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Simvastatin pharmacokinetics in healthy Chinese subjects and its relations with CYP2C9, CYP3A5, ABCB1, ABCG2 and SLCO1B1 polymorphisms

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A pharmacokinetic study of simvastatin (single oral dose, 40 mg) was conducted in 17 healthy Chinese volunteers. Plasma concentrations of simvastatin were determined by an LC-ESI-MS-MS method. The pharmacokinetic parameters of simvastatin were derived with a non-compartmental method. The polymorphisms of CYP2C9, CYP3A5, ABCB1 (encoding P-gp), ABCG2 (encoding BCRP) and SLCO1B1 (encoding OATP1B1) were determined by TaqMan® genotyping assay and the impacts of these SNPs on the pharmacokinetics of simvastatin were analyzed. Major pharmacokinetic parameters were as follows: T_{max} 1.44 ± 0.39 h, C_{max} 9.83 ± 2.41 μg·L^{-1}, $t_{1/2}$ 4.85 ± 1.23 h and $AUC_{(0-\infty)}$ 40.32 ± 6.82 μg·h·L^{-1}. Effect of ABCB1 G2677T/A SNP on dispostion of simvastatin was observed. Significant differences in $t_{1/2}$, but not C_{max} and $AUC_{(0-\infty)}$, were observed between G/A, G/T or G/G carriers and non-G carriers (5.65 ± 0.50 h *vs* 4.41 ± 1.31 h, $P < 0.05$). There were no significant effects on simvastatin pharmacokinetics by SNPs such as CYP2C9*3 (1075A > C), CYP3A5*3 g.6986A > G, ABCB1 C3435T, ABCG2 c.34G > A, ABCG2 c.421C > A, SLCO1B1 c.388 A > G, SLCO1B1 c.521 T > C, SLCO1B1 g.11187 G > A, SLCO1B1 c.571 T > C and SLCO1B1 c.597 C > T. We conclude here that there is a small inter-subject variation in simvastatin pharmacokinetics in healthy Chinese volunteers. P-gp, OATP1B1 and BCRP seem unlikely to play an important role in the pharmacokinetics of simvastatin. The gene-dose effects of ABCG2 c.421 C > A and CYP3A5*3 g.6986A > G on simvastatin pharmacokinetics are not strong enough in Chinese subjects.

1. Introduction

Simvastatin, administered in an inactive lactone form, was the first HMG-CoA reductase inhibitor. It significantly reduces low density lipoprotein cholesterol (LDL-C) and consistently increases high density lipoprotein cholesterol (HDL-C), thus being prescribed for hypercholesterolaemia and the prevention of cardiovascular diseases.

Simvastatin undergoes extensive first-pass metabolism in the intestinal wall and liver, which is primarily mediated by CYP3A4/5. A study in Korea revealed that the polymorphic CYP3A5 gene could affect the disposition of simvastatin (Kim et al. 2007). *In vitro* studies indicated that simvastatin was not a substrate for P-gp (encoded by ABCB1) and BCRP (encoded by ABCG2) (Li et al. 2011). However, associations between the ABCB1 gene variants and efficacy or tolerance of simvastatin therapy were reported (Bceker et al. 2009). Genetic variability in ABCG2 markedly affects the pharmacokinetics of fluvastatin and simvastatin lactone, but has no significant effect on pravastatin or active simvastatin acid (Keskitalo et al. 2009). There were several studies with controversial conclusions with repect to SLCO1B1 c.388A > G and 521T > C polymorphisms on clinical response of simvastatin in different populations (Sortica et al. 2012a; Hu et al. 2012b).

Up to now, there is little knowledge about the effects of genetic polymorphism in drug transporters and metabolizing enzymes on pharmacokinetics of simvastatin in the Chinese population. This study aimed to explore the effects of polymorphisms in ABCB1, ABCG2, SLCO1B1, CYP2C9 and CYP3A5 on the pharmacokinetics of simvastatin in healthy male Chinese volunteers after the administration of a single dose of simvastatin.

