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ABCB1 Gene polymorphisms, ABCB1 haplotypes and ABCG2 c.421C > A are determinants of inter-subject variability in rosuvastatin pharmacokinetics

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A randomized cross-over pharmacokinetic study of rosuvastatin calcium (single dose: 5 mg, 10 mg and 20 mg; multiple doses: 10 mg once daily for 7 days) was conducted in 12 healthy Chinese volunteers. Plasma concentrations of rosuvastatin were determined by an LC-ESI-MS-MS method. Single-nucleotide polymorphisms (SNPs) in ABCB1, ABCG2, SLCO1B1, CYP2C9 and CYP3A5 were determined by TaqMan®(MGB) genotyping assay. An impact of the aforementioned SNPs on steady state pharmacokinetic parameters [average steady state concentration ($C_{av,ss}$) and area under the plasma concentration versus time curve during the dosing interval at steady state (AUC_{ss})], dose-normalized (based on 5 mg) pharmacokinetic parameters of single-dose rosuvastatin were further analyzed. Rosuvastatin exhibited linear pharmacokinetics and great inter-subject variability. $C_{av,ss}$, AUC_{ss} and dose-normalized peak plasma concentration (C_{max}) and $AUC_{(0-\infty)}$ of single-dose rosuvastatin were significantly related with ABCB1 C1236T, G2677T/A and C3435T polymorphisms and ABCB1 haplotypes. Compared to homozygous wild type and heterozygous mutation gene carriers, subjects carrying the variant ABCB1 1236TT, 2677 non-G or 3435TT genotype had higher $C_{av,ss}$, AUC_{ss}, C_{max} and $AUC_{(0-\infty)}$ ($p < 0.05$). ABCB1 haplotype (1236TT-2677TT-3435TT) had significant influence on dose-normalized pharmacokinetics of single-dose rosuvastatin. ABCB1 haplotype (1236TT-2677TT-3435TT) carriers ($n = 12$) had obvious higher C_{max} (11.16 ± 3.10 μg·L^{-1} *vs* 8.35 ± 3.31 μg·L^{-1}, $p < 0.05$) and $AUC_{(0-\infty)}$ (86.61 ± 24.32 μg·h·L^{-1} *vs* 62.60 ± 26.19 μg·h·L^{-1}, $p < 0.05$) compared to non-1236TT-2677TT-3435TT carriers ($n = 24$). ABCG2 c.421C > A had a significant impact on rosuvastatin pharmacokinetics. Homozygotes (AA) carriers had obvious higher C_{max} (12.20 ± 4.09 μg·L^{-1} *vs* 8.70 ± 3.09 μg·L^{-1}, $p < 0.05$) and $AUC_{(0-\infty)}$ (98.74 ± 25.36 μg·h·L^{-1} *vs* 64.97 ± 24.90 μg·h·L^{-1}, $p < 0.05$) values compared to heterozygotes (CA) and homozygotes (CC) carriers.There were no significant effects on single-dose and steady-state pharmacokinetics of rosuvastatin by CYP2C9*3 (1075A > C), CYP3A5*3 g.6986A > G, ABCG2 c.34G > A, SLCO1B1 c.521 T > C, c.388 A > G, g.11187 G > A, c.571 T > C and c.597 C > T. In addition, no difference in rosuvastatin pharmacokinetics was observed among subjects of different genders. We conclude that ABCB1 C1236T, G2677T/A and C3435T polymorphism, ABCB1 haplotypes and ABCG2 c.421C > A are determinants of inter-subject variability in rosuvastatin pharmacokinetics in healthy Chinese volunteers, and potentially affect the efficacy and toxicity of statin therapy.

1. Introduction

Rosuvastatin, a potent HMG-CoA reductase inhibitor, is minimally metabolized by the cytochrome P450 isozymes (CYPs). It is eliminated mainly by biliary excretion (Kostapanos et al. 2010). It is also a substrate of some important drug transporters such as breast cancer resistance protein (BCRP) and organic anion transporting polypeptide 1B1 (OATP1B1). The hepatic uptake of rosuvastatin is mainly mediated by OATP1B1 whereas the biliary excretion is related with BCRP (Kitamura et al. 2008; Hua et al. 2011). Whether P-glycoprotein (P-gp) is the efflux transporter of rosuvastatin is contradictory (Liyue et al. 2006; Cooper et al. 2003; Hua et al. 2012; Li et al. 2011).

It has been reported that the pharmacokinetics of rosuvastatin exhibit substantial inter-subject variability. The BCRP 421C > A polymorphism may play an important role in the pharmacokinetics of rosuvastatin in healthy Chinese males and healthy Finnish volunteers (Zhang et al. 2006; Keskitalo et al. 2009). As compared to Chinese hypercholesterolemia patients with the BCRP c.421CC genotype, those with the c.421AA genotype showed a 6.9% greater reduction in LDL-C level, which would be equivalent to the effect obtained by doubling the dose of rosuvastatin (Tomlinson et al. 2010).

