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CYP2C9*3(1075A > C), ABCB1 and SLCO1B1 genetic polymorphisms and gender are determinants of inter-subject variability in pitavastatin pharmacokinetics

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A pharmacokinetics study was conducted in 12 Chinese volunteers following a single dose of 1 mg, 2 mg and 4 mg of pitavastatin calcium in an open-label, randomized, three-period crossover design. Plasma concentrations of pitavastatin acid and pitavastatin lactone were determined by a HPLC method. Single-nucleotide polymorphisms (SNPs) in ABCB1, ABCG2, SLCO1B1, CYP2C9 and CYP3A5 were determined by TaqMan® (MGB) genotyping assay. An analysis was performed on the relationship between the aforementioned SNPs and dose-normalized (based on 1 mg) area under the plasma concentration-time curve extrapolated to infinity [$AUC_{(0\text{-}\infty)}$] and peak plasma concentration (C_{max}) values of the acid and lactone forms of pitavastatin. Pitavastatin exhibited linear pharmacokinetics and great inter-subject variability. Compared to CYP2C9*1/*1 carriers, CYP2C9*1/*3 carriers had higher $AUC_{(0\text{-}\infty)}$ and C_{max} of pitavastatin acid and $AUC_{(0\text{-}\infty)}$ of pitavastatin lactone ($P < 0.05$). With respect to ABCB1 G2677T/A, non-G carriers had higher C_{max} and $AUC_{(0\text{-}\infty)}$ of pitavastatin acid, and C_{max} of pitavastatin lactone compared to GT, GA or GG genotype carriers ($P < 0.05$). Gene-dose effects of SLCO1B1 c.521T > C and g.11187G > A on pharmacokinetics of the acid and lactone forms were observed. Compared to non-SLCO1B1*17 carriers, SLCO1B1*17 carriers had higher C_{max} and $AUC_{(0\text{-}\infty)}$ of the acid and lactone forms ($P < 0.05$). Significant sex difference was observed for pharmacokinetics of the lactone. Female SLCO1B1 521TT subjects had higher C_{max} and $AUC_{(0\text{-}\infty)}$ of pitavastatin lactone compared to male 521TT subjects, however, such gender difference disappeared in 521TC and 521CC subjects. Pitavastatin pharmacokinetics was not significantly affected by ABCB1 C1236T, ABCB1C3435T, CYP3A5*3, ABCG2 c.34G > A, c.421C > A, SLCO1B1 c.388A > G, c.571T > C and c.597C > T. We conclude that CYP2C9*3, ABCB1 G2677T/A, SLCO1B1 c.521T > C, SLCO1B1 g.11187G > A, SLCO1B1*17 and gender contribute to inter-subject variability in pitavastatin pharmacokinetics. Personalized medicine should be necessary for hypercholesterolaemic patients receiving pitavastatin.

1. Introduction

Pitavastatin, administrated in the pitavastatin calcium form, is a potent HMG-CoA reductase inhibitor. It can significantly reduce low density lipoprotein cholesterol (LDL-C) and consistently increase high density lipoprotein cholesterol (HDL-C). Thus it is often prescribed to treat hypercholesterolaemia and to prevent cardiovascular disease.

Pitavastatin is minimally metabolized by the cytochrome P450 isozymes (CYPs). It is eliminated mainly by biliary excretion (Mukhtar et al. 2005). The main biotransformation pathway is lactonization. Pitavastatin is firstly glucuronidated by uridine 5-diphosphate glucuronosyltransferases (UGT1A3 and UGT2B7) and then transforms into the lactone form through molecular rearrangement (Fujino et al. 2003). The lactone form can be hydrolyzed and converted into the acid form, so there are two major forms (i.e., acid form and lactone form) in plasma after administration (Fig. 1).

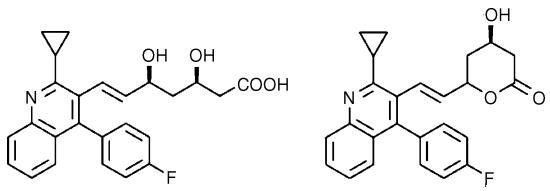

Fig. 1: Chemical structures of pitavastatin (left) and pitavastatin lactone (right)

The solute carrier (SLC) and ATP binding cassette (ABC) transporters exhibit different substrate specificity to pitavastatin acid and pitavastatin lactone. The acid form is a breast cancer resistance protein (BCRP/ABCG2) substrate whereas the lactone is a P-glycoprotein (P-gp/ABCB1) substrate (Fujino et al. 2005). The hepatic uptake of pitavastatin is mainly mediated by organic anion transporting polypeptide 1B1 (OATP1B1/SLCO1B1), whereas the biliary excretion mechanism is related with BCRP and P-gp (Hirano et al. 2005).

Although it has been reported that the pharmacokinetics of pitavastatin exhibit substantial inter-subject variability and

SLCO1B1 388A > G and SLCO1B1 variant haplotypes can affect the pharmacokinetics of pitavastatin significantly (Chung. et al. 2005; Wen et al. 2010), there is little knowledge about the effects of genetic polymorphism in multiple transporters and CYPs on pharmacokinetics of pitavastatin.

