL/F (L/h)
<i>ABCC2</i>							
G1249A	GG (n = 33)	32.7 ± 12.1	89.6 ± 32.8	103.0 ± 37.3	0.8 [0.5–2.0]	10.0 ± 3.1	22.0 ± 8.0
	GA (n = 11)	37.1 ± 11.9	98.6 ± 30.8	113.0 ± 36.0	1.0 [0.8–1.3]	9.4 ± 4.1	19.9 ± 8.3
	AA (n = 1)	102.9	248.1	275.9	1.0	11.2	7.3
	p-value ^a	0.127	0.147	0.170	0.056	0.488	0.170
C3972T	CC (n = 29)	38.7 ± 17.9	103.2 ± 43.1	118.5 ± 47.9	0.6 [0.5–0.8]	10.0 ± 3.4	19.4 ± 7.4
	CT (n = 14)	28.4 ± 7.2	80.2 ± 22.9	91.5 ± 27.1	0.8 [0.5–1.3]	9.8 ± 3.1	23.7 ± 7.4
	TT (n = 2)	35.8 ± 16.4	86.5 ± 67.2	99.6 ± 75.3	1.0 [0.5–2.0]	8.8 ± 3.2	28.1 ± 21.3
	p-value ^b	0.097	0.174	0.154	0.045	0.738	0.154
C–24T	CC (n = 31)	38.1 ± 17.5	103.4 ± 42.2	118.7 ± 47.3	1.0 [0.5–2.0]	10.0 ± 3.4	19.3 ± 7.2
	CT (n = 13)	29.6 ± 9.2	80.2 ± 23.8	91.5 ± 27.0	0.8 [0.5–1.3]	9.8 ± 3.2	23.8 ± 7.6
	TT (n = 1)	24.2	39.0	46.3	0.5	6.5	43.2
	p-value ^a	0.057	0.027	0.028	0.034	0.411	0.028
G1549A	GG (n = 30)	38.4 ± 17.7	104.2 ± 42.7	119.8 ± 47.7	1.0 [0.5–2.0]	10.1 ± 3.4	19.2 ± 7.3
	GA (n = 13)	28.3 ± 7.5	76.0 ± 17.4	86.3 ± 19.7	0.6 [0.5–1.3]	9.6 ± 3.2	24.6 ± 6.9
	AA (n = 2)	35.8 ± 16.4	86.5 ± 67.2	99.6 ± 75.3	0.6 [0.5–0.8]	8.8 ± 3.2	28.1 ± 21.3
	p-value ^b	0.103	0.063	0.057	0.028	0.581	0.057
G1774T	GG (n = 21)	37.6 ± 19.1	96.1 ± 44.3	110.7 ± 50.2	1.0 [0.5–1.3]	10.5 ± 3.6	21.2 ± 8.8
	GT (n = 21)	31.6 ± 9.6	87.9 ± 28.2	100.8 ± 33.1	0.8 [0.5–2.0]	9.3 ± 3.0	22.1 ± 7.6
	TT (n = 3)	45.2 ± 23.4	141.7 ± 53.2	157.8 ± 53.6	1.0 [1.0–1.3]	9.7 ± 2.2	13.8 ± 5.0
	p-value ^b	0.591	0.183	0.218	0.097	0.607	0.218
<i>SLCO1B1</i>							
w/o *15 (n = 37)		30.7 ± 9.0	84.7 ± 25.7	98.5 ± 32.1	0.8 [0.5–2.0]	9.9 ± 3.5	22.7 ± 8.0
w *15 (n = 8)		56.9 ± 22.3	144.1 ± 55.3	159.1 ± 60.1	0.9 [0.8–1.3]	9.7 ± 2.0	14.0 ± 4.6
	p-value ^a	0.0002	0.002	0.004	0.527	0.929	0.004
<i>ABCG2</i>							
C421A	CC (n = 24)	34.7 ± 12.6	95.6 ± 38.2	108.8 ± 41.3	0.8 [0.5–1.3]	9.6 ± 3.1	21.1 ± 8.4
	CA (n = 16)	34.1 ± 20.2	92.3 ± 46.1	106.8 ± 53.6	0.8 [0.5–2.0]	9.7 ± 4.0	22.1 ± 8.7
	AA (n = 5)	21.1 ± 8.4	103.2 ± 25.5	118.9 ± 30.4	0.8 [0.5–1.3]	11.5 ± 1.3	17.9 ± 5.3
	p-value ^b	0.296	0.650	0.620	0.900	0.235	0.620