With respect to ABCB1 polymorphism, ABCB1 c.1236C-c.2677G-c.3435C and c.1236T-c.2677T-c.3435T haplotypes play no significant role to the interindividual variability in the

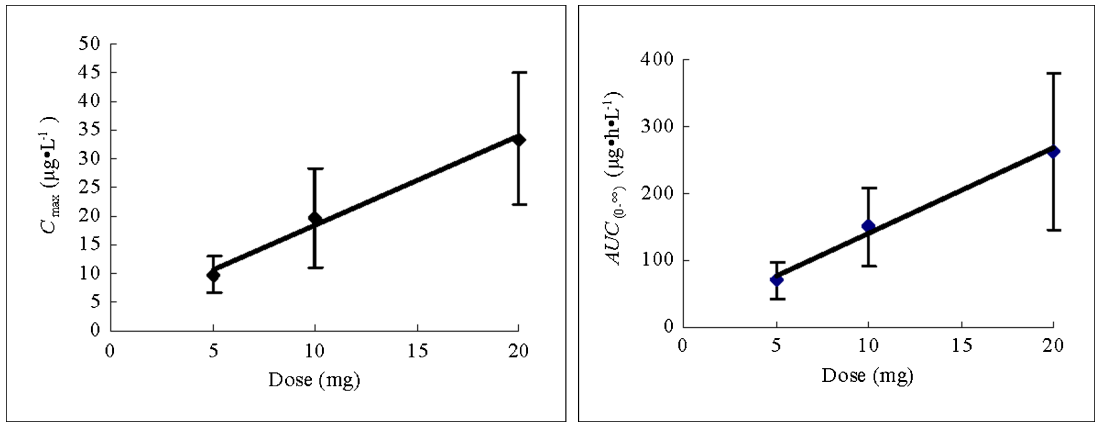

Fig. 1: Linear pharmacokinetics of rosuvastatin. The regression coefficients of C_{max} and $AUC_{(0-\infty)}$ on dose (r^2) were 0.991 and 0.991 for rosuvastatin, respectively

pharmacokinetics of rosuvastatin in healthy Finnish volunteers (Keskitalo et al. 2009).

The pharmacokinetic exposure of rosuvastatin was higher in the OATP1B1*15/*15 subjects than in the others, suggesting a potential association between the OATP1B1 genetic polymorphisms and altered rosuvastatin pharmacokinetics in Korean populations (Choi et al. 2008). However, the study by Lee et al. (2005) investigated rosuvastatin pharmacokinetics and pharmacogenetics in Caucasian and Asian subjects residing in the same environment. It was found that SLCO1B1 polymorphisms (c.388A > G and c.521T > C) showed no association with $AUC_{0\text{-}t}$ or C_{max} in any of the Asian groups whereas it had significant impacts on rosuvastatin pharmacokinetics in the Caucasian volunteers (Bailey et al. 2010).

CYP3A4 and CYP3A5 are the most abundant CYP3A enzymes in adult human liver and small intestine, and they share 83% identity in amino acid sequence. CYP3A5 accounts for > 50% of CYP3A activity and thus may be the most important determinant of genetic differences in CYP3A-dependent drug clearance. Rosuvastatin is a non-CYP3A4-metabolized statin, thus CYP3A5 polymorphism seems unlikely to have an impact on rosuvastatin pharmacokinetics and its clinical response. However, enhanced response to rosuvastatin was seen in patients with variant genotypes of either CYP3A5 or BCRP. Furthermore, multivariate logistic-regression analysis revealed that patients with at least 1 variant CYP3A5 and/or BCRP allele were more likely to achieve the LDL cholesterol target (odds ratio: 2.289) (Bailey et al. 2010).

So, there is still lack of knowledge about the effects of genetic polymorphisms in multiple transporters and CYPs on pharmacokinetics of rosuvastatin. Therefore, we explored the effects of polymorphisms in ABCB1, ABCG2, SLCO1B1, CYP2C9 and CYP3A5 on the pharmacokinetics of rosuvastatin, then to interpret the genetic and molecular mechanism of interindividual variability in drug response, and further provide basis for dose rationalization.

2. Investigations and results

2.1. Pharmacokinetics

After administration of 5, 10 and 20 mg rosuvastatin calcium, we found that there was a linear correlation between rosuvastatin dose and C_{max} or $AUC_{(0-\infty)}$ (Fig. 1). Rosuvastatin exhibited a linear pharmacokinetic characteristics. Dose-normalized (based on 5 mg) C_{max} and $AUC_{(0-\infty)}$ values were further investigated to analyze the impact of SNPs on the pharmacokinetics of rosuvastatin.

