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The influences of *SLCO1B1* and *ABCB1* genotypes on the pharmacokinetics of simvastatin, in relation to CYP3A4 inhibition

Aim: To investigate the combined effects of *SLCO1B1* and *ABCB1* genotypes on the pharmacokinetics of simvastatin and its active metabolite simvastatin acid, in relation to CYP3A4 inhibition. **Methods:** We conducted a single-dose pharmacokinetic study of simvastatin in 26 healthy volunteers screened for their *SLCO1B1* c.521T>C and *ABCB1* c.1236C>T–2677G>T–3435C>T genotypes, with and without amlodipine pretreatment. The genetic effects and drug-interaction effect on simvastatin pharmacokinetic parameters were analyzed using a linear-mixed model. **Results:** The *SLCO1B1* c.521T>C variant significantly increased exposure to simvastatin acid by around 40% ($p < 0.05$), similar to that caused by the amlodipine pretreatment. The *ABCB1* gene showed no influence on exposure to simvastatin or simvastatin acid. **Conclusion:** Only *SLCO1B1*, not *ABCB1* genotype, is likely to be associated with simvastatin-induced myopathy. *SLCO1B1* genotyping may be particularly beneficial in simvastatin users who are co-administered CYP3A4 inhibitors.

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Simvastatin is one of the world's most frequently prescribed drugs due to its excellent hypolipidemic performance and relatively low price as a generic drug. However, simvastatin is known for its higher association with myopathy than other statins [1], and this adverse effect is pharmacokinetics related [2]. The pharmacokinetics of simvastatin is mainly determined by its disposition (i.e., distribution and metabolism) rather than its absorption (which occurs rapidly and almost completely in the GI tract) (Figure 1). Simvastatin, as a prodrug, undergoes extensive metabolism (including first-pass metabolism) to the extent that its absolute bioavailability is less than 5% [3]. Metabolism, including nonenzymatic reversible hydrolysis into its active metabolite simvastatin acid and enzymatic Phase-I metabolism which is primarily mediated by the CYP enzyme CYP3A4 [4], predominantly takes place in the liver cells.

Distribution (e.g., influx and efflux across the liver cell membrane) of simvastatin is mainly through passive diffusion due to its lipophilic property [5]. Contrastingly, simvastatin acid is hydrophilic and therefore subject to active transport. The influx of simvastatin acid is mainly mediated by the OATP1B1 transporter, which is encoded by the *SLCO1B1* gene. Numerous genetic mutations, particularly the *SLCO1B1* c.521T>C variant (SNP ID: rs4149056; present in *SLCO1B1**5, *15, *16 and *17 haplotypes) have been confirmed to be associated with simvastatin's pharmacokinetics [6] and myopathy [7–9]. On the other hand, it is reported that the efflux of simvastatin acid is mediated by the P-gp transporter [10] which is encoded by the *ABCB1* gene, and the c.1236C>T–2677G>T(A)–3435C>T (SNP ID: rs1128503, rs2032582 and rs1045642, respectively) haplotype in this gene are associated with simvastatin's

Figure 1. Pathway of simvastatin disposition in the human gastrointestinal system based on previous reports.

M: Phase-I metabolites of simvastatin in lactone form; MA: Phase-I metabolites of simvastatin in acid form; SV: Simvastatin; SVA: Simvastatin acid.

pharmacokinetics [11] and adverse reactions [12], but study results have been conflicting [13,14]. Although the effects of *SLCO1B1* genotype and *ABCB1* haplotype on simvastatin disposition have been studied separately or (recently) within one study [14,15], their combined effects on the pharmacokinetics of simvastatin are unknown, particularly with the presence of a CYP3A4 inhibitor. And hence the combined risk of myopathy these factors pose is undetermined.