Therefore, we want to explore the effects of polymorphisms in SLCO1B1, ABCB1, ABCG2, CYP2C9 and CYP3A5 on the pharmacokinetics of pitavastatin, then to interpret the genetic and molecular mechanism of interindividual variability in drug response, further provide basis for dose rationalization.

2. Investigations and results

2.1. Validation of the bioanalytical method

The assay method was specific and endogenous compounds did not interfere with analysis of pitavastatin acid and pitavastatin lactone (Fig. 2). The calibration curves were linear in the range of 1∼100 $\mu g \cdot L^{-1}$ (pitavastatin acid) and 1∼80 $\mu g \cdot L^{-1}$ (pitavastatin lactone), with the lowest limit of quantitation (LLOQ) of 1 $\mu g \cdot L^{-1}$ for both substances (RSD<10%, $n = 6$). The within- and between-batch coefficients of variation of QC samples of pitavastatin acid at 3.0, 20.0 and 80.0 $\mu g \cdot L^{-1}$ concentrations were less than 11%. The average method recovery was 82.0%∼111.3%. The average absolute recovery was 96.6%∼97.5%. The within- and between-batch coefficients of variation of QC samples of pitavastatin lactone at 3.0, 15.0 and 60.0 $\mu g \cdot L^{-1}$ concentrations were less than 6.8%. The average method recovery was 95.0%∼107.8%. The average absolute recovery was 96.0%∼106.3%. The stability results indicated that pitavastatin acid and pitavastatin lactone were stable in plasma samples. The specificity, sensitivity, accuracy, precision and stability of the bioanalytical method all met the requirement for pharmacokinetic study.

2.2. Pharmacokinetics

There were linear correlations between pitavastatin dose and C_{max} and $AUC_{(0-\infty)}$ of pitavastatin acid and pitavastatin lactone (Fig. 3). So dose-normalized C_{max} and $AUC_{(0-\infty)}$ values were further investigated to analyze the impact of SNPs in ABCB1, ABCG2, SLCO1B1, CYP2C9 and CYP3A5 on the pharmacokinetics of pitavastatin.

2.3. Impacts of SNPs in drug transporters and CYPs on the pharmacokinetics of pitavastatin

CYP2C9*3(1075A > C) significantly affected the pharmacokinetics of pitavastatin acid. Heterozygous variant carriers had higher C_{max} and $AUC_{(0-\infty)}$ of pitavastatin acid compared to homozygous wild type carriers (Table 1). With respect to the pitavastatin lactone, C_{max} of pitavastatin lactone was not affected in carriers of heterozygous mutation while $AUC_{(0-\infty)}$ increased by 72% compared to wild-type individuals.

ABCB1 G2677T/A also had a great influence on the pharmacokinetics of pitavastatin acid. Compared to subjects with GT, GA or GG genotype, non-G carriers had higher C_{max} and $AUC_{(0-\infty)}$ of pitavastatin acid. ABCB1 G2677T/A had a significant influence on C_{max} of pitavastatin lactone. Compared to subjects with GT, GA or GG genotype, heterozygous non-G carriers had a higher C_{max}.

SLCO1B1 c.521T > C had a significant effect on C_{max} and $AUC_{(0-\infty)}$ of pitavastatin acid and very obvious gene-dose effect of SLCO1B1 c.521T > C was observed in homozygous wild type TT, heterozygous CT and homozygous CC carriers (CC > CT > TT). SLCO1B1 c.521T > C also had a statistically significant influence on C_{max} and $AUC_{(0-\infty)}$ of pitavastatin lactone. Obvious gene-dose effect was observed among homozygous wild type TT, heterozygous CT and homozygous CC carriers (CC > CT, TT). In 521CC genotype