Values are presented as mean ± SD or median [min-max].

w/o *15 = *1a/*1a (n = 3) + *1a/*1b (n = 18) + *1b/*1b (n = 16); w *15 = *1b/*15 (n = 7) + *15/*15 (n = 1).

C_{max} = maximum plasma concentration, AUC_{last} = area under the plasma concentration-time curve from time 0 to the last quantifiable timepoint, AUC_{inf} = area under the plasma concentration-time curve from time 0 to infinity, t_{max} = time of occurrence of C_{max}, t_{1/2} = mean elimination half-life, CL = plasma clearance, F = bioavailability.

^aWilcoxon rank sum test; For G1249A, GG vs. GA+AA, C–24T, CC versus CT+TT.

^bKruskal-Wallis test.

liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) system. The system consisted of a HPLC (Thermo Surveyor, Thermo Fisher Scientific, Waltham, MA) and a triple quadrupole mass spectrometry (TSQ Quantum Discovery, Thermo Fisher Scientific). To each 300-μL plasma sample, 20 μL internal standard (IS) solution (1 mg/mL rosuvastatin in 50% methanol), 75 μL 0.1 M ammonium acetate (pH 4.0 adjusted with acetic acid), and 2.5 mL methyl *tert*-butyl ether were added, and the tube was vigorously mixed for 20 min. Each sample was centrifuged at 3,000 rpm for 5 min. The organic layer was transferred to a clean glass tube and evaporated to dryness under a stream of nitrogen gas. The residue was dissolved in 500 μL acetonitrile/water (6:4; v/v), and 10 μL was injected for HPLC/MS/MS analysis. A mobile phase of 10 mM ammonium acetate/acetonitrile/acetic acid (50:50:0.1 [v/v/v], pH 4.0) was used at a flow rate of 0.8 mL/min through a Unison UK-C₁₈ column (75 × 2.0 mm internal diameter, 3.0-μm particle size, Imtakt, Japan). The Xcalibur software package (version 2.0.7, Thermo Fisher Scientific) was used for instrument control, data acquisition, and processing. The lower limit of quantification was 0.5 ng/mL, and calibration curves were linear over the concentration range 0.5 to 200 ng/mL (r² > 0.99). The intra-day accuracy and precision of this assay were within the range of 87.6–98.9% and 3.1–7.8%, and the inter-day accuracy and precision were 91.7–104.4% and 6.3–8.8%, respectively.

PK parameters were calculated using actual sampling times. C_{max} was determined from the observed values. Plasma concentrations of the terminal phase were fitted to a log-linear line by the least squares method to obtain the terminal half-life. The area under the time-concentration curve (AUC) was calculated using a combination of the trapezoidal rule and extrapolation to infinity using the elimination rate constant. The apparent oral clearance (CL/F) was calculated as dose/AUC_{inf}. WinNonlin version 6.2 (Pharsight, Mountain View, CA) was used for the PK analysis.

Statistical analysis: To explore the potential association of each *ABCC2*, *SLCO1B1*, and *ABCG2* genotype with pitavastatin PK parameters, the Kruskal-Wallis test for three-group comparisons and the Wilcoxon rank sum test for two-group comparisons were made nonparametrically. The recessive homozygous groups having only one subject were included in heterozygotes in the statistical analysis. After dividing participants into two groups based on the presence of *SLCO1B1**15, the mean PK parameter values were compared according to the *ABCC2* C–24T allele, which was a significant factor together with the *SLCO1B1* haplotype in the analysis in **Table 1**. The effects of these two different genotypes on AUC and C_{max} were analyzed by two-way analysis of covariance (ANCOVA) using body weight as a covariate. Values of p < 0.05 were considered statistically significant. Statistical analyses were conducted using SAS 9.2 (SAS Institute Inc., Cary, NC).