The primary objective of this study was to determine the influences of the *SLCO1B1* c.521T>C variant and the *ABCB1* c.1236C>T-2677G>T-3435C>T haplotype on the pharmacokinetics of simvastatin in

relation to CYP3A4 inhibition, in healthy volunteers. To analyze the *in vivo* functions of the minor alleles in both the *SLCO1B1* and *ABCB1* genes, we adopted a genetic polymorphism enrichment study design. To produce a CYP3A4 inhibitory effect, we pretreated subjects with amlodipine which is a known weak CYP3A4 inhibitor and the most commonly co-prescribed calcium-channel blocker with simvastatin [16]. To evaluate the influence of baseline CYP3A4 activity on the genetic effects of interest, we measured and compared all study subjects' CYP3A4 activity using the urinary 6 β -hydroxycortisol/cortisol index that has been adopted in other studies [17,18].

Methods

Subject selection based on *SLCO1B1*–*ABCB1* combined genotypes

We genotyped four polymorphic alleles, including the c.521T>C in the *SLCO1B1* gene, the c.1236C>T, c.2677G>T and the c.3435C>T in the *ABCB1* gene, in 489 unrelated Korean individuals. Four combined genotypes were selected as candidate genotypes: T/T-CGC/CGC, T/C-CGC/CGC, T/T-TTT/TTT and T/C-TTT/TTT (the T/T and T/C before the dash represent the genotypes of *SLCO1B1* c.521T>C; the CGC/CGC and TTT/TTT after the dash represent the haplotypes of *ABCB1* c.1236C>T–2677G>T–3435C>T). The genotyping methods have been described previously [19–21]. Individuals with the four candidate genotypes were contacted for further screening through physical examination and laboratory tests. It was calculated that a minimum of ten individuals would be required for each *SLCO1B1* and *ABCB1* genotype group to demonstrate a 30% difference in simvastatin pharmacokinetic parameters at a level of significance of $p = 0.05$ and power of 80%. In the end, 26 healthy volunteers (T/T-CGC/CGC, $n = 6$; T/T-TTT/TTT, $n = 10$; T/C-CGC/CGC, $n = 7$ and T/C-TTT/TTT, $n = 3$) were enrolled into the study. From 1 week prior to commencement and throughout the clinical trial, volunteers abstained from all the drugs and nutritional supplements, tobacco, alcohol, caffeine and grapefruit juice.

Clinical trial

The clinical trial was a fixed-sequence two-period study, as a substrate (S) period was followed by a substrate + inhibitor (S + I) period. The fixed-sequence design is popularly adopted in drug–drug interaction studies and supported by the US FDA and EMA. In the first period (designated as the control period), all study participants were admitted overnight to the clinical trial center and urine was collected for 12 h (from 8 PM to 8 AM). After fasting overnight, at 8 AM each participant received a single oral dose of 40 mg simvastatin (Chong Kun Dang Pharm, Seoul, Republic of Korea) with 240 ml of tap water. Twelve blood samples (7 ml draw volume of each sample) were collected from each participant via intravenous catheter before and at 0.33, 0.67, 1, 1.5, 2, 3, 4, 5, 7, 9 and 12 h post drug administration (sampling time was determined based on reported half-life for simvastatin and simvastatin acid which are around 3 h). The second period (designated as the amlodipine period) was similar to the first period in design. Following a 7-day washout period, participants took a pretreatment oral dose of 5 mg amlodipine (Ilhwa Co., Ltd, Gyeonggi-Do, Republic of Korea) at 8 AM for three consecutive days. Subject were then admitted

and fasted overnight, with urine collected from 8 PM to 8 AM. At 8 AM, each participant received an oral dose of amlodipine 5 mg together with a single oral dose of 40 mg simvastatin (Chong Kun Dang Pharm). The second period then followed the same procedures as the first. Vital signs and electrocardiograph were monitored during the trial and all adverse reactions were recorded. The study was approved by the Institutional Review Board of the Korean National Institute of Food and Drug Safety Evaluation and carried out in accordance with the Declaration of Helsinki. A written informed consent was obtained from each study participant. The clinical trial was conducted in the Clinical Trial Center of Samsung Hospital, Seoul, Republic of Korea. The Clinical Research Information Service, Republic of Korea (a primary registry in the WHO Registry Network) identifier is KCT0001119.