2. Investigations and results

2.1. Concentration-time curves and pharmacokinetics of simvastatin

The average concentration-time curve is shown in Fig. 1. The main pharmacokinetic parameters including C_{max}, $AUC_{(0-\infty)}$, t_{max} and $t_{1/2}$ were as follows: C_{max} 9.83 ± 2.41 μg·L^{-1}, t_{max} 1.44 ± 0.39 h, $T_{1/2}$ 4.85 ± 1.23 h, $AUC_{(0-\infty)}$ 40.32 ± 6.82 μg· h·L^{-1}.

2.2. Effects of SNPs in drug transporters and CYPs on the pharmacokinetics of simvastatin

Significant differences in $t_{1/2}$, but not C_{max} and $AUC_{(0-\infty)}$, were observed between G/A, G/T or G/G carriers and non-G carriers (5.65 ± 0.50 h *vs* 4.41 ± 1.31 h, $P<0.05$). There were no statistically significant effects on simvastatin pharmacokinetics by ABCB1 halotype derived from C1236T, G2677T/A

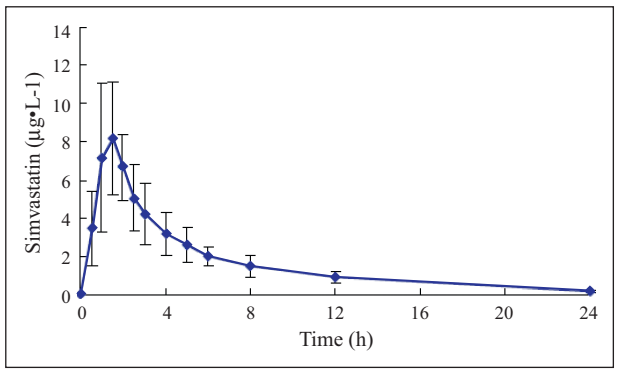

Fig. 1: Mean plasma concentration-time curves of simvastatin after a single dose of 40 mg simvastatin tablets to 17 healthy Chinese volunteer. Values are represented as mean±SD

and C3435T and SNPs such as CYP2C9*3(1075A > C), CYP3A5*3 g.6986A > G, ABCB1 C3435T, ABCG2 c.34G > A, ABCG2 c.421C > A, SLCO1B1 c.388 A > G, SLCO1B1 c.521 T > C, SLCO1B1 g.11187 G > A, SLCO1B1 c.571 T > C and SLCO1B1 c.597 C > T (Tab.).

3. Discussion

Becker et al. (2009) reported associations between the ABCB1 variants and reductions in cholesterol levels after the start of simvastatin therapy. Male simvastatin users with the ABCB1 1236/2677/3435 TTT haplotype showed larger reductions in total cholesterol and LDL-C compared with the wild-type CGC haplotype. The simvastatin acid $AUC_{(0\text{-}12h)}$ was 60% larger in subjects with the ABCB1 TTT/TTT genotype ($n = 12$) than in those with the CGC/CGC genotype ($n = 12$) ($P < 0.05$), but there was no difference between the two genotypes with respect to the pharmacokinetics of the lactones of simvastatin (Keskitalo et al. 2008). Li et al. (2011) investigated the *in vitro* efflux of statin drugs using transporter knockdown Caco-2 cells and revealed that atorvastatin, fluvastatin and rosuvastatin were transported by P-gp, BCRP, and MRP2 whereas neither the lactone nor the acid of lovastatin and simvastatin was transported by P-gp, BCRP or MRP2. Our study also found no significant differences in AUC and C_{max} values of simvastatin when volunteers were grouped according to ABCB1 halotype derived from C1236T, G2677T/A and C3435T or disparate SNPs.

P-gp is an efflux transporter. Previous studies have shown that the ABCB1 G2677T and ABCB1 C3435T genes are significantly associated with decreased expression of P-gp protein (Chen et al. 2009; Gonzalez et al. 2008). Our study strongly indicates that P-gp does not play an important role in the pharmacokinetics of simvastatin. Although the effect of ABCB1 G2677T/A SNP on $t_{1/2}$ of simvastatin was statistically significant, it seemed to be not clinically significant.