2.2. Effects of SNPs in drug transporters and CYPs on the dose-normalized single-dose pharmacokinetics of rosuvastatin

As indicated in Table 1, ABCB1 C1236T, G2677T/A and C3435T polymorphism had a significant influence on the dose-normalized pharmacokinetics of rosuvastatin. Compared to subjects with 1236CT genotype, 1236TT genotype carriers had a higher C_{max} and $AUC_{(0-\infty)}$ of rosuvastatin. ABCB1 G2677T/A significantly affected C_{max} and $AUC_{(0-\infty)}$ of rosuvastatin. Compared to subjects with GT, GA or GG genotype, non-G carriers had a higher C_{max} and $AUC_{(0-\infty)}$. Compared to subjects with 3435TC and 3435CC genotype, 3435TT carriers had a higher C_{max} and $AUC_{(0-\infty)}$. With respect to ABCB1 haplotypes, 1236TT-2677TT-3435TT carriers obviously had a higher C_{max} and $AUC_{(0-\infty)}$, when compared to non-1236TT-2677TT-3435TT carriers (Fig. 2).

Table 1: Impacts of ABCB1 and ABCG2 polymorphism on dose-normalized (5 mg) pharmacokinetics of rosuvastatin

Genotype	*n*	C_{max}(μg· L^{-1})	$AUC_{(0-\infty)}$(μg·h·L^{-1})
ABCB1 C1236T			
C/T	18	7.94 ± 3.67	57.18 ± 22.95
TT	15	10.81 ± 2.86	80.92 ± 24.61
p		0.020	0.007
ABCB1 G2677T/A			
Non-G	12	11.16 ± 3.10	86.61 ± 24.32
G/T + G/A + G/G	24	8.35 ± 3.31	62.60 ± 26.19
p		0.019	0.012
ABCB1 C3435T			
C/C	18	8.67 ± 3.58	66.07 ± 29.17
T/C	6	7.38 ± 2.32	52.17 ± 9.40
T/T	12	11.16 ± 3.10	86.61 ± 24.32
CC + CT	24	8.35 ± 3.31	62.60 ± 26.19
p(T/C *vs* T/T)		0.018	0.004
p(TT *vs* CC, CT)		0.019	0.012
ABCG2 c.421C > A			
CC	15	8.65 ± 3.75	62.51 ± 26.68
CA	15	8.76 ± 2.39	67.44 ± 23.66
AA	6	12.20 ± 4.09	98.74 ± 25.36
CC + CA	30	8.70 ± 3.09	64.97 ± 24.90
p (AA *vs* CA)		0.025	0.015
p (CA *vs* CC)		0.923	0.596
p (AA *vs* CC)		0.070	0.010
p (AA *vs* CC, CA)		0.022	0.005

Note: Data were dose normalised to 5 mg prior to statistical analysis.

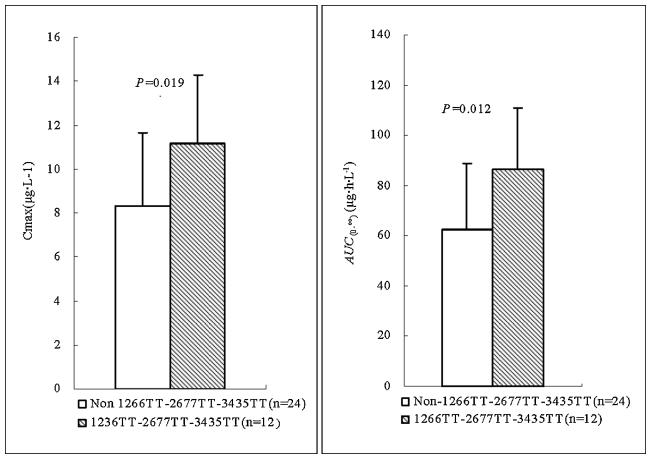

Fig. 2: Effects of ABCB1 halotypes on rosuvastatin pharmacokinetics. Note: Data were dose normalised to 5 mg prior to statistical analysis

ABCG2 c.421C > A SNP had a significant effect on C_{max} and $AUC_{(0-\infty)}$ of rosuvastatin. C_{max} and $AUC_{(0-\infty)}$ increased by 40.2% and 52.0% in 421AA genotype carriers, when compared to 421CC and 421CA genotype carriers.

There were no significant effects on pharmacokinetics of rosuvastatin by CYP2C9*3(1075A > C), CYP3A5*3 g.6986A > G, ABCG2 c.34G > A, SLCO1B1 c.388 A > G, SLCO1B1 c.571 T > C and SLCO1B1 c.597 C > T($p > 0.05$).