Drug analysis

Investigators who conducted the drug analysis were blinded to the subjects' treatments and genotypes. All samples were prepared and analyzed in triplicate, and mean values were used for data analysis. The plasma concentration of simvastatin and its active metabolite simvastatin acid were determined by ACQUITY ultra performance liquid chromatography (UPLC)/Xevo TQ Tandem mass (MS/MS) system (Waters Corp., MA, USA). A total of 0.5 ml of plasma was spiked with 50 μ l of lovastatin (0.25 mg/ml) as an internal standard, buffered with 0.2 ml of 0.02 M ammonium formate ($pH = 4.5$) and extracted with 3 ml of methyl tertiary butyl ether. After centrifugation (4000 rpm, 4°C, 5 min), the organic phase was evaporated to dryness by a gentle flow of nitrogen heated to 40°C. The residue was reconstituted in 0.2 ml of reconstitution solvent (0.1% formic acid: acetonitrile = 20:80, v/v) and 5 μ l of the sample was injected into an LC–MS/MS system. Chromatographic separation of the compounds was accomplished by ACQUITY UPLC BEH C18 Column (1.7 μ m, 2.1 mm $\times$ 50 mm, Waters Corp.). For the isocratic mobile phase, a mixture of 0.02 M ammonium formate ($pH = 4.5$) and acetonitrile was delivered at a flow rate of 0.3 ml/min. MS positive ion mode was chosen. Precursor-to-product ion reactions were monitored for simvastatin, simvastatin acid and lovastatin. The mass-to-charge ratios were 419.32/199.15, 437.3/285.2 and 405.3/199.15, respectively. The lower limits of quantification for simvastatin and simvastatin acid were 0.2 and 0.1 ng/ml, respectively. The interassay variation was less than 20%.

Urinary cortisol and its metabolite 6 β -hydroxycortisol were determined by ACQUITY UPLC/Xevo TQ MS/MS system (Waters Corp.). For each urine sample, 0.25 ml was transferred to an E-P tube and

then 0.25 ml phosphate-buffered solution and 20 μ l internal standard (6 α -methylprednisol) was added. After mixing, 50 μ l of the sample was loaded to an SPE cartridge which was preconditioned and equilibrated with methanol followed by water. The analyte was eluted with 1 ml methanol and the eluate was then evaporated to dryness by a gentle flow of nitrogen heated to 45°C. The residue was reconstituted in 50 μ l reconstitution solvent (30% methanol) and then 5 μ l of the sample was injected into an LC–MS/MS system. Chromatographic separation of the compounds was accomplished by an ACQUITY UPLC BEH C18 column (1.7 μ m, 2.1 mm $\times$ 50 mm, Waters Corp.). The gradient mobile phase consisted of 5 mM ammonium formate (pH = 3.0) and 0.1% formic acid in acetonitrile delivered at a flow rate of 0.3 ml/min. MS negative ion mode was chosen. For cortisol, 6 β -hydroxycortisol and 6 α -methylprednisolone, the precursor-to-product ion reactions were monitored and their mass-to-charge ratios were 407.4/331.35, 423.4/347.3 and 419.4/343.3, respectively. The lower limits of quantification for cortisol and 6 β -hydroxycortisol were 2.5 and 10 ng/ml, respectively. The interassay variation was less than 20%.