Results

The 45 study participants were divided into two groups according to the *SLCO1B1**15 allele (*15 carriers and non-carriers) and into three groups based on the five *ABCC2* alleles (G1249A, C3972T, C-24T, G1549A, and G1774T) or *ABCG2* C421A SNPs to investigate the effects of these genotypes on the PK of pitavastatin. The mean age of all participants was 25.5 ± 3.7 years. Age, height, and body weight were not significantly different among the genotype groups for *SLCO1B1*, *ABCC2*, or *ABCG2* alleles (data not shown).

Systemic exposure and clearance of pitavastatin were significantly different among the genotype groups based on *SLCO1B1* and *ABCC2* C-24T. The mean AUC_{last} and C_{max} values in participants with *SLCO1B1**15 were 144.06 h-ng/mL and 56.88 ng/mL, respectively, which were 1.7 and 1.9-fold higher than values (84.73 h-ng/mL and 30.68 ng/mL, respectively) in those without *SLCO1B1**15. The mean clearance/bioavailability (CL/F) values were lower in participants with *15. Tendencies toward a decrease in AUC, as well as an increasing trend in CL/F, were observed on moving from *ABCC2* C-24T genotype group CC to CT to TT in sequence. No significant difference was observed with respect to the distribution of other genotypes compared in the present study (Table 1). Representative pitavastatin plasma concentration-time profiles versus genotypes are shown in Figure 1. When participants were divided into two groups for *SLCO1B1* based on the presence of *15, the mean AUC_{last} and C_{max} values varied significantly according to the *ABCC2* C-24T genotype in subjects without *15 (AUC_{last} , $p = 0.012$ and C_{max} , $p = 0.011$; Table 2), but not in those with *15 (AUC_{last} , $p = 0.371$ and C_{max} , $p = 0.233$; Table 2). The C_{max} and AUC_{last} of pitavastatin were also significantly different according to *ABCC2* G1549A genotype in participants without *SLCO1B1**15 (AUC_{last} , $p = 0.024$ and C_{max} , $p = 0.043$). *ABCC2* C-24T and *ABCC2* G1549A polymorphisms were in fairly strong linkage disequilibrium ($D' = 1.00$; $R^2 \geq 0.80$) using Haploview, version 4.2 (Daly Lab at the Broad Institute, Cambridge, MA).

ANCOVA with body weight as the covariate was used to ascertain whether *SLCO1B1* or *ABCC2* C-24T genotypes affected the PK of pitavastatin. AUC_{last} values were significantly dependent on *SLCO1B1* and *ABCC2* C-24T genotype (*SLCO1B1*, $p < 0.001$ and *ABCC2* C-24T, $p = 0.003$), but not on body weight ($p = 0.422$) (two-way ANCOVA, $R^2 = 0.4919$, $p < 0.001$). C_{max} values were also significantly associated with *SLCO1B1* and *ABCC2* C-24T genotype (*SLCO1B1*, $p < 0.001$ and *ABCC2* C-24T, $p =$

0.003), but not with body weight ($p = 0.516$) (two-way ANCOVA; $R^2 = 0.5376$, $p < 0.001$). There was no significant interaction between *SLCO1B1* and *ABCC2* C-24T genotype (AUC_{last} , $p = 0.154$ and C_{max} , $p = 0.116$).