2.3. Effects of SNPs in drug transporters and CYPs on the steady state pharmacokinetics of 10 mg rosuvastatin

ABCB1 C1236T, G2677T/A and C3435T polymorphism had a significant influence on the steady state pharmacokinetics of rosuvastatin (10 mg) (Table 2). Compared to subjects with 1236CT and 1236CC genotype, 1236TT genotype carriers had a higher $C_{av,ss}$ and AUC_{ss} of rosuvastatin. ABCB1 G2677T/A significantly affected $C_{av,ss}$ and AUC_{ss} of rosuvastatin. Compared to subjects with GT, GA or GG genotype, non-G carriers had a higher $C_{av,ss}$ and AUC_{ss}. Compared to subjects with 3435TC and 3435CC genotype, 3435TT carriers had a higher $C_{av,ss}$ and AUC_{ss}. With respect to ABCB1 haplotypes, 1236TT-2677TT-3435TT carriers obviously had a higher $C_{av,ss}$ and AUC_{ss} compared to non-1236TT-2677TT-3435TT carriers.

Table 2: Influence of ABCB1 gene polymorphism and ABCB1 haplotypes on steady state pharmacokinetics of 10 mg rosuvastatin (10 mg once daily for 7 days)

Genotype	*n*	$C_{av,ss}$ ($\mu g \cdot L^{-1}$)	AUC_{ss} ($\mu g \cdot h \cdot L^{-1}$)
ABCB1 C1236T			
C/T + C/C	7	4.46 ± 0.93	106.95 ± 22.42
T/T	5	7.45 ± 1.40	178.69 ± 33.50
p		0.038	0.038
ABCB1 G2677T/A			
T/T	4	7.88 ± 1.15	189.18 ± 27.63
GG + G/A + G/T	8	4.27 ± 0.98	102.49 ± 23.63
p		0.024	0.024
ABCB1 C3435T			
C/C + T/C	8	4.85 ± 2.09	116.34 ± 50.16
T/T	4	7.88 ± 1.15	189.18 ± 27.63
p		0.024	0.024
ABCB1 haplotypes			
1236TT-2677TT-3435TT	4	7.88 ± 1.15	116.34 ± 50.16
Non-1236TT-2677TT-3435TT	8	4.85 ± 2.09	189.18 ± 27.63
p		0.024	0.024

ABCG2 c.421C > A had an insignificant impact on $C_{av,ss}$ and AUC_{ss} of rosuvastatin ($p > 0.05$).

There were no significant effects on the steady state pharmacokinetics of 10 mg rosuvastatin by CYP2C9*3(1075A > C), CYP3A5*3 g.6986A > G, ABCG2 c.34G > A, SLCO1B1 c.388 A > G, c.521 T > C, c.571 T > C and c.597 C > T($p > 0.05$).

2.4. Gender effects on pharmacokinetics of rosuvastatin

We found no gender difference in C_{max} ($8.43 \pm 3.56\ \mu g \cdot L^{-1}$ *vs* $10.14 \pm 3.25\ \mu g \cdot L^{-1}$, $p < 0.05$) and $AUC_{(0-\infty)}$ ($68.67 \pm 31.23\ \mu g \cdot h \cdot L^{-1}$ *vs* $72.54 \pm 24.48\ \mu g \cdot h \cdot L^{-1}$, $p < 0.05$) of rosuvastatin.

3. Discussion

Li et al. (2011) investigated the *in vitro* efflux of rosuvastatin using transporter knockdown Caco-2 cells and results indicated that rosuvastatin was transported by P-gp, BCRP and MRP2. The *ABCB1* c.3435T allele has been associated with reduced expression and function of ABCB1, especially when combined with either the c.1236T or c.2677T allele, or both (Hoffmeyer et al. 2000; Kimchi-Sarfaty et al. 2007). P-gp is an efflux transporter. Decrease in efflux transporter-mediated elimination may result in elevated C_{max} and $AUC_{(0-\infty)}$ of rosuvastatin. In our study, the pharmacokinetic parameters of rosuvastatin were significantly affected by the ABCB1 C1236T, G2677T/A and C3435T polymorphism as well as ABCB1 haplotypes. To our knowledge, this is the first report regarding the influence of ABCB1 polymorphism on rosuvastatin pharmacokinetics in the Chinese population. Keskitalo et al. (2009) investigated possible effects of ABCB1 genotype on fluvastatin, pravastatin, lovastatin, and rosuvastatin pharmacokinetics in Caucasian volunteers. The data of their study suggested that the ABCB1 c.1236C-c.2677G-c.3435C and c.1236T-c.2677T-c.3435T haplotypes play no significant role in the interindividual variability in the pharmacokinetics of fluvastatin, pravastatin, lovastatin, and rosuvastatin.