Data analysis

Genetic information obtained from the 489 Koreans was tested for linkage disequilibrium using the haplotype analysis software Haploview 4.2 (Broad Institute, MA, USA). The pharmacokinetic parameters of simvastatin and simvastatin acid were derived from plasma concentration data by a noncompartmental method using Phoenix WinNonlin 6.3 (Pharsight Corp., CA, USA). And for statistical analysis, all pharmacokinetic parameters were log-transformed for further analysis. Effects of *SLCO1B1* and *ABCB1* genotypes and CYP3A4 inhibition, as well as their interactions were analyzed by a linear-mixed model using SPSS software 21.0 (SPSS Inc., IL, USA). The effect of CYP3A4 inhibition was estimated as a repeated variable, the *SLCO1B1* and *ABCB1* genotypes and CYP3A4 inhibition were set as fixed effects, and subject ID was set as a random effect. The comparison of exposure of simvastatin acid between subjects of the *SLCO1B1* c.521T/T genotype in control period and subjects of the *SLCO1B1* c.521T/C genotype in amlodipine period was conducted using an independent samples *t*-test. $p < 0.05$ was considered statistically significant.

Results

SLCO1B1–*ABCB1* combined genotypes in 489 unrelated Koreans

A total of 49 *SLCO1B1*–*ABCB1* combined genotypes were identified in 489 unrelated Korean individuals: 23 of the combined genotypes showed a frequency of

more than 1%, and the remaining 26 combined genotypes accounted for 9.2% (Table 1). In the *ABCB1* gene, the c.1236C>T, c.2677G>A(T) and c.3435C>T alleles were in strong linkage disequilibrium. However, the *SLCO1B1* c.521T>C allele was not linked with any of the three alleles in the *ABCB1* gene (data were not shown). Four combined genotypes were chosen for study: the 3rd (T/T-TTT/TTT), the 8th (T/T-CGC/CGC), the 14th (T/C-TTT/TTT) and the 18th (T/C-CGC/CGC). Due to difficulty in recruiting individuals with the 18th (T/C-CGC/CGC) genotype, two individuals from the 15th (T/C-CGC/TGC) genotype were included instead (the two genotypes differed by one *ABCB1* c.1236C>T allele).

Baseline CYP3A4 activity

Baseline CYP3A4 activity measured before the first control period and before the second period was significantly correlated ($r^2 = 0.61$; $p = 0.002$) but not statistically different ($p = 0.86$; paired *t*-test). Baseline CYP3A4 activity was significantly correlated with simvastatin $C_{\max}$ and AUC_{0-12} in control period, but showed no correlation with any pharmacokinetic parameters of simvastatin or simvastatin acid in amlodipine period. Baseline CYP3A4 activity is presented in Supplementary Table 1.

Effects of CYP3A4 inhibition

After pretreatment of amlodipine, exposure (AUC_{0-12} and $C_{\max}$) to simvastatin was increased by around 80% ($p < 0.01$) and exposure to simvastatin acid was increased by around 40% ($p < 0.01$). The $t_{1/2}$ of simvastatin was increased by 40% ($p < 0.01$), whereas the $t_{1/2}$ of simvastatin acid showed no significant change. Results are shown in Figure 2 and Table 2.

Effects of *SLCO1B1* & *ABCB1* genotypes

Exposure (AUC_{0-12} and $C_{\max}$) to simvastatin was not different between the *SLCO1B1* genotypes. The $t_{1/2}$ of simvastatin and exposure to simvastatin acid were around 40% higher in the *SLCO1B1* T/C group than in the T/T group ($p < 0.05$; Table 2). No pharmacokinetic parameters were significantly different between the *ABCB1* genotypes, other than the $t_{1/2}$ of simvastatin, which was about 30% higher in the *ABCB1* TTT/TTT group than in the CGC/CGC group (Table 2). The pharmacokinetic parameters of simvastatin and simvastatin acid in each subgroup are shown in Supplementary Table 2.

Interactions between *SLCO1B1* & *ABCB1* genotypes & CYP3A4 inhibition

The linear-mixed model analyses showed that the *SLCO1B1* and *ABCB1* genetic effects, as well as the

Table 1. Frequencies of *SLCO1B1*–*ABCB1* combined genotypes in 489 unrelated Korean individuals.