Discussion

This study investigated the functional significance of the genetic variations of *SLCO1B1*, *ABCG2*, and *ABCC2* on the PK of pitavastatin. OATP1B1 was reported as the most important transporter that mediates hepatic uptake of pitavastatin in humans,¹² and the *SLCO1B1* T521C variant is the most extensively

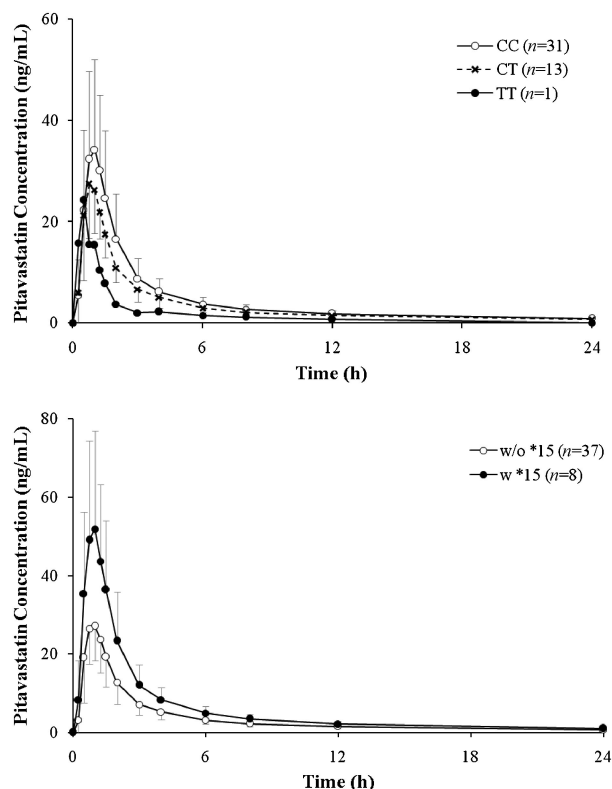


Fig. 1. Representative mean plasma concentration-time profiles of pitavastatin by *ABCC2* C-24T (top) or *SLCO1B1* haplotypes (bottom) after oral administration of 2 mg pitavastatin

w/o *15 = *1a/*1a + *1a/*1b + *1b/*1b; w/o *15 = *1b/*15 + *15/*15.

Table 2. Pharmacokinetic parameters of pitavastatin according to *SLCO1B1* and *ABCC2* C-24T genotypes

Genotype (n)		C_{max} (ng/mL)	AUC_{last} (h-ng/mL)	AUC_{inf} (h-ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	CL/F (L/h)
<i>SLCO1B1</i>	<i>ABCC2</i> C-24T						
w/o *15 (n = 37)	CC (n = 26)	32.9 ± 9.5	91.6 ± 25.5	106.6 ± 32.5	0.5 [1.0-2.0]	10.1 ± 3.5	20.7 ± 6.9
	CT (n = 10)	25.5 ± 5.2	71.5 ± 16.8	82.5 ± 20.3	0.8 [0.5-1.3]	9.8 ± 3.6	25.8 ± 7.4
	TT (n = 1)	24.2	39.0	46.3	0.5	6.5	43.2
	p-value ^a	0.011	0.012	0.018	0.134	0.455	0.018
w *15 (n = 8)	CC (n = 5)	65.0 ± 25.5	164.9 ± 60.5	181.7 ± 65.2	1.0 [0.8-1.3]	9.7 ± 2.4	12.3 ± 4.6
	CT (n = 3)	43.4 ± 3.5	109.3 ± 21.7	121.5 ± 27.3	0.8 [0.8-0.8]	9.7 ± 1.5	17.0 ± 3.4
	p-value ^a	0.233	0.371	0.233	0.077	1.000	0.233

Values are presented as mean \pm SD or median [min-max].

w/o *15 = *1a/*1a + *1a/*1b + *1b/*1b; w *15 = *1b/*15 + *15/*15.

C_{max} = maximum plasma concentration, AUC_{last} = area under the plasma concentration-time curve from time 0 to the last quantifiable timepoint, AUC_{inf} = area under the plasma concentration-time curve from time 0 to infinity, t_{max} = time of occurrence of C_{max} , $t_{1/2}$ = mean elimination half-life, CL = plasma clearance, F = bioavailability.