Many SNPs have been identified in ABCG2. ABCG2 c.34G > A and ABCG2 c.421C > A are the most common nonsynonymous mutations, the frequencies for homozygous mutation are 8% and 5.95%, respectively. Keskitalo et al. (2009) reported that the $AUC_{(0\text{-}\infty)}$ of simvastatin lactone derived from a single dose of 40 mg simvastatin was 111% ($P = 0.005$) larger in participants with the ABCG2 c.421 A/A ($n = 5$) genotype than in participants with the ABCG2 c.421 C/C ($n = 23$) genotype whereas genetic variability in ABCG2 has no significant effects on the pharmacokinetics of active simvastatin acid. Compared to participants with hemozygous wild type ABCG2 c.421 C/C, participants with heterozygous variant ABCG2 c.421 C/A ($n = 4$) did not show obvious effects on pharmacokinetics of simvastatin. In our study, although we have only a volunteer with hemozygous variant ABCG2 c.421 A/A, there was no significant difference in pharmacokinetics of simvastatin between carriers with 421 C/C ($n = 8$) and carriers with 421 C/A ($n = 8$) or 421A/A ($n = 1$). This indicates that the gene-dose effect of ABCG2 c.421 C > A is not strong enough in Chinese subjects. In addition, our study firstly describes that ABCG2 c.34G > A SNP does not have a significant effect on the pharmacokinetics of simvastatin.

OATP1B1 is mainly expressed on the basolateral membrane of the liver and mediates the uptake of a diverse set of substrates, especially HMG-CoA reductase inhibitors. So far, more than 40 SNPs have been indentified in SLOC1B1. The SLCO1B1 c.521 T > C SNP is relatively common in Chinese and the frequency is about 14% (Xu et al. 2007). This SNP could impair the transport activity of OATP1B1 and thus reduce the clearance of statins (Tirona et al. 2001; Niemi et al. 2004). The SLCO1B1 g.-11187 G > A SNP is strongly linked with SLCO1B1 c.521 T > C SNP and it also might affect the pharmacokinetics of statins. It was reported that C_{max} and $AUC_{(0\text{-}12)}$ of pravastatin in SLCO1B1 g.-11187G/A genotype carriers were significantly higher when compared with those in SLCO1B1 g.-11187G/G genotype carriers (Niemi et al. 2004). SLCO1B1 c.521T > C decreases the ability of OATP1B1 to transport active simvastatin acid from portal circulation into the liver, resulting in markedly increased plasma concentrations of simvastatin acid and an enhanced risk of simvastatin-induced myopathy (Pasanen et al. 2006). Sortica et al. (2012) reported that SLCO1B1 c.388A > G polymorphism could play a role in the inter-individual variation of clinical response to simvastatin in Brazilians whereas c.521T > C SNPs were not associated with simvastatin treatment. The results of our study showed that there were no significant impacts on simvastatin pharmacokinetics by SLCO1B1 c.388 A > G, SLCO1B1 c.521 T > C, SLCO1B1 g.11187 G > A, SLCO1B1 c.571 T > C and SLCO1B1 c.597 C > T. Our results may be providing pharmacokinetic evidence for the finding that the 388A > G and 521T > C polymorphisms were not associated with the LDL-C response to simvastatin in Chinese patients with hyperlipidaemia (Hu et al. 2012). Also, the effects of SLCO1B1 polymorphism on pharmacokinetics of statins were dependent on the kind of statin.