Combined genotype number	SLCO1B1		ABCB1		Counts (frequency)
	T521C	C1236T	G2677T(A)	C3435T	
1	T/T	C/T	G/T	C/T	58 (11.9%)
2	T/T	T/T	G/T	C/T	45 (9.2%)
3	T/T	T/T	T/T	T/T	41 (8.4%)
4	T/T	C/T	G/G	C/C	40 (8.2%)
5	T/T	C/T	A/T	C/T	35 (7.2%)
6	T/T	C/C	G/A or G/T	C/C	32 (6.5%)
7	T/T	C/T	G/A	C/C	24 (4.9%)
8	T/T	C/C	G/G	C/C	18 (3.7%)
9	T/C	C/T	G/T	C/T	18 (3.7%)
10	T/T	T/T	G/G	C/C	17 (3.5%)
11	T/C	C/T	A/T	C/T	17 (3.5%)
12	T/C	T/T	G/T	C/T	14 (2.9%)
13	T/T	T/T	T/T	C/T	12 (2.5%)
14	T/C	T/T	T/T	T/T	11 (2.2%)
15	T/C	C/T	G/G	C/C	9 (1.8%)
16	T/T	C/C	A/A	C/C	8 (1.6%)
17	T/T	C/T	A/A or A/T	C/C	8 (1.6%)
18	T/C	C/C	G/G	C/C	8 (1.6%)
19	T/C	C/C	G/A	C/C	8 (1.6%)
20	T/C	C/T	G/A	C/C	6 (1.2%)
21	T/T	C/C	G/A or G/T	C/T	5 (1.0%)
22	T/T	C/T	G/T	T/T	5 (1.0%)
23	T/C	T/T	G/G	C/C	5 (1.0%)
24–49	All the others				45 (9.2%)
Total					489 (100%)
Allele frequencies					
T (87%)		C (42%)	G (47%) [†]	C (63%)	
C (13%)		T (58%)	T (37%)	T (37%)	
Genotype frequencies					
T/T (1%)		C/C (18%)	G/G (22%)	C/C (40%)	
T/C (23%)		C/T (49%)	G/T (32%)	C/T (45%)	
C/C (76%)		T/T (33%)	T/T (14%)	T/T (14%)	
[†] Only the frequencies of G and T alleles (but not A allele) were presented.					

[†]Only the frequencies of G and T alleles (but not A allele) were presented.

CYP3A4 inhibitory effect, were independent from each other and no significant interaction was detected. The additive effects of two significant factors, the *SLCO1B1* genotype and CYP3A4 inhibition, on the exposure of simvastatin acid were compared between two extreme conditions: *SLCO1B1* c521T/T in control period versus *SLCO1B1* c521T/C in amlodipine period, the $C_{\max}$ and AUC_{0-12} of simvastatin acid were

around 90% higher in the latter than in the former (Figure 3).

Discussion

In this study, we evaluated the pharmacokinetic characteristics of simvastatin and simvastatin acid in healthy volunteers, with the presence of two established risk factors (*SLCO1B1* genetic mutation and

Figure 2. Mean concentration–time curves of simvastatin and simvastatin acid following administration of a single oral dose of 40 mg simvastatin, displayed by control period (hollow) and amlodipine pretreatment (5 mg/day for 4 days) period (solid).

CYP3A4 inhibition) and one risk factor with conflicting reports (*ABCB1* genetic mutation) of simvastatin-induced myopathy. This study provides an insight into the general pharmacokinetic mechanisms behind simvastatin-induced myopathy related to various factors. The frequencies of the *SLCO1B1* c.521C allele (13.0%) and the *ABCB1* c.1236T–2677T–3435T haplotype (33%) observed in our study were similar to those previously reported [22,23]. Despite the sample sizes being unbalanced between the two genes (i.e., the number of *SLCO1B1* T/T vs T/C = 16:10; *ABCB1* CGC/CGC vs TTT/TTT = 13:13), their effects can be measured and compared using a linear-mixed model [24]. In addition, baseline CYP3A4 activity measurement