BCRP is another important ABC transporter. It mediates the biliary excretion of rosuvastatin. BCRP and MRP2 appear to represent the primary efflux mechanisms for rosuvastatin in the rat. It has been reported that rosuvastatin is excreted into the bile mediated by BCRP (Liyue et al. 2006; Li et al. 2011). ABCG2 c.421C > A is the most common nonsynonymous mutations which could decrease drug elimination via biliary excretion. The frequencies of the c.421A variant allele are 9.5% in Caucasian and 29.8% in Asian, respectively (Keskitalo et al. 2009; Kim et al. 2010). The BCRP 421C > A polymorphism may play an important role in the pharmacokinetics of rosuvastatin in healthy Chinese males and healthy Finnish volunteers (Zhang et al. 2006; Keskitalo et al. 2009). It also affected the clinical response in Chinese hypercholesterolemia patients (Tomlinson et al. 2010). In our study, we also observed a significant effect of ABCG2 c.421C > A on C_{max} and $AUC_{(0-\infty)}$ of rosuvastatin.

OATP1B1 is mainly expressed on the basolateral membrane of liver and it mediate the uptake of a diverse set of substrates, especially HMG-CoA reductase inhibitors. The SLCO1B1 c.521 T > C is relatively common in the Chinese and the frequency is about 14% (Xu et al. 2007). This SNP could impair the transport activity of OATP1B1 (Tirona et al. 2001) and thus reduce the clearance of statins (Niemi et al. 2004; Mwinyi et al. 2004). Among the OATP family transporters, OATP1B1 contributes predominantly to the hepatic uptake of rosuvastatin (Kitamura et al. 2008). Rosuvastatin exposure was significantly increased in transplant recipients receiving an antirejection regimen containing cyclosporine. Cyclosporine inhibits OATP-C-mediated

rosuvastatin hepatic uptake and thus leads to sigfinicant drug-drug interaction (Simonson et al. 2004). Choi et al. (2008) reported that the pharmacokinetic exposure of rosuvastatin was higher in the OATP1B1*15/*15 subjects than in group 1 (*1a/*1a, *1a/*1b, *1b/*1b) and group 2 (*1a/*15, *1b/*15). This suggested a potential association between the OATP1B1 genetic polymorphisms and altered rosuvastatin pharmacokinetics in Korean populations. Pasanen et al. (2007) reported that rosuvastatin $AUC_{(0\text{-}48h)}$ and C_{max} were 65% ($p = 0.002$) and 79% ($p = 0.003$) higher in Caucasian subjects with the c.521CC genotype than in those with the c.521TT genotype. Lee et al. (2005) investigated rosuvastatin pharmacokinetics and pharmacogenetics in Caucasian and Asian subjects residing in the same environment. No effect of the SLCO1B1 T521 > C genotype on systemic exposure of rosuvastatin was evident in the Chinese, Malay, and Asian-Indian subjects. The A388 > G genotype also showed no association with $AUC_{0\text{-}t}$ or C_{max} in any of the Asian groups. Among caucasian subjects, there was a significant effect of T521 > C genotype on $AUC_{0\text{-}t}$ ($p < .001$). $AUC_{0\text{-}t}$ was higher in 521C homozygotes (CC) than in heterozygotes (TC) and in 521T homozygotes (TT), but $AUC_{0\text{-}t}$ in heterozygotes did not differ significantly from that of 521T homozygotes (TT). The T521 > C genotype has a significant effect on C_{max} of rosuvastatin ($p < 0.0001$) in Caucasian subjects. There was a marginally significant effect of the SLCO1B1 c.388A > G genotype on C_{max} ($p = 0.0456$), with a higher mean in homozygotes (GG) than in homozygotes (AA) (Lee et al. 2005). Bailey et al. (2004) examined the influence of common variants of hepatic metabolism and transporter genes on the lipid-lowering response to statin therapy. There were no differences for patients with variants of CYP2C9, CYP2C19, or SLCO1B1 in comparison with their respective wild types. In our study, we also found no significant relationship between rosuvastatin pharmacokinetics and CYP2C9*3(1075A > C), SLCO1B1 c.521 T > C, c.388 A > G,. c.571 T > C and c.597 C > T polymorphism.

Pharmacokinetic differences may exist between men and women because of their different physiological features. Niemi et al. (2006) found that in women with c.521TT genotype had a 147% greater C_{max} and a 142% greater $AUC_{(0\text{-}\infty)}$ than men with the c.521TT genotype and thus concluded that gender could affect the pharmacokinetics of pravastatin and possibly the function consequences of SLCO1B1 polymorphism. Gender had no effect on the pharmacokinetics of rosuvastatin (Martin et al. 2002; Li et al. 2009).