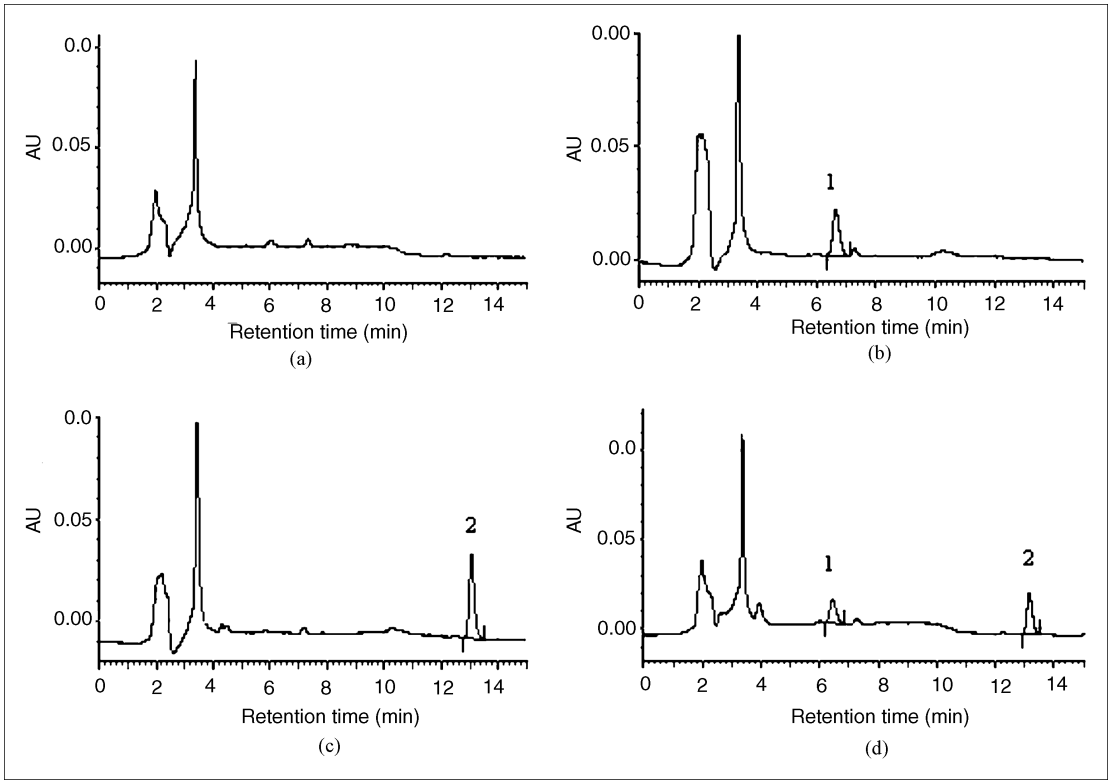

Fig. 2: Chromatograms (a) blank plasma samplel; (b) Blank plasma spiked with pitavastatin acid (30 $\mu g \cdot L^{-1}$); (c) Blank plasma spiked with pitavastatin lactone (20 $\mu g \cdot L^{-1}$); (d) Plasma sample 2 hours after a single dose of 1 mg pitavastatin tablet in one volunteer. Peak 1: pitavastatin acid; Peak 2: pitavastatin lactone

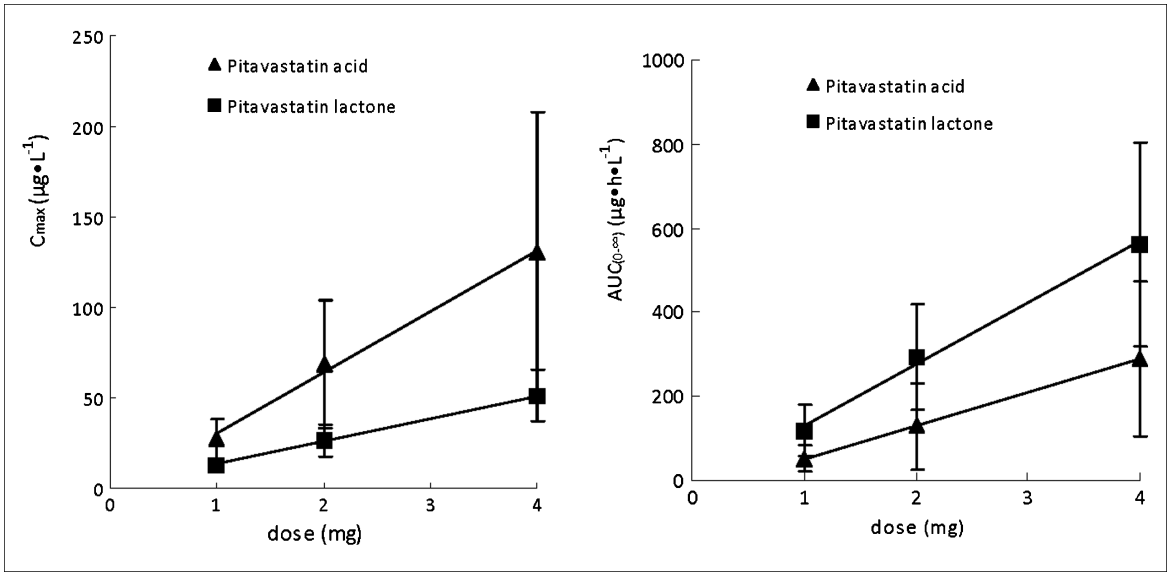

Fig. 3: Linear pharmacokinetics of pitavastatin acid and pitavastatin lactone. The regression coefficient of c_{max} on dose (r^2) was 0.997 and 1 for pitavastatin acid and pitavastatin lactone, respectively. The regression coefficient of AUC(0-∞) on dose (r^2) was 1 and 0.999 for pitavastatin acid and pitavastatin lactone, respectively