ruled out possible false genetic relationships caused by unbalanced CYP3A4 activity between genotypes. The effects of CYP3A4 inhibitors on the pharmacokinetics of simvastatin have been previously well characterized. Co-administration of simvastatin with strong inhibitors of CYP3A4 (such as itraconazole, clarithromycin, grapefruit juice, among others) is contraindicated. On the contrary, co-administration of simvastatin and CYP3A4 weak inhibitors is very common in the clinical setting. To minimize the risk of adverse effects, we selected a low-dose (5 mg), short-term (4 day) pretreatment regimen of weak CYP3A4 inhibitor amlodipine to produce drug interactions. In our study, amlodipine increased the exposure (AUC_{0-12}

Table 2. Effects of <i>SLCO1B1</i> , <i>ABCB1</i> genotypes and amlodipine pretreatment on the pharmacokinetic parameters of simvastatin and simvastatin acid, using linear-mixed model analyses.				
Pharmacokinetic parameters		Geometric mean ratio (95% CIs)		
		<i>SLCO1B1</i> genotype T/C (n = 10; T/T as Ref.; n = 16)	<i>ABCB1</i> genotype TTT/TTT (n = 13; CGC/CGC as Ref.; n = 13)	Amlodipine period (n = 26; control as Ref.; n = 26)
Simvastatin				
AUC_{0-12}	ng ml ⁻¹ h	0.87 (0.54–1.39)	1.15 (0.73–1.82)	1.78 (1.51–2.12)**
C_{max}	ng ml ⁻¹	0.72 (0.44–1.16)	0.68 (0.43–1.08)	1.77 (1.40–2.24)**
$t_{1/2}$	h	1.41 (1.10–1.82)**	1.32 (1.03–1.69)*	1.37 (1.10–1.70)**
Simvastatin acid				
AUC_{0-12}	ng ml ⁻¹ h	1.41 (1.03–1.94)*	0.95 (0.69–1.29)	1.37 (1.19–1.57)**
C_{max}	ng ml ⁻¹	1.43 (1.03–2.00)*	0.89 (0.64–1.23)	1.40 (1.18–1.65)**
$t_{1/2}$	h	1.00 (0.74–1.36)	1.07 (0.80–1.43)	1.16 (0.93–1.45)
*p < 0.05. **p < 0.01.

Figure 3. Box plots of exposure ($C_{\max}$ and AUC_{0-12}) of simvastatin acid, compared between subjects of *SLCO1B1* c.521T/T genotype in control period (white box) and subjects of *SLCO1B1* c.521T/C genotype in amlodipine period (gray box), following administration of a single oral dose of 40 mg of simvastatin. The black lines across each box represent the median. The bottom and the top edge represent the first and the third quartile, respectively. The horizontal lines connected to the whiskers denote the 1.5-times interquartile range. The circles indicate outliers from the 1.5-times interquartile range. The p-values were obtained from independent samples t-test. GMR and 95% CI were used to indicate the folds of difference between the two compared groups. GMR: Geometric mean ratio.

and $C_{\max}$) and $t_{1/2}$ of simvastatin by around 80 and 40%, respectively, which is in agreement with previous studies [25,26]. We estimate that about half of the 80% increased exposure of simvastatin can be attributed to decreased clearance because of the inhibition

of hepatic CYP3A4 (indicated by the 40% increased $t_{1/2}$). The remaining 40% increase of exposure is likely due to a lowered first pass elimination following the inhibition of intestinal CYP3A4. Despite simvastatin acid being a CYP3A4 substrate [27], in our study we

only observed a 40% increase in its exposure but no change in its $t_{1/2}$ after pretreatment of amlodipine. The different effects of CYP3A4 inhibition on simvastatin and simvastatin acid are likely because simvastatin rapidly enters the liver cells via passive diffusion and is extensively metabolized by the CYP3A4 enzyme, but the entering of simvastatin acid is mainly subject to OATP1B1 transport activity and hence its clearance is less likely to be influenced by CYP3A4 inhibition.