In conclusion, our study for the first time reported ABCB1 C1236T, G2677T/A and C3435T polymorphism, ABCB1 haplotypes and ABCG2 c.421C > A are determinants of inter-subject variability in rosuvastatin pharmacokinetics in healthy Chinese volunteers. Rosuvastatin pharmacokinetics showed no difference between male and female subjects. Our findings provided abundant data and information on the interindividual variability in drug response. However, studies with a large sample size may be needed to further confirm our discoveries.

4. Experimental

4.1. Materials and instruments

dNTP-mix (Invitrogen), Platinum-DNA-polymerase (Invitrogen), AxyPrep DNA gel extraction kit (Axygen), AxyPrep blood genomic DNA mini preparation kit (Axygen). Standard rosuvastatin calcium (purity 99.5%) was supplied by Zhejiang Yongning Pharmaceutical Co. Ltd (Zhejiang, China). Standard cilostazol (purity 99.7%) was used as internal standard (I.S.) for chromatographic analysis and purchased from National Institute For Food And Drug Control (Beijing, China). Blank human plasma was obtained from healthy volunteers who had not taken any medication for 1 month, at the Blood Center of Hangzhou (Hangzhou, China). HPLC grade methanol and formic acid were purchased from Fisher Scientific (Pittsburgh, PA, USA). Ultrapure water was obtained from a Milli-Q Plus water purification system (Millipore, Bedford, MA, USA).

A liquid chromatography, electrospray ionization, tandem mass spectrometry (LC-ESI-MS/MS) system consists of a waters 2695 HPLC system, Waters Micromass Quattro Ultima Pt triple-quadruple mass spectrometer (microMass, UK), ESI interface (microMass) and MassLynx 4.0 software. 5810R/5415D centrifuge (Eppendorf), MyCycler Thermal Cycler (Bio-Rad), Electrophoresis apparatus (Bio-Rad), ABI PRISM® 7000 Sequence Detection System (Applied Biosystems).

4.2. Assay of plasma concentrations of rosuvastatin

Plasma concentrations of rosuvastatin were determined by an LC-ESI-MS/MS approach which was established and validated in our laboratory (Xu et al. 2006). Briefly, a volume of 50 μL of the working solution of the I.S. (20 $\mu g \cdot L^{-1}$ in methanol) was added to each tube containing 250 μL of plasma and 250 μL of 0.1 M acetate buffer (pH 4.0) and then 5 mL of ether were added to the samples. The samples were shaken for 10 min and centrifuged for 10 min at 735 × g (AllegraTM 6R centrifuge; Beckman Coulter, USA). Then 4 mL of the upper ethereal layer were transferred into another tube where it was evaporated to complete dryness under a gentle stream of nitrogen gas at 40 °C. Samples were reconstituted with 100 μL of methanol/water (50:50, v/v), vortexed for 30 *s*, and centrifuged for 5 min at 735 × g. Twenty microliter of the clear supernatant was directly injected into the LC/MS/MS system for analysis. Separation was achieved on a 5 μm (2.1 mm × 150 mm, i.d.) Atlantis C_{18} column (Waters, Milford, MA, USA), protected by a security guard cartridge ODS C_{18} (4 mm × 3.0 mm i.d.) (Phenomenex. Inc., USA). The mobile phase was a mixture of water (containing 0.2% formic acid)- methanol (30:70, v/v) pumped at a flow-rate of 0.2 mL/min. Column oven was kept at 35 °C. Autosampler was set at 4 °C. Total run time was 6.0 min for each injection. Mass spectrometric analysis was performed in the positive-ion mode (ESI) and multiple reaction monitoring (MRM) acquisition mode. The mass spectrometer was set to monitor the transitions of the precursors to the product ions as the followings: m/z 482→258 for rosuvastatin and m/z 370→288 for the I.S. Nitrogen was used as the cone gas (50 $L \cdot h^{-1}$) and the desolvation gas (400 $L \cdot h^{-1}$). The capillary temperature was 35 °C. The spray voltage was set at 3.7 kV. Argon was used as the collision gas with a collision cell gas pressure of 3.0×10^{-3} mPa. The optimized collision energy was 30 eV for rosuvastatin and 14 eV for the I.S.