Fiegenbaum et al. (2005) reported that no significant associations were observed between the CYP3A5*3 allele variants and the efficacy or tolerability of simvastatin in 116 hypercholesterolemic patients. However, Kim et al. (2007) reported that the polymorphic CYP3A5 gene affects the AUC of simvastatin whereas C_{max} and $t_{1/2}$ did not show any difference between genotype groups. The AUC in the CYP3A5*1/*1 carriers ($n = 4$, 4.94 ± 2.25 μg·h·L^{-1}) was significantly lower than that of the CYP3A5*3/*3 ($n = 10$, 16.35 ± 6.37 μg·h·L^{-1}) carriers ($P = 0.013$). The potential difference between CYP3A5*1/*3 ($n = 8$, 11.54 ± 6.44 μg·h·L^{-1}) carriers and CYP3A5*3/*3 carriers did not reach statistical significance. The findings by Kim et al. strongly suggested that the CYP3A5*3 genotype was a substantial factor in increasing the systemic exposure of simvastatin and is a cause of interindividual variability in simvastatin pharmacokinetics in humans. In our study, there were no significant differences in AUC, C_{max} and $t_{1/2}$ values of simvastatin between with CYP3A5*3/*3 ($n = 7$) carriers and carriers with genotypes of CYP3A5*1/*3 ($n = 8$). Due to low frequency of CYP3A5*1/*1 in Chinese populations and difficulty in recruiting this genotype carriers, we only recruit two CYP3A5*1/*1 carriers and thus it might not have met the statistical requirements. However, it seems impossible to exhibit an obvious difference between CYP3A5*1/*1 and CYP3A5*3/*3 genotype carriers in that one CYP3A5*1/*1 carrier has an AUC value of 45.26 μg·h·L^{-1} and the other CYP3A5*1/*1 carrier has an AUC value of 43.20 μg·h·L^{-1} whereas seven CYP3A5*3/*3 carriers had similar AUC values

($37.40 \pm 5.71\ \mu g \cdot h \cdot L^{-1}$). So our study indicates no enough gene-dose effect of CYP3A5*3 g.6986A > G on pharmacokinetics of simvastatin in Chinese subjects.

In conclusion, there was a small inter-subject variation in simvastatin pharmacokinetics in healthy Chinese volunteers. There were no significant effects on simvastatin pharmacokinetics by SNPs such as CYP2C9*3 (1075A > C), CYP3A5*3 g.6986A > G, ABCB1 C3435T, ABCG2 c.34G > A, ABCG2 c.421C > A, SLCO1B1 c.388 A > G, SLCO1B1 c.521 T > C, SLCO1B1 g.11187 G > A, SLCO1B1 c.571 T > C and SLCO1B1 c.597 C > T. P-gp, OATP1B1 and BCRP seem unlikely to play an important role in the pharmacokinetics of simvastatin. The gene-dose effects of ABCG2 c.421 C > A and CYP3A5*3 g.6986A > G on simvastatin pharmacokinetics are not strong enough in Chinese subjects.

4. Experimental

4.1. Reagents and instruments

dNTP-mix (Invitrogen), Platinum-DNA-polymerase (Invitrogen), AxyPrep DNA gel extraction kit (Axygen), AxyPrep blood genomic DNA mini preparation kit (Axygen). Standard simvastatin and lovastatin were supplied by National Institute For Food And Drug Control (China).

A liquid chromatography, electrospray ionization, tandem mass spectrometry (LC-ESI-MS/MS) system consists of a Surveyor HPLC system, Surveyor autosampler, Finnigan TSQ Quantum discovery MS, ESI interface and Xcalibur software. 5810R/5415D centrifuge (Eppendorf), MyCycler Thermal Cycler (Bio-Rad), Electrophoresis apparatus (Bio-Rad), ABI PRISM® 7000 Sequence Detection System (Applied Biosystems), Allegra centrifuge 64R,6R (Beckman Coulter).