Our study, together with previous reports, showed that the *SLCO1B1* c.521T>C variant only increased the exposure to simvastatin acid, but not simvastatin [6,15]. In addition, the relationship between the *SLCO1B1* c.521T>C variant and simvastatin-induced myopathy has been well established at different dosage levels (ranging from 30 to 80 mg/day), the relative risk of one copy variant is around 3 [7–9]. The established association resulted in the Clinical Pharmacogenetics Implementation Consortium Guideline which recommends a lower dose of simvastatin for patients carrying the variant [2]. While an increased exposure to simvastatin acid (but not simvastatin) may appear to be the key pharmacokinetic mechanism behind the myopathy among the *SLCO1B1* c.521T>C carriers, less than one-fifth of these carriers will go on to develop this adverse effect [9] which suggests the presence of other nonpharmacokinetic contributing factors. A recent *in vitro* study suggests that decreased body fluid pH may play an important role in myotoxicity of simvastatin and simvastatin acid, particularly at higher drug concentrations [28], such are the cases in *SLCO1B1* c.521T>C carriers and patients taking CYP3A4 inhibitors. It is known that diseases causing metabolic acidosis, such as diabetes and renal impairment are risk factors for simvastatin-induced-rhabdomyolysis [29]. A few documented cases of simvastatin-induced rhabdomyolysis reported incidences of metabolic acidosis [30–32]. Future studies in patients are needed to investigate the relationship between the *SLCO1B1* genotype, CYP3A4 inhibition and other nonpharmacokinetic contributing factors, such as blood pH value, on simvastatin-induced myopathy.

To date, *SLCO1B1* genotyping is not routinely performed because cost-effectiveness has not been established. To investigate the cost-effectiveness, it would be prudent to start from patients who are most likely to benefit from the *SLCO1B1* genotyping, for instance, simvastatin users who are co-administered CYP3A4 inhibitors. In our study, amlodipine pretreatment and the *SLCO1B1* c.521T>C variant (one copy) showed similar effects on the exposure of simvastatin acid, they both increased the exposure of simvastatin acid by around 40% and subjects with the two risk factors showed around 90% higher exposure of simvastatin acid, compared with those without. We estimate that

patients with both risk factors are likely to have a much higher chance of developing myopathy. In addition, it is worth noting that both the FDA and the UK Medicines and Healthcare products Regulatory Agency propose a 50% lower dose of simvastatin for patients who coadminister amlodipine – it is unknown how this practice will influence the clinical outcomes of simvastatin treatment, particularly in patients carrying the *SLCO1B1* c.521T>C variant, who are less responsive to simvastatin [33,34]. In conclusion, *SLCO1B1* genotyping may be critical for simvastatin users who unavoidably take CYP3A4 inhibitors such as amlodipine, in order to lower the risk of adverse effects and impaired drug efficacy. Given the current dosage recommendation needs more evidence, if genotyping results identify a patient as an *SLCO1B1* c.521C carrier, changing prescription to an alternative statin (such as atorvastatin) may be preferable than lowering the simvastatin dosage.

Controversy over the roles of P-gp in the transport of simvastatin acid is long standing, arising from *in vitro* [10,13,35] and *in vivo* [11,14,15] studies which disagree on whether simvastatin acid is a P-gp substrate, and whether *ABCB1* genetic polymorphisms influence the pharmacokinetics and pharmacodynamics of simvastatin. Both of these positive and negative studies are well-cited, making the controversy more confusing. Our study and more recent studies [13–15] tend to report negative results, suggesting simvastatin acid is not subject to significant intestinal transport and *ABCB1* genotype does not impact the pharmacokinetics of simvastatin acid. In addition, the replicated *SLCO1B1* genetic effect and CYP3A4 inhibitory effect in this study serve as a study quality control for our conclusion on the *ABCB1*. Furthermore, a widely overlooked fact is that little simvastatin acid is present in human bile following oral administration [36,37], which rules out any significant impact of hepatic efflux transporter activity on the pharmacokinetics of simvastatin acid. Collectively, P-gp activity, as well as the genetic polymorphisms in the *ABCB1* gene, is unlikely to be important to the pharmacokinetics of simvastatin acid. *ABCB1* genotyping may have little value in optimizing simvastatin treatments.