carriers when compared with those in 521TT genotype carriers, C_{max} increased by 48.8% and $AUC_{(0-\infty)}$ increased by 80%.
SLCO1B1 g.11187G > A had a significant effect on C_{max} and $AUC_{(0-\infty)}$ of pitavastatin acid and obvious gene-dose effect was observed in homozygous wild type GG, heterozygous AG and homozygous AA carriers (AA > AG, GG). Compared to GG carriers, the mean C_{max} and $AUC_{(0-\infty)}$ in AA carriers increased by 143% and 223%, respectively. SLCO1B1 g.11187G > A also had a statistically significant influence on C_{max} and $AUC_{(0-\infty)}$ of pitavastatin lactone. Obvious gene-dose effect was observed among homozygous wild type GG, heterozygous AG and homozygous AA carriers (AA > AG, GG). In homozygous AA carriers when compared with those in homozygous wild type, C_{max} increased by 38% and $AUC_{(0-\infty)}$ increased by 58.1%.
There were no significant effects on pharmacokinetics of pitavastatin acid and pitavastatin lactone by SNPs such as ABCB1 C1236T, ABCB1 C3435T, CYP3A5*3 g.6986A > G, ABCG2 c.34G > A, ABCG2 c.421C > A, SLCO1B1 c.388 A > G, SLCO1B1c.571T > C and SLCO1B1 c.597C > T.

2.4. Effects of SLCO1B1 haplotypes on the pharmacokinetics of pitavastatin acid and pitavastatin lactone

Compared to non-SLCO1B1*17 carriers, SLCO1B1*17 (c.388G- c.521C-11187A) carriers had higher C_{max} and $AUC_{(0-\infty)}$ of pitavastatin acid and pitavastatin lactone (Fig. 4, Table 2).

2.5. Gender effects on pharmacokinetics of pitavastatin acid and pitavastatin lactone

We found gender differences in the pharmacokinetics of pitavastatin acid and pitavastatin lactone (Table 3). For pitavastatin acid, $AUC_{(0-\infty)}$ between women and men was statistically different while C_{max} was observed with only marginal significant difference. As to pitavastatin lactone, there was significant difference between women and men for both $AUC_{(0-\infty)}$ and C_{max}.
In SLCO1B1 c.521TT subjects, C_{max} values of pitavastatin lactone in women were higher than those in men and $AUC_{(0-\infty)}$ values of pitavastatin lactone in women were higher than in men. However, no significant difference existed in the pharmacokinetic parameters of pitavastatin acid between women and men (Table 4). Interestingly, in SLCO1B1c.521TC and 521CC carriers, there was no gender difference in pharmacokinetic parameters of pitavastatin acid as well as pitavastatin lactone.

3. Discussion

CYP2C9 makes up approximately 20% of the CYPs in liver microsomes (Guo et al. 2005). Three alleles of CYP2C9 were identified globally: CYP2C9*1, CYP2C9*2, and CYP2C9*3. The CYP2C9*3 allele frequencies differ among racial groups (Caucasians: 0.06–0.10; Asians: less than 0.01). CYP2C9*3 variant is less than 5% as efficient as the wild-type enzyme (Takanashi et al. 2000), thus leads to reduced intrinsic clearance of CYP2C9 substrates. Although CYP2C9 is the major enzyme responsible for the hydroxylation of pitavastatin in humans (Fujino et al. 2002), pitavastatin undergoes a minimal metabolism by CYPs and thus CYP2C9 seems unlikely to have an obvious influence on the pharmacokinetics of pitavastatin. However, we observed a significant effect of CYP2C9*3 on the pharmacokinetics of pitavastatin. C_{max} and $AUC_{(0-\infty)}$ of pitavastatin acid as well as $AUC_{(0-\infty)}$ of pitavastatin lactone increased significantly in the mutated heterozygote carriers. Vormfelde et al. (2008) investigated the effects of SLCO1B1 polymorphisms (c.388A > G, c.521T > C) and CYP2C9*3 on torsemide pharmacokinetics and pharmacodynamics. They hypothesized significant gene–gene interactions between CYP2C9 and SLCO1B1 polymorphisms; and SLCO1B1 alleles may impact on CYP2C9 substrates. It needs to be investigated in the future whether CYP2C9*3 has an influence on the activities of drug transporters which participate in disposition of pitavastatin.
P-gp is an efflux transporter. As reported, pitavastatin lactone was a substrate of P-gp and pitavastatin acid was not (Fujino et al. 2003). Shirasaka et al. (2011) reported that the plasma concentration of pitavastatin after oral administration was significantly increased by grapefruit juice due to inhibition of P-gp. In our study, the pharmacokinetic parameters of both pitavastatin acid and pitavastatin lactone were affected by ABCB1 G2677T/A, which is probably due to the mutual

Table 1: Influence of genotype on the pharmacokinetics of pitavastatin acid and pitavastatin lactone