4.2. Assay of plasma concentrations of simvastatin

Plasma concentrations of simvastatin were determined by an LC-ESI-MS/MS. Plasma samples (1.0 mL) were mixed with 20 μL of lovastatin solution (100 $mg \cdot L^{-1}$ in acetonitrile), extracted with 10 ml ethyl acetate. Supernatant (5 ml) was taken and then evaporated to dryness under a gentle stream of nitrogen at 35 °C. The residue was reconstituted with 100 μL acetonitrile and the supernatant was directly injected into the LC system. Separation was achieved on a 3-μm reverse phase column (Discovery ODS C_{18} column, 2.1 mm × 100 mm), with a mixture of acetonitrile-0.1% formic acid (65:35, v/v) as mobile phase. The flow rate was 0.2 ml/min. The column was maintained at 30 °C, while the autosampler temperature was set at 10 °C. Detection of analytes and IS was done by MS/MS with an ESI interface operating in positive ion and selective reaction monitoring (SRM) acquisition mode. The SRM was performed at m/z 441.3→325.0 for simvastatin, 427.2→325.0 for lovastatin (internal standard). The collision energy for simvastatin and lovastatin were 27 eV and 25 eV, respectively. Spray Voltage: 4300 eV; Sheath Gas Pressure: 45 Arb; Aux Gas Pressure: 30 Arb; Capillary Temperature: 350 °C.

The assay method was specific, endogenous chemicals and metabolites did not interfere with determining simvastatin and internal standard (Fig. 2). The calibration curves were linear in the range of 0.2∼20.0 $\mu g \cdot L^{-1}$, with the lowest limit of quantification (LLOQ) of 0.2 $\mu g \cdot L^{-1}$ (RSD< 10%, $n = 6$). The within- and between-day coefficient of variation of quality-control samples at high-, medium- and low-concentrations were less than 15%. The average method recovery was 98.0%∼112.6%. The average absolute recovery was 90.0%∼92.4%. The stability of simvastatin in plasma was evaluated with four studies: a short term stability study, a long-term stability study, a freeze-thaw study and stability in the processed sample. The specificity, sensitivity, accuracy, precision and stability all met the requirement for pharmacokinetic study.

4.3. Clinical study

4.3.1. Subjects

The study protocol was approved by the Ethics Committee of the 2nd Affiliated Hospital, School of Medicine, Zhejiang University. Seventeen healthy Chinese Han volunteers participated in this study (age range, 20–28 years; BMI range, 19–25 kg/m^2). All volunteers gave their written informed consent. They were determined to be healthy by a medical history, a physical examination, electrocardiogram, and laboratory tests (including complete blood count, blood biochemistry testing and urinalysis) before starting this study. Participants were excluded for the following reasons: any significant medical history; history of any localized or systemic infectious within 4 weeks before admission; use of prescription or over-the counter medication or alcohol within 2 weeks before enrollment; history of smoking, alcohol or drug abuse; donation of blood within the past 2 months.

4.3.2. Study design

A pharmacokinetic study was conducted in 17 Chinese volunteers. All volunteers were not allowed to take any other medications, coffee and flavonoids-enriched beverage and food 2 weeks before study and during the study period. After a 12 h overnight fast, the volunteers received a single oral dose (40 mg) of simvastatin tablets (Hangzhou MSD Pharmaceutical