There are several limitations in the current study. First, due to our limited sample size, it was impossible to include more genetic polymorphisms in other less-studied genes (such as *SCLO1B3*, *CYP2D6*, among others) [14,38]. Second, due to the low frequency of the *SLCO1B1* c.521C/C genotype in the Korean population, we did not include the c.521C/C genotype and therefore we were unable to observe the potential gene-dose effect. Our genetic polymorphism enrichment study design also made subject recruitment more challenging and, because we assumed it was unlikely to impact the results greatly, two subjects with the

SLCO1B1 T/C and *ABCB1* CGC/TGC genotype were recruited and placed into the same group as the T/C-CGC/CGC genotype.

Conclusion

Despite these limitations, we hope this study will contribute to our understanding of simvastatin-induced myopathy from a pharmacokinetic perspective. Our results indicate increased exposure to simvastatin acid as a general pharmacokinetic characteristic of two independent risk factors (the *SLCO1B1* c.521T>C variant and CYP3A4 inhibition) for simvastatin-induced-myopathy. However, more studies are needed to understand the contribution of nonpharmacokinetic factors to the myotoxicity of simvastatin acid. *SLCO1B1* genotyping may be particularly beneficial in simvastatin users who are co-administered CYP3A4 inhibitors, to avoid risks of myopathy. On the contrary, *ABCB1* genotyping may have little value in optimizing simvastatin treatments.

Financial & competing interests disclosure

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Ethical conduct of research

The study was approved by the Institutional Review Board of the Korean National Institute of Food and Drug Safety Evaluation and carried out in accordance with the Declaration of Helsinki. A written informed consent was obtained from each study participant. The clinical trial was conducted in the Clinical Trial Center of Samsung Hospital, Seoul, Republic of Korea. The Clinical Research Information Service (CRIS), Republic of Korea (a primary registry in the WHO Registry Network) identifier is KCT0001119.

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Executive summary

- Drug metabolizing enzyme CYP3A4 and drug transporters OATP1B1 and P-gp have been studied extensively for their associations with simvastatin's pharmacokinetics and pharmacodynamics.
- CYP3A4 inhibition and the *SLCO1B1* (OATP1B1 encoding gene) c.521T>C variant are established risk factors for simvastatin-induced myopathy, whereas reports on the *ABCB1* (P-gp encoding gene) genetic mutations have been conflicting.
- This study investigated the combined effects of the *SLCO1B1* c.521T>C variant and the *ABCB1* c.1236C>T–2677G>T–3435C>T haplotype on simvastatin's pharmacokinetics in healthy volunteers, with and without a pretreatment of a CYP3A4 inhibitor amlodipine.
- Our study showed that the *SLCO1B1* c.521T>C variant caused a 40% increase ($p < 0.05$) in the exposure of simvastatin acid (but not simvastatin).
- Amlodipine showed a similar effect on simvastatin acid as the *SLCO1B1* genetic effect, but it also significantly increased the exposure of simvastatin by 80% ($p < 0.01$).
- The *ABCB1* haplotype did not show significant effect on the exposure of simvastatin and simvastatin acid.
- Increased exposure of simvastatin acid is the general pharmacokinetic characteristics underlying the two established risk factors, meanwhile, contributions by other nonpharmacokinetic factors to the adverse effect are suggested.
- *SLCO1B1* genotyping may be particularly beneficial in simvastatin users who are co-administered CYP3A4 inhibitors, to avoid risks of myopathy and lower efficacy.
- *ABCB1* genotyping may have little value in optimizing simvastatin treatments.

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