The assay method was specific and endogenous compounds did not interfere with analysis of rosuvastatin and I.S. The calibration curves were linear in the range of 0.2∼50 $\mu g \cdot L^{-1}$, with the lowest limit of quantitation (LLOQ) of 0.2 $\mu g \cdot L^{-1}$ (RSD<10.57%, $n = 6$). The deviations from the nominal concentrations were between −6.0 and 12.0% for all concentrations. CV values were < 10.57% for all concentrations. Quality control samples (0.4, 8, 25 and 40 $\mu g \cdot L^{-1}$) in six replicates from three different runs of analysis demonstrated an intra-assay precision of 7.97∼15.94%, an inter-assay precision of 3.19∼15.27%, and an overall accuracy (relative error) of < 3.7%. The average absolute recoveries were 81.52, 64.83, 61.96 and 63.55% for quality control samples at 0.4, 8.0, 25.0 and 40.0 $\mu g \cdot L^{-1}$, respectively. The stability of rosuvastatin in plasma was evaluated with four studies: short term stability in the light at room temperature for 2 h in plasma, 30 days' stability of QC samples at −20 °C, three freeze-thaw cycles of QC samples and stability of processed QC samples in the autosampler for 12 h. The specificity, sensitivity, accuracy, precision and stability all met the requirement for pharmacokinetic studies.

4.3. Clinical study

4.3.1. Study design

A pharmacokinetics study was conducted in 12 Chinese volunteers following a single dose of 5 mg, 10 mg and 20 mg of rosuvastatin calcium in an open-label, randomized, three-period crossover design, with a 1 week washout between periods. The study protocol was approved by the Ethics Committee of the 2nd Affiliated Hospital, School of Medicine, Zhejiang University (Zhejiang, China) and Chinese Clinical Trial Register (registration number: ChiCTR-TTRCC-12002289, ChiCTR-TTRCC-12002290).

4.3.2. Subjects

Twelve healthy Chinese volunteers (6 women, 6 men) participated in this study (age range, 20–30 years; BMI range, 19–24 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 enrolled in the 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.

4.3.3. Drug

Rosuvastatin calcium tablets were manufactured by Zhejiang Yongning Pharmaceutical Co. Ltd (Zhejiang, China). Each tablet contains 5 mg rosuvastatin calcium. Approved by State Food and Drug Administration (Beijing, China), the drug (approval number: 2005L04753) should undergo phase I clinical trial to evaluate its pharmacokinetics.

4.3.4. Sample collection

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 10 h overnight fast, the volunteers received a single oral dose of rosuvastatin calcium 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 (4.5 mL) were drawn into Vacutainer^{TM} tubes containing K2EDTA from a forearm vein using an indwelling catheter before drug intake and at 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 24.0, 36.0, 48.0 and 72.0 h after dosing. Blood samples were centrifuged at 3000 × g for 10 min, and plasma was separated and stored at −80 °C until assay. For genetic analysis, a 5 mL blood sample was drawn from each subject and stored at −80 °C until DNA extraction.

4.3.5. 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.388 A > G (rs2306283), SLCO1B1 c.521 T > C(rs4149056), SLCO1B1 g.11187 G > A (rs4149015), SLCO1B1 c.571 T > C (rs4149057) and SLCO1B1 c.597 C > T (rs2291075).

TaqMan drug metabolism genotyping assay (20X) was 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) and SLCO1B1 c.463C > A (rs11045819) were determined by sequencing. The primers for rs2231137 genotyping were 5′-CTCTCCAGATGTCTTCCAGTAATGTC-3′ (forward) and 5′-TCAGTAAATGCCTTCAGGTCATTG-3′ (reverse). The primers for rs11045819 genotyping were 5′-TCAACATCGACCTTATCCACTTGT-3′ (forward) and 5′-TGTTAATGGGCGAACTGTGTATATTAA-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 were shown as mean values ± SD. Statistical comparisons between two groups were made with Student's *t*-test for unpaired values. Differences in continuous variables between more than two groups were compared by one-way ANOVA and post hoc tests (LSD). Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software program for Windows (version 13.0). A *P*-value of less than 0.05 was considered statistically significant.

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References

Bailey KM, Romaine SP, Jackson BM, Farrin AJ, Efthymiou M, Barth JH, Copeland J, McCormack T, Whitehead A, Flather MD, Samani NJ, Nixon J, Hall AS, Balmforth AJ; SPACE ROCKET Trial Group (2010) Hepatic metabolism and transporter gene variants enhance response to rosuvastatin in patients with acute myocardial infarction: the GEOSTAT-1 Study. Circ Cardiovasc Genet 3: 276–285.

Choi JH, Lee MG, Cho JY, Lee JE, Kim KH, Park K (2008) Influence of OATP1B1 genotype on the pharmacokinetics of rosuvastatin in Koreans. Clin Pharmacol Ther 83: 251–257.

Cooper KJ, Martin PD, Dane AL, Warwick MJ, Raza A, Schneck DW (2003) Lack of effect of ketoconazole on the pharmacokinetics of rosuvastatin in healthy subjects. Br J Clin Pharmacol 55: 94–99.

Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmöller J, Johne A, Cascorbi I, Gerloff T, Roots I, Eichelbaum M, Brinkmann U (2000) Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity *in vivo*. Proc Natl Acad Sci U S A 97: 3473–3478.