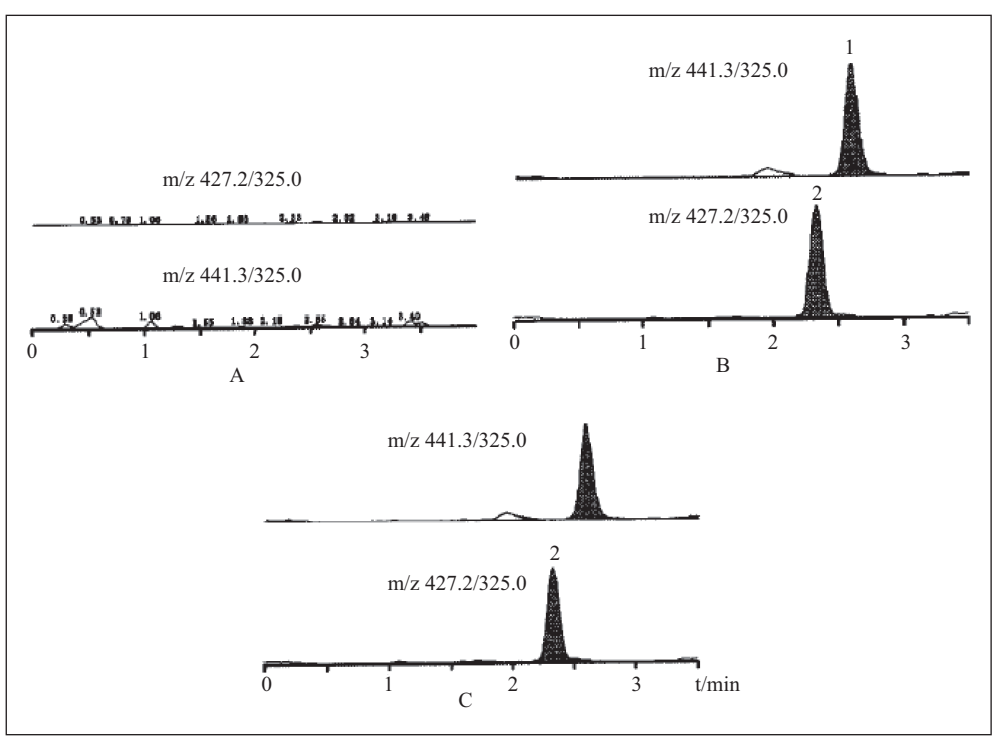

Fig. 2: Ionization chramatograms of the blank plasma (A), blank plasma spiked with simvastatin (10 $\mu g \cdot L^{-1}$) and internal standard (B), typical plasma sample (plasma sample at 1.5 h after intake of 40 mg simvastatin tablets (C)

Table: Influence of genotype on pharmacokinetic parameters for simvastatin (40 mg)

Genotype	*n*	$AUC_{(0-\infty)}$ ($\mu g \cdot h \cdot L^{-1}$)	C_{max} ($\mu g \cdot L^{-1}$)	$T_{1/2}$ (h)
ABCB1 G2677T/A				
Non-G (A/T + T/T)	6	37.25 ± 2.19	8.52 ± 1.56	5.65 ± 0.50*
G/A + G/T + G/G	11	42.00 ± 7.96	10.55 ± 2.54	4.41 ± 131
ABCB1 C3435T				
C/C	9	40.53 ± 8.85	10.28 ± 2.87	4.57 ± 1.08
T/C + T/T	8	40.41 ± 5.21	9.33 ± 1.81	5.16 ± 1.39
ABCB1 halotype derived from C1236T, G2677T/A and C3435T				
TTT	3	35.98 ± 2.35	8.07 ± 1.02	6.01 ± 0.37
Non-TTT	14	41.25 ± 7.15	10.21 ± 2.47	5.35 ± 0.49
ABCG2 c.34G > A				
A/G	7	41.06 ± 5.42	9.81 ± 2.85	5.25 ± 0.53
G/G	10	39.81 ± 7.90	9.85 ± 2.21	4.57 ± 1.52
ABCG2 c.421C > A				
C/C	8	38.87 ± 5.26	9.66 ± 2.07	4.70 ± 1.31
C/A + A/A	9	41.62 ± 8.05	9.99 ± 2.79	4.98 ± 1.22
SLCO1B1 c.388 A > G				
A/A + A/G	9	39.03 ± 7.03	9.27 ± 1.89	4.66 ± 1.53
G/G	8	41.47 ± 6.83	10.46 ± 2.88	5.06 ± 0.84
SLCO1B1 c.521 T > C				
T/T	13	40.15 ± 6.99	9.81 ± 2.46	4.62 ± 1.30
C/T + C/C	4	40.88 ± 7.24	9.90 ± 2.60	5.61 ± 0.57
SLCO1B1 g.11187 G > A				
G/G	13	39.51 ± 6.94	9.72 ± 2.49	4.67 ± 1.36
A/G	4	42.97 ± 6.58	10.20 ± 2.43	5.41 ± 0.42
SLCO1B1 c.571 T > C				
T/T	8	41.70 ± 7.27	9.19 ± 1.85	5.20 ± 0.97
C/T + C/C	9	39.10 ± 6.58	10.56 ± 2.79	4.54 ± 1.41
SLCO1B1 c.597 C > T				
C/C	8	38.43 ± 7.36	9.74 ± 2.15	4.24 ± 1.48
C/T + T/T	9	42.00 ± 6.24	9.92 ± 2.74	5.39 ± 0.65
CYP2C9*3(1075A > C)				
C/A	3	41.34 ± 3.62	9.06 ± 0.48	4.88 ± 0.33
A/A	14	40.10 ± 7.42	10.00 ± 2.63	4.84 ± 1.37
CYP3A5*3 g. 6986A > G				
G/G	7	37.40 ± 5.71	8.72 ± 1.30	4.85 ± 1.23
A/G + A/A	10	42.37 ± 7.05	10.62 ± 2.74	4.85 ± 1.30