Genotype	*n*	Pitavastatin acid		Pitavastatin lactone	
		C_{max} ($\mu g \cdot L^{-1}$)	$AUC_{(0-\infty)}$ ($\mu g \cdot h \cdot L^{-1}$)	C_{max} ($\mu g \cdot L^{-1}$)	$AUC_{(0-\infty)}$ ($\mu g \cdot h \cdot L^{-1}$)
CYP2C9*3(1075A > C)					
CA	6	53.15 ± 25.08	138.57 ± 48.06	15.66 ± 3.92	206.66 ± 52.15
AA	30	26.96 ± 9.48	47.36 ± 20.33	12.53 ± 3.46	120.16 ± 52.55
P		0.000	0.000	0.064	0.001
ABCB1 C1236T					
CC	3	18.37 ± 4.23	27.73 ± 4.00	9.58 ± 1.05	71.24 ± 29.14
CT	18	37.39 ± 19.07	74.07 ± 49.54	13.59 ± 3.82	142.23 ± 60.42
TT	15	26.64 ± 10.24	55.71 ± 34.50	13.09 ± 3.60	138.06 ± 62.15
ABCB1 G2677T/A					
Non-G	6	47.12 ± 30.18	106.76 ± 75.56	15.86 ± 3.80	160.84 ± 82.18
GT + GA + GG	30	28.16 ± 9.87	53.72 ± 27.87	12.49 ± 3.44	129.32 ± 56.44
P		0.007	0.004	0.038	0.255
ABCB1 C3435T					
CC	18	35.47 ± 20.23	67.98 ± 52.30	12.86 ± 3.97	128.01 ± 63.98
TC	18	27.17 ± 9.72	57.13 ± 32.16	13.24 ± 3.46	141.14 ± 59.40
CYP3A5*3 g.6986A > G					
GG	27	29.93 ± 18.25	56.72 ± 46.44	13.10 ± 4.13	127.05 ± 65.62
AG	9	35.51 ± 6.25	80.07 ± 25.95	12.90 ± 1.94	157.16 ± 40.35
ABCG2c.34G > A					
AG	18	31.72 ± 21.30	64.54 ± 53.79	12.59 ± 3.73	119.81 ± 65.20
GG	15	30.92 ± 9.86	62.87 ± 32.91	12.93 ± 3.56	146.04 ± 57.09
A/A	3	30.98 ± 6.98	49.09 ± 9.43	16.42 ± 3.28	125.17 ± 89.95
ABCG2c.421OA					
CC	15	26.09 ± 10.38	47.61 ± 23.06	13.76 ± 4.25	138.15 ± 64.84
CA	21	35.06 ± 18.66	73.23 ± 50.97	12.54 ± 3.37	132.02 ± 56.78
SLCO1B1 c.388 A > G					
A/G	12	33.16 ± 9.87	69.0U32.77	13.28 ± 3.98	148.76 ± 66.77
G/G	24	30.41 ± 18.71	59.33 ± 47.81	12.94 ± 3.60	127.49 ± 58.40
SLCO1B1 c.521 T > C					
TT	21	25.86 ± 10.52	44.03 ± 21.98	12.58 ± 3.86	123.61 ± 56.77
CT	12	31.29 ± 6.37	68.23 ± 28.24	12.46 ± 2.13	131.66 ± 52.27
CC	3	69.68 ± 27.21	169.51 ± 48.96	18.72 ± 3.11	223.01 ± 72.50
P (CC *vs* CT)		0.000	0.000	0.007	0.017
P (CT *vs* TT)		0.189	0.017	0.925	0.696
P (CC *vs* TT)		0.000	0.000	0.005	0.007
SLC01Blg.-11187G > A					
GG	18	28.63 ± 10.62	52.47 ± 22.57	13.57 ± 3.98	141.05 ± 56.96
GA	15	26.89 ± 8.20	53.27 ± 31.89	11.29 ± 1.61	109.12 ± 47.43
AA	3	69.68 ± 27.21	169.51 ± 48.96	18.72 ± 3.11	223.01 ± 72.50
P (AA *vs* GG)		0.000	0.000	0.013	0.021
P(AAwAG)		0.000	0.000	0.001	0.002
P(GGvsAG)		0.667	0.938	0.046	0.102
SLCO1B1 c.571 T > C					
TT	24	30.41 ± 18.71	59.33 ± 47.81	12.94 ± 3.60	127.49 ± 58.40
CT	12	33.16 ± 9.87	69.01 ± 32.77	13.28 ± 3.98	148.76 ± 66.77
SLCO1B1 C.597OT					
CC	6	35.52 ± 6.88	58.73 ± 13.65	14.26 ± 3.24	145.27 ± 37.30
CT	12	28.04 ± 10.27	60.95 ± 36.99	12.77 ± 4.22	143.14 ± 71.55
T/T	18	32.11 ± 21.05	64.91 ± 53.65	12.83 ± 3.54	125.31 ± 61.70

Note: Data were dose normalised to 1 mg prior to statistical analysis. $AUC_{(0-\infty)}$: plasma concentration-time curve; C_{max}: peak plasma concentration.

transformation between pitavastatin acid and pitavastatin lactone. Some researchers found that ABCB1 G2677T/A did not affect the activity of P-gp (Morita et al. 2003), but this SNP did influence the *AUC* of the P-gp probe drug talinolol (Siegmund et al. 2002) and the pharmacokinetics of repaglinide in healthy Chinese volunteers (Xiang et al. 2012).