Hua WJ, Fang HJ, Hua WX (2012) Transepithelial transport of rosuvastatin and effect of ursolic acid on its transport in Caco-2 monolayers. Eur J Drug Metab Pharmacokinet 37: 225–231.

Hua WJ, Hua WX, Fang HJ (2012) The Role of OATP1B1 and BCRP in Pharmacokinetics and DDI of novel statins. Cardiovasc Ther 30: e234–241.

Keskitalo JE, Zolk O, Fromm MF, Kurkinen KJ, Neuvonen PJ, Niemi M (2009) ABCG2 polymorphism markedly affects the pharmacokinetics of atorvastatin and rosuvastatin. Clin Pharmacol Ther 86: 197–203.

Keskitalo JE, Kurkinen KJ, Neuvonen M, Backman JT, Neuvonen PJ, Niemi M (2009) No significant effect of ABCB1 haplotypes on the pharmacokinetics of fluvastatin, pravastatin, lovastatin, and rosuvastatin. Br J Clin Pharmacol 68: 207–213.

Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV, Gottesman MM (2007) A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science 315: 525–528.

Kim KA, Joo HJ, Park JY (2010) ABCG2 polymorphisms, 34G > A and 421C > A in a Korean population: analysis and a comprehensive comparison with other populations. J Clin Pharm Ther 35: 705–712.

Kitamura S, Maeda K, Wang Y, Sugiyama Y (2008) Involvement of multiple transporters in the hepatobiliary transport of rosuvastatin. Drug Metab Dispos 36: 2014–2023.

Kostapanos MS, Milionis HJ, Elisaf MS (2010) Rosuvastatin-associated adverse effects and drug-drug interactions in the clinical setting of dyslipidemia. Am J Cardiovasc Drugs 10: 11–28.

Lee E, Ryan S, Birmingham B, Zalikowski J, March R, Ambrose H, Moore R, Lee C, Chen Y, Schneck D (2005) Rosuvastatin pharmacokinetics and pharmacogenetics in white and Asian subjects residing in the same environment. Clin Pharmacol Ther 78: 330–341.

Liyue Hu, Yi W, Scott G (2006) ATP-dependent transport of rosuvastatin in membrane vesicles expressing breast cancer resistance protein. Drug Metab Dispos 34: 738–742.

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.

Li Y, Jiang X, Lan K, Jiang Q (2009) Comparative single- and multiple-dose pharmacokinetics of rosuvastatin following oral administration in Chinese volunteers. Eur J Drug Metab Pharmacokinet 34: 221–227.

Martin PD, Dane AL, Nwose OM, Schneck DW, Warwick MJ (2002) No effect of age or gender on the pharmacokinetics of rosuvastatin: a new HMG-CoA reductase inhibitor. J Clin Pharmacol 42: 1116–1121.

Mwinyi J, Johne A, Bauer S, Roots I, Gerloff T (2004) Evidence for inverse effects of OATP-C (SLC21A6) 5 and 1b haplotypes on pravastatin kinetics. Clin. Pharmacol Ther 75: 415–421.

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.

Niemi M, Pasanen MK, Neuvonen PJ (2006) SLCO1B1 polymorphism and sex affect the pharmacokinetics of pravastatin but not fluvastatin. Clin Pharmacol Ther 80: 356–366.

Pasanen MK, Fredrikson H, Neuvonen PJ, Niemi M (2007) Different effects of SLCO1B1 polymorphism on the pharmacokinetics of atorvastatin and rosuvastatin. Clin Pharmacol Ther 82: 726–733.

Simonson SG, Raza A, Martin PD, Mitchell PD, Jarcho JA, Brown CD, Windass AS, Schneck DW (2004) Rosuvastatin pharmacokinetics in heart transplant recipients administered an antirejection regimen including cyclosporine. Clin Pharmacol Ther 76: 167–177.

Tomlinson B, Hu M, Lee VW, Lui SS, Chu TT, Poon EW, Ko GT, Baum L, Tam LS, Li EK (2010) ABCG2 polymorphism is associated with the low-density lipoprotein cholesterol response to rosuvastatin. Clin Pharmacol Ther 87: 558–562.

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 Biol Chem 276: 35669–35675.

Xu DH, Ruan ZR, Zhou Q, Yuan H, Jiang B (2006) Quantitative determination of rosuvastatin in human plasma by liquid chromatography with electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrom 20: 2369–2375.

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.

Zhang W, Yu BN, He YJ, Fan L, Li Q, Liu ZQ, Wang A, Liu YL, Tan ZR, Fen-Jiang, Huang YF, Zhou HH (2006) Role of BCRP 421C > A polymorphism on rosuvastatin pharmacokinetics in healthy Chinese males. Clin Chim Acta 373: 99–103.