* $P = 0.04287$ (ABCB1 2677Non-G *vs* ABCB1 2677 G/A, G/T, G/G)

Co. Ltd) with 200 mL water. No food was allowed until 4 h after dose administration. Water intake was allowed after 2 h of dose; water, lunch and dinner were given to all volunteers according to a time schedule. Blood samples (5 mL) were drawn into Vacutainer™ tubes containing K2EDTA from a forearm vein using an indwelling catheter before drug intake and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0 and 24.0 h after dosing. Blood samples were centrifugated at 3000 × g for 10 min, and plasma was separated and stored at −80 °C until assay. For genetic analysis, a 5 mL EDTA blood sample was drawn from each subject and stored at −80 °C until DNA extraction.

4.3.3. SNP Genotyping

DNA was extracted using standard methods (AxyPrep blood genomic DNA mini preparation kit, Axygen). The SNPs determined by TaqMan® (MGB) genotyping assay included CYP2C9*1, CYP2C9*2 (rs1799853), CYP2C9*3 c.1075A > C (rs1057910), ABCB1 C1236T (rs1128503), ABCB1 G2677T/A (rs2032582), ABCB1 C3435T (rs1045642), CYP3A5*3 g.6986A > G(rs776746), ABCG2 c.421C > A(rs2231142), SLCO1B1 c.388A > G(rs2306283), SLCO1B1 c.521 T > C(rs4149056), SLCO1B1 g.11187G > A (rs4149015), SLCO1B1 c.571T > C(rs4149057) and SLCO1B1 c.597C > T (rs2291075).

TaqMan drug metabolism genotyping assay (20X) were obtained from Life Technologies (Foster City, CA, USA). Each genotyping reaction was performed in a final volume of 25 μL containing 12.5 μL of TaqMan Universal PCR mastermix (2X) (Life Technologies), 1.25 μL of TaqMan drug metabolism genotyping assay (20X), 1.0 μL de DNA (20 ng) and nuclease-free water as dilution solvent. The reactions were submitted to thermal cycling (95 °C for 10 min and 40 cycles with 95 °C for 10 s and 60 °C for 1 min) in a MyCycler Thermal Cycler. End-point fluorescence, corresponding to cleavage of the allele-specific probe (allelic discrimination) was measured using an ABI PRISM® 7000 Sequence Detection System.

ABCG2 c.34G > A (rs2231137) was determined by sequencing. The primers used for rs2231137 genotyping were 5′-CTCTCCAGATGTCTTCCAGTAATGTC-3′ (forward) and 5′-TCAGTAAATGCCTTCAGGTCATTG-3′ (reverse). The PCR conditions were one cycle at 95 °C for 3 min, followed by 35 cycles (95 °C for 30 s, 58 °C for 30 s and 72 °C for 50 s) and one cycle at 72 °C for 10 min. Detections were duplicated at separate times for reconfirmation.

4.4. Statistics

Pharmacokinetic parameters were calculated by the use of software DAS 2.0 with non-compartmental method. Maximal plasma concentrations (C_{max}) and the times at which they occurred (t_{max}) were determined by inspection of the plasma concentration-time profile. The terminal elimination rate constant (λ_z) was determined by linear regression of the terminal portion of the log concentration-time profile. The elimination half-life ($t_{1/2}$) was calculated as $0.693/\lambda_z$. Area under the plasma concentration-time curve (AUC) was determined by trapezoidal rule and extrapolated to infinity by calculation of C_t/λ_z. Data are shown as means ± SD. Statistical significance between groups was evaluated using a two-tailed Student's t test. A *P*-value of less than 0.05 was considered statistically significant.