^aWilcoxon rank sum test; TT was included in CT of *ABCC2* C-24T in the group of w/o *15.

characterized polymorphism reducing transport activity,²⁷⁾ with a frequency of approximately 15% in Asian and Caucasian populations.²⁸⁾ In our study, significant PK differences were observed between participants carrying different *SLCO1B1* T521C genotypes. The AUC_{last} and C_{max} of pitavastatin were 1.7 and 1.9-fold higher, respectively, in individuals carrying the *SLCO1B1**15 allele than those without this allele. These results reaffirm similar results in previous studies that investigated healthy Japanese and Korean populations.^{7,29)}

As for *ABCC2* C-24T was significantly associated with reduced AUC_{last} and C_{max} of pitavastatin among *SLCO1B1**15 non-carriers. Although the results were not significant in the *SLCO1B1**15 group, possibly due to small sample size, a trend towards reduced mean PK parameters was also evident in this group, and *ABCC2* C-24T was a significant factor on ANCOVA. The findings related to *ABCC2* C-24T in this study differ from the results of an earlier study of methotrexate, which reported that pediatric acute lymphoblastic leukemia patients carrying the -24T allele showed higher exposure.²¹⁾ However, the patients with the -24T allele ($n = 4$) were all female, and a marked interaction between gender and the C-24T polymorphisms was shown with gender being a significant factor affecting methotrexate plasma concentration.²¹⁾ Other studies have reported that the -24T allele was associated with higher exposure of mycophenolic acid²²⁾ and irinotecan.^{24,25)} Although the substrates were different, these findings were clearly in contrast to our findings. At present, we speculate 2 possibilities for this discrepancy. Firstly, the C-24T variant could affect the pharmacokinetics of pitavastatin with increased *ABCB1* mRNA expression levels rather than *ABCC2* expression. *ABCC2* mRNA levels did not differ between C-24T genotype groups, but *ABCB1* mRNA levels showed a gene-dose-dependent increase according to the C-24T polymorphism in surgical specimens of the hippocampus of epileptic patients,³⁰⁾ and pitavastatin as a MDR1 substrate was demonstrated by Shirasaka et al.⁹⁾ In their investigations, the secretory (basal to apical) permeability of pitavastatin was about 3 fold that of absorptive (apical to basal) permeability in LLC-PK1 cells expressing rat *Mdr1a* (LLC-PK1/*Mdr1a*), and its transport was inhibited by P-glycoprotein (P-gp) inhibitor. The presence of P-gp in the biliary canalicular membrane in human tissues is well known.³¹⁾ Further investigation is needed to evaluate whether the -24T allele affects intestinal absorption or biliary excretion of pitavastatin. Secondly, the reabsorption of pitavastatin by enterohepatic circulation (EHC) could explain the unique result that the *ABCC2* C-24T allele was associated with lower plasma concentrations. Pitavastatin undergoes extensive EHC³⁾; thus, the effect of *ABCC2* C-24T on biliary excretion and intestinal efflux by increasing MDR1 expression possibly would be more prominent during the conditions of continuous recycling. In addition, in an experiment using oocytes of *Xenopus laevis*, pitavastatin lactone exhibited ATPase hydrolysis of canalicular P-gp, but the acid form did not.³²⁾ Hence, further study of whether the -24T allele will increase canalicular P-gp expression or activity, with measurements of pitavastatin lactone would be helpful in understanding the underlying mechanism.

There was no significant effect of *ABCG2* C421A on PK variability, a finding consistent with earlier studies. *ABCG2* C421A was not associated with pitavastatin PK in healthy Japanese volunteers.⁷⁾ Our study has some limitations, mostly stemming from its relatively small sample size and retrospective design. Data for *ABCC2* -24TT were obtained from only one participant due

to the low frequency of the allele in the Korean population,²⁰⁾ which makes it difficult to draw a firm conclusion. The insignificant results of G1249A, C3972T, and G1549A associated with pitavastatin PK could be introduced for the same reason. Nevertheless, the effects of *SLCO1B1**15 and *ABCC2* -24T genotype on the PK of pitavastatin were statistically significant.