BCRP is another important ABC transporter, it mediates the biliary excretion of pitavastatin (Hirano et al. 2005). Pitavastatin acid was a substrate of BCRP whereas pitavastatin lactone was not (Fujino et al. 2003). ABCG2 c.34G > A and ABCG2 c.421C > A were the most commonly implicated SNPs and the frequencies for homozygous mutation were 8% and 5.95%, respectively. In our study, we did not find any effect of the two SNPs on pharmacokinetics of pitavastatin acid and pitavastatin lactone, the possible reason was that the homozygous mutants recruited were too few.

OATP1B1 is mainly expressed on the basolateral membrane of liver and it mediates the uptake of a diverse set of substrates, especially HMG-CoA reductase inhibitors. In our study, we found that SLCO1B1 c.521T > C and SLCO1B1 g.-11187G > A exerted obvious gene-dose effects on the pharmacokinetics of pitavastatin acid as well as pitavastatin lactone. So far, more than

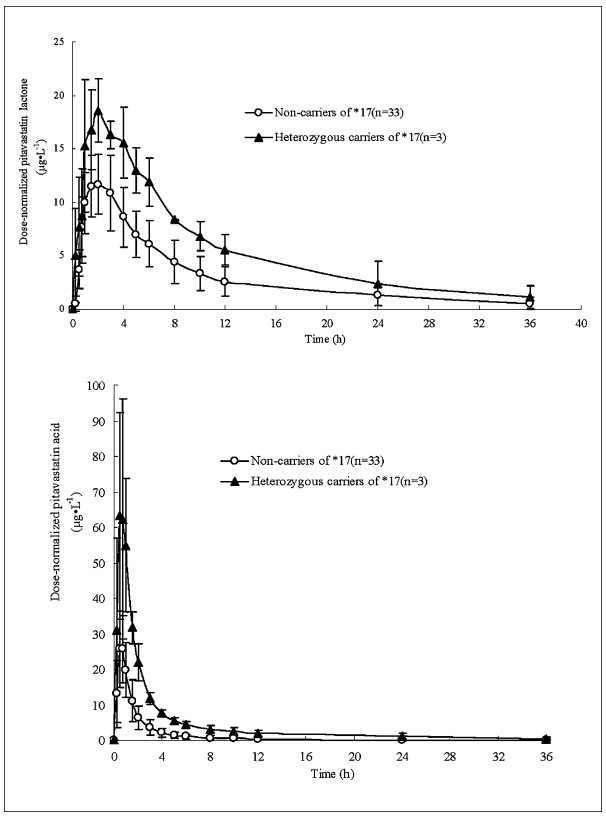

Fig. 4: Impact of SLCO1B1 polymorphisms and haplotypes on the disposition of pitavastatin acid and pitavastatin lactone after dose normalization to 1 mg

40 SNPs have been indentified in SLOC1B1. The SLCO1B1 c.521T > C is relatively common in 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). The SLCO1B1 g.-11187G > A is strongly linked with SLCO1B1 c.521T > C in the promoter region of SLCO1B1. Although no amino acid substitution or activity change is associated with this SNP, it also affects the pharmacokinetics of statins. It was reported that C_{max} and *AUC* of pravastatin in SLCO1B1 g.-11187G/A genotype carriers were significantly higher than those in SLCO1B1 g.-11187G/G genotype carriers (Kivistö et al. 2007). The influence of this SNP on the transcriptional regulation of OATP1B1 or the linkage with SLCO1B1 c.521T > C might be explanations for the results.

In vitro studies showed that SLCO1B1 c.388 A > G did not impact the transport activity of OATP1B1 (Kameyama et al. 2005). In addition, it did not influence the lipid-lowering efficacy of pitavastatin *in vivo* (Yang et al. 2010). To the contrary, one research revealed that C_{max} and $AUC_{(0\text{-}\infty)}$ of pitavastatin elevated obviously in the 388GA and 388GG group when compared with those in the wild type group (Hirano et al. 2005; Chung et al. 2005). In our study, we found this SNP did not have any impact on the disposition of pitavastatin, the possible reason was that the wild type carriers were not identified in our study.

SLCO1B1*1b (388A > G) and SLCO1B1*15 (c.388G-c.521C) are common haplotypes in Chinese population, the frequencies are 59.9% and 14.0%, respectively (Xu et al. 2007). Choi et al. (2012) found that pitavastatin exposure was significantly increased in subjects with the SLCO1B1*15/*15 genotype due to reduced hepatic absorption. SLCO1B1*17 haplotype (containing the -11187G > A, 388A > G and 521T > C SNPs) is more prevalent than *15 in Japanese subjects and the haplotype frequencies for *1b, *15 and *17 were 0.469, 0.037 and 0.133, respectively (Kim et al. 2007). Niemi et al. (2005) concluded that uptake of pravastatin into hepatocytes was impaired in carriers of the SLCO1B1*17 haplotype, resulting in higher plasma pravastatin concentrations but lower concentrations of pravastatin in hepatocytes and thereby in a smaller inhibitory effect on cholesterol synthesis. In our study, we observed that SLCO1B1*17 carriers had higher C_{max} and $AUC_{(0\text{-}\infty)}$ values of pitavastatin than those of non-SLCO1B1*17 carriers. Considering the impaired transport activity of OATP1B1 in SLCO1B1*17 carriers, cautions should be warranted with respect to both adverse drug reactions and efficacy of pitavastatin.