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References

Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH (2009) Common genetic variation in the ABCB1 gene is associated with the cholesterol-lowering effect of simvastatin in males. Pharmacogenomics 10: 1743–1751.

Chen B, Fang J, Zhang W, Jin Z, Yu Z, Cai W (2009) Detection of C1236T, G2677T/A, and C3435T polymorphism of MDR1 by amplification refractory mutation system PCR. J Clin Lab Anal 23: 110–116.

Fiegenbaum M, da Silveira FR, Van der Sand CR, Van der Sand LC, Ferreira ME, Pires RC, Hutz MH (2005) The role of common variants of ABCB1, CYP3A4, and CYP3A5 genes in lipid-lowering efficacy and safety of simvastatin treatment. Clin Pharmacol Ther 78: 551–558.

Gonzalez TP, Mucenic T, Brenol JC, Xavier RM, Schiengold M, Chies JA (2008) ABCB1 C1236T, G2677T/A and C3435T polymorphisms in systemic lupus erythematosus patients. Braz J Med Biol Res 41: 769–772.

Hu M, Mak VW, Tomlinson B (2012) Intronic variants in SLCO1B1 related to statin-induced myopathy are associated with the low-density lipoprotein cholesterol response to statins in Chinese patients with hyperlipidaemia. Pharmacogenet Genomics.

Keskitalo JE, Kurkinen KJ, Neuvoneni PJ, Niemi M (2008) ABCB1 haplotypes differentially affect the pharmacokinetics of the acid and lactone forms of simvastatin and atorvastatin. Clin Pharmacol Ther 84: 457–461.

Keskitalo JE, Pasanen MK, Neuvonen PJ, Niemi M (2009) Different effects of the ABCG2 c.421C > A SNP on the pharmacokinetics of fluvastatin, pravastatin and simvastatin. Pharmacogenomics 10: 1617–1624.

Kim KA, Park PW, Lee OJ, Kang DK, Park JY (2007) Effect of polymorphic CYP3A5 genotype on the single-dose simvastatin pharmacokinetics in healthy subjects. J Clin Pharmacol 47: 87–93.

Li J, Volpe DA, Wang Y, Zhang W, Bode C, Owen A, Hidalgo IJ (2011) Use of transporter knockdown Caco-2 cells to investigate the *in vitro* efflux of statin drugs. Drug Metab Dispos 39: 1196–1202.

Niemi M, Schaeffeler E, Lang T, Fromm MF, Neuvonen M, Kyrklund C, Backman JT, Kerb R, Schwab M, Neuvonen PJ, Eichelbaum M, Kivistö KT (2004) High plasma pravastatin concentrations are associated with single nucleotide polymorphisms and haplotypes of organic anion transporting polypeptide-C (OATP-C, SLCO1B1). Pharmacogenetics 14: 429–440.

Pasanen MK, Neuvonen M, Neuvonen PJ, Niemi M (2006) SLCO1B1 polymorphism markedly affects the pharmacokinetics of simvastatin acid. Pharmacogenet Genomics 16: 873–879.

Sortica VA, Fiegenbaum M, Lima LO, Van der Sand CR, Van der Sand LC, Ferreira ME, Pires RC, Hutz MH (2012) SLCO1B1 gene variability influences lipid-lowering efficacy on simvastatin therapy in Southern Brazilians. Clin Chem Lab Med 50: 441–448.

Tirona RG, Leake BF, Merino G, Kim RB (2001) Polymorphisms in OATP-C identification of multiple allelic variants associated with altered transport activity among European-and African-Americans. J Bio Chem 276: 35669–35675.

Xu LY, He YJ, Zhang W, Deng S, Li Q, Zhang WX, Liu ZQ, Wang D, Huang YF, Zhou HH, Sun ZQ (2007) Organic anion transporting polypeptide-1B1 haplotypes in Chinese patients. Acta Pharmacol Sin 28: 1693–1697.