Additionally, we did not determine pitavastatin lactone concentrations and the genotype of MDR1 which could be an additional confounder. This would support the understanding of the transport mechanisms of pitavastatin if these data were analyzed together. However, the relationship between *ABCB1* genotypes and the MDR1 expression has not been clearly established. The known alleles of *ABCB1* (C3435T or G2677T/A) might influence mRNA expression in different human tissues,³³⁻³⁵⁾ but the results have been discordant.³⁶⁾ As for OATP, we did not analyze the 1B3 allele, which is known to account for about 10% of total hepatic clearance of pitavastatin in an *in vitro* study.¹²⁾ We did not exclude the possible compensatory effect of OATP1B3 for hepatic uptake, but OATP1B1 is well known to be most important and contributing much more than OATP1B3 to hepatic uptake of pitavastatin.¹²⁾

However, to the best of our knowledge, this is the first report on the association between *ABCC2* C-24T and the PK of pitavastatin in humans. The C-24T polymorphism is located in the 5'-untranslated region (UTR),³⁶⁾ but this variant combined with the G1549A decreased MRP2 promoter activity by 39% in a functional molecular study.²⁰⁾ In a recent report, the genetic variants C-24T, G1249A, and C3972T appeared to influence transport capacity as a haplotype,³⁷⁾ and C3972T is highly linked with C-24T in all ethnic populations.⁶⁾ Even though G1249A, C3972T, and G1549A did not influence the PK of pitavastatin in our results (**Table 1**), the 1249AA group showed about 2.5-fold higher C_{max} and systemic exposure compared to the GG or GA. C_{max} of the 3972CC group was also higher than the value in the CT+TT group (38.7 ± 17.9 ng/mL and 29.3 ± 8.3 ng/mL, respectively; $p = 0.045$), and AUC_{last} in the 1549GG group was significantly higher than the value in the GG+AA group (104.2 ± 42.7 h-ng/mL and 77.4 ± 24.4 h-ng/mL, respectively; $p = 0.022$). Therefore, the dominant or recessive effect of these SNPs needs to be investigated further based on an exact functional study. *In vitro* and *in vivo* data on the transport function of the C-24T SNP with the use of pitavastatin as a substrate have not been available. Further functional studies also might elucidate the mechanisms that underlie this contribution of *ABCC2* C-24T polymorphisms to the inter-individual variability of pitavastatin PK.

Additionally, studies are needed in the primary care setting with respect to the clinical implications of these alleles. A lower therapeutic response in carriers of the -24T allele was reported in epileptic patients,³⁰⁾ but later reports showed controversial results.^{38,39)} In another interesting report, the *ABCC2* C-24T genotype was associated with significant dose decreases or switch to another cholesterol-lowering drug in simvastatin users, but not atorvastatin users.⁴⁰⁾ *SLCO1B1* polymorphism has also been reported to be associated with intolerance or pharmacodynamic response change after treatment with atorvastatin,⁴¹⁾ simvastatin,⁴¹⁻⁴³⁾ or pravastatin,^{41,44)} but a recent report showed no significant effect on the lipid-lowering response of pitavastatin in Chinese patients.⁴⁵⁾

To conclude, the *ABCC2* C-24T polymorphism was significantly associated with reduced plasma concentrations of pitavastatin when the known effect of *SLCO1B1**15 was also considered.

Both *SLCO1B1**15 and *ABCC2* C-24T genetic variants were significant factors accounting for about half of the inter-individual variability in systemic exposure and C_{max} of pitavastatin. Therefore, these genetic polymorphisms of OATP1B1 and MRP2 transporters may provide an explanation for the heterogeneity of pitavastatin PK in humans. A future study with a large sample will confirm the effect of *ABCC2* polymorphisms on the efficacy and adverse events in pitavastatin users.

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