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 concluded that sex could affect the pharmacokinetics of pravastatin and possibly the function consequences of SLCO1B1 polymorphism. To our knowledge, there is no report on sex effect on pitavastatin till now. In our study, sex did affect the pharmacokinetics of pitavastatin. C_{max} and $AUC_{(0\text{-}\infty)}$ of pitavastatin lactone were much higher in women with SLCO1B1c.521TT whereas this gender difference disappeared in SLCO1B1c.521TC and 521CC carriers. In addition to the study of pravastatin (Niemi et al. 2006), the results of our study further indicate that sex may affect the activity or phenotype of OATP1B1 and thus alter the pharmacokinetics of pitavastatin lactone. With respect to pitavastatin acid, sex also had a significant effect on the $AUC_{(0\text{-}\infty)}$. However, among SLCO1B1 c.521TT, 521TC and 521CC genotype carriers there were no obvious sex effects on the pharmacokinetics of pitavastatin acid. There may be other mechanisms responsible for sex difference in the pharmacokinetics of pitavastatin acid.

In conclusion, to our knowledge, this is the first study reporting that CYP2C9*3(1075A > C), ABCB1 G2677T/A, SLCO1B1 c.521T > C, SLCO1B1 g.-11187G > A, SLCO1B1*17 (c.388G-c.521C-11187A) and sex are determinants of inter-subject variability in pitavastatin pharmacokinetics in Chinese population. Sex may affect the activity or phenotype of OATP1B1 and thus alter the pharmacokinetics of pitavastatin lactone. Our findings provided abundant data and information on the interindividual variability in drug response. Personalized medicine may be necessary for hypercholesterolaemic patients receiving pitavastatin.

Table 2: Influence of halotypes of SLCO1B1 on pharmacokinetics of pitavastatin acid and pitavastatin lactone

SLCO1B1 polyphism	*n*	Pitavastatin acid		Pitavastatin lactone	
		C_{max} ($\mu g \cdot L^{-1}$)	$AUC_{(0\text{-}\infty)}$ ($\mu g \cdot h \cdot L^{-1}$)	C_{max} ($\mu g \cdot L^{-1}$)	$AUC_{(0\text{-}\infty)}$ ($\mu g \cdot h \cdot L^{-1}$)
Non-carriers of *17	33	27.84 ± 9.49	52.83 ± 26.75	12.53 ± 3.30	126.54 ± 54.49
SLCOlBl*17(c.388G-c.521C-11187A)	3	69.68 ± 27.21	169.5U48.96	18.72 ± 3.11	223.0 ± 72.50
P (SLCO1B1*17 *vs* Non-carriers of *17)		0.000	0.000	0.004	0.007

Note: Data were dose normalised to 1 mg prior to statistical analysis. $AUC_{(0\text{-}\infty)}$: plasma concentration-time curve; C_{max}: peak plasma concentration.

Table 3: Gender effects on dose-normalized pharmacokinetic parameters for pitavastatin acid and pitavastatin lactone

Substance	C_{max} ($\mu g \cdot L^{-1}$)			$AUC_{(0-\infty)}$ ($\mu g \cdot h \cdot L^{-1}$)		
	Male (n=18)	Female (M = 18)	P (male *vs* female)	Male (n=18)	Female (n=18)	P (male *vs* female)
Pitavastatin acid	26.14 ± 10.28	36.50 ± 19.43	0.054	45.34 ± 20.86	79.78 ± 52.61	0.014
Pitavastatin lactone	11.16 ± 2.09	14.94 ± 3.98	0.001	104.34 ± 39.08	164.81 ± 65.18	0.002

Note: $AUC_{(0-\infty)}$: plasma concentration-time curve; C_{max}: peak plasma concentration.

Table 4: Gender differences in dose normalized C_{max} and $AUC_{(0-\infty)}$ for pitavastatin lactone and pitavastatin acid in SLCO1B1 521TT, 521TC and 521CC carriers

Substance	Genotype	C_{max} ($\mu g \cdot L^{-1}$)			$AUC_{(0-\infty)}$ ($\mu g \cdot h \cdot L^{-1}$)		
		Male	Female	P (male *vs* female)	Male	Female	P (male *vs* female)
Pitavastatin lac tone	521TT (male 12, female 9)	10.68 ± 1.44	15.11 ± 4.67	0.006	97.09 ± 30.35	158.96 ± 65.79	0.009
	521TC + 521CC (male 6, female 9)	12.12 ± 2.93	14.77 ± 3.44	0.146	118.84 ± 52.81	170.66 ± 67.99	0.140
Pitavastatin Acid	521TT (male 12, female 9)	23.22 ± 10.91	29.38 ± 9.39	0.190	37.68 ± 20.03	52.51 ± 22.70	0.129
	521TC + 521CC (male 6, female 9)	32.00 ± 5.96	43.60 ± 24.50	0.281	60.66 ± 13.24	107.04 ± 60.78	0.092

Note: $AUC_{(0-\infty)}$ plasma concentration-time curve; C_{max}: peak plasma concentration.

However, it still has certain limitations; a larger sample size including more participants is preferred to confirm some of the results.

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 pitavastatin calcium and pitavastatin lactone were supplied by Zhejiang Jingxin Pharmaceutical Co. Ltd (Zhejiang, China).

The high performance liquid chromatography (HPLC) system consisted of a Beckman Coulter 125 pump, a Jasco FP-2020 Plus fluorescence detector and software of Beckman System Gold. 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. Bioanalytical method

Plasma concentrations of pitavastatin acid and pitavastatin lactone were determined by a reversed phase HPLC with fluorescence detection which was established and validated by our laboratory. Briefly, plasma samples (100 μL) were deproteinized by precipitation with 300 μL of acidified methanol (glacial acetic acid: methanol = 0.19: 100, v/v), centrifuged and 50 μL of the supernatant was directly injected into the HPLC system. Separation was achieved on a 5 μm reverse phase column (Diamonsil C_{18} ODS 250 mm × 4.6 mm), protected by a security guard cartridge ODS C_{18} 4 mm × 3.0 mm ID (Phenomenex. Inc., USA). The mobile phase was a mixture of 0.1% phosphoric acid solution-methanol (63:27, v/v) pumped at a flow-rate of 1.2 mL/min. Column oven was kept at 30 °C. Autosampler was set at 4 °C. The fluorescence detector was set at excitation wavelength of 350 nm and emission wavelength of 415 nm. The external standard method was used to quantify pitavastatin acid and pitavastatin lactone.

Validation of this assay method was performed according to guideline on bioanalytical method validation recommended by Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA). Calibrations were constructed from a blank sample and six standard plasma samples covering the range (pitavastatin acid: 1∼100 $\mu g \cdot L^{-1}$; pitavastatin lactone: 1∼80 $\mu g \cdot L^{-1}$) on six consecutive days. Peak areas were recorded and plotted against the corresponding concentration of the analyte by linear regression analysis. The selectivity was studied by analyzing six different sources of blank human plasma. Absolute recovery of pitavastatin acid was evaluated by comparing the analyte peak area of five processed low (3.0 $\mu g \cdot L^{-1}$), medium (20.0 $\mu g \cdot L^{-1}$) and high (80.0 $\mu g \cdot L^{-1}$) quality control (QC) samples to the peak area obtained from standard solutions at the same theoretical concentrations of pitavastatin acid. Within- batch, between-batch accuracy and precision evaluations were performed by repeated analysis of pitavastatin acid in QC samples. The stability of pitavastatin acid in plasma was evaluated at different storage conditions: immediately, after 2 h at ambient temperature, after sample processing and being on the autosampler at 4 °C for 24 h, after three freeze/thaw cycles and after 40 days stored at –20 °C. Accuracy, precision and stability of the assay method for pitavastatin lactone were similarly validated with QC samples at concentrations of 3.0, 15.0 and 60.0 $\mu g \cdot L^{-1}$.

4.3. Clinical study

4.3.1. Study design

A pharmacokinetics study was conducted in 12 Chinese volunteers following a single dose of 1 mg, 2 mg and 4 mg of pitavastatin 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-12002290, ChiCTR-TTRCC-12002291, http://www.chictr.org/en/proj/search.aspx)

4.3.2. Subjects

Twelve healthy Chinese Han 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

Pitavastatin calcium dispersible tablets were provided by Zhejiang Jingxin Pharm Co. Ltd (Zhejiang, China). Each tablet contained 1 mg pitavastatin calcium. Approved by State Food and Drug Administration (Beijing, China), the drug should undergo phase I clinical trial to evaluate the pharmacokinetics of pitavastatin acid and pitavastatin lactone.

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 pitavastatin 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 (1.5 mL) were drawn into Vacutainer™ tubes containing K2EDTA from a forearm vein using an indwelling catheter before drug intake and at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 24.0 and 36.0 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.521T > C(rs4149056), SLCO1B1 g.11187G > A (rs4149015), SLCO1B1 c.571T > C (rs4149057) and SLCO1B1 c.597C > 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 DAS 2.0 software with a 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 extrapolated to infinity [$AUC_{(0\text{-}\infty)}$] 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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Author contribution Quan ZHOU, Zou-rong RUAN and Su ZENG conceived and designed research; Quan ZHOU and Hong YUAN analyzed clinical samples; Qiu-xia CHEN and Hui-min XU performed data analysis; and Quan ZHOU and Su ZENG wrote the paper.

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