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The effect of *SLCO1B1* polymorphism on the pharmacokinetics of atorvastatin and 2-hydroxyatorvastatin in healthy Chinese people

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Received November 29, 2016, accepted December 30, 2016

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Pharmazie 72: 365–368 (2017) *doi: 10.1691/ph.2017.6944*

The pharmacokinetics of statins show substantial inter-subject variability. Increasing systemic exposure of statins may lead to adverse drug reactions such as myopathy. The variation in statin pharmacokinetics is partly explained by genetic factors. OATP1B1, coded by *SLCO1B1* transports a large number of therapeutic drugs, such as atorvastatin. Here we investigated the effect of *SLCO1B1* polymorphism on the pharmacokinetics of atorvastatin and its metabolites. Two pharmacokinetic studies were conducted in Chinese Han volunteers and 132 volunteers were enrolled in our study as 72 in trial 1 and 60 in trial 2. A LC-MS/MS method was developed for the identification and quantification of atorvastatin acid and its metabolites. S*LCO1B1* c.521T>C (rs4149056) was identified by the MALDI-TOF MS and Sequenom MassARRAY system. The distribution frequencies of *SLCO1B1* c.521T>C were in agreement with Hardy-Weinberg equilibrium both in trial 1 and trial 2. In subjects with 521C allele the mean C_{max}, $AUC_{0\text{-}24h}$ and $AUC_{0\text{-}\infty}$ of atorvastatin acid and 2-hydroxyatorvastatin acid were significantly higher than subjects with 521TT genotype, while the mean CL was lower. In conclusion, our results suggested that *SLCO1B1* c.521T>C had an effect on the pharmacokinetics of atorvastatin and 2-hydroxyatorvastatin in Chinese Han population. Subjects with 521C allele have an increased risk of toxic effects caused by atorvastatin.

1. Introduction

Statins are synthetic 3-hydroxy-3-methylglutary coenzyme A (HMG-CoA) reductase inhibitors that are widely used for reducing the circulating low-density lipoprotein cholesterol (LDL-C) level (Endo 1992). The pharmacokinetics of statins was extensively studied in its clinical development program, indicating that their serum concentration and systemic exposure exhibited substantial inter-subject variability (Lins et al. 2003; Superko et al. 2012; Zineh 2005). Increasing systemic exposure of statins would lead to adverse drug reactions such as myopathy (Neuvonen et al. 2006). The variation of statins in systemic exposure is partly explained by genetic factors. Single nucleotide polymorphisms (SNPs) in genes involved in the uptake, metabolism, or clearance of statins may increase the systemic exposure as well as the risk of developing adverse drug reactions (Mangravite et al. 2007, 2008).

OATP1B1, coded by *SLCO1B1*, is a member of organic anion transporting polypeptides (OATPs). OATP1B1 is expressed in the sinusoidal membrane of hepatocytes and transports a large number of therapeutic drugs, such as atorvastatin (Lau et al. 2006). Many SNPs of *SLCO1B1* have been identified, among which c.521T>C (rs4149056) is studied extensively (Pasanen et al. 2008). Atorvastatin is metabolized by cytochrome P450 (CYP) 3A4 to 2-hydroxyatorvastatin and 4-hydroxyatorvastatin, which are approximately equal in activity to the parent compound (Borek-Dohalský et al. 2006; Lilja et al. 1999). The effect of *SLCO1B1* variants on the pharmacokinetics of atorvastatin has been studied well, but information for its metabolites is limited (Lee et al. 2015). In this study, we evaluated the effect of *SLCO1B1* c.521T>C on the pharmacokinetics of atorvastatin and its metabolites in Chinese Han population.

2. Investigations and results

2.1. Demographic characteristics

132 subjects were enrolled in our study as 72 in trial 1 and 60 in trial 2. The distribution frequencies of *SLCO1B1* c.521T>C were in agreement with Hardy-Weinberg equilibrium in two trials. The baseline demographic characteristics of the population shown in

Table 1: Baseline demographic characteristics of the population in this study based on c.521T>C genotype

Characteristic	Trial 1			Trial 2		
	All	521TT	521TC	ALL	521TT	521TC+CC
Age (years)	22±2	22±2	22±3	23±3	23±3	23±3
Weight (kg)	1.70±0.06	1.71±0.05	1.69±0.06	1.71±0.06	1.70±0.06	1.72±0.07
Hight (m)	62.16±7.36	62.21±7.45	61.92±6.90	62.28±6.82	61.66±6.36	64.00±7.68
BMI (kg/m^2)	21.38±1.71	21.33±1.67	21.62±1.87	21.30±1.66	21.20±1.62	21.58±1.75

BMI: body mass index

Table 1 indicated that no significant difference was found in age, height, weight among the genotypes.

2.2. The effect of SLCO1B1 polymorphism on the pharmacokinetics of atorvastatin acid

When making statistical analysis, we combined TC and CC genotypes because the frequency of TT genotype was too small (n=2) to make comparison in trial 2. The pharmacokinetic parameters of atorvastatin acid are presented in Table 2 according to the *SLCO1B1* genotypes and the mean plasma concentration-time profiles are shown in the Fig. 1.

In subjects with 521TC genotype in trial 1, the mean C$_{max}$, AUC$_{0-24h}$ and AUC$_{0-\infty}$ of atorvastatin acid were 34.4%, 46.5% and 41.5% higher than subjects with 521TT genotype (*p*=0.024; *p*=0.000; *p*=0.000), while the mean CL was 34.2% lower (*p*=0.000). Excitingly, the results of trial 2 were almost consistent with the former. Compared with 521TT carriers, the mean C$_{max}$, AUC$_{0-24h}$ and AUC$_{0-\infty}$ of atorvastatin acid in subjects with 521C allele (TC+CC) were 49.1%, 52.6% and 48.4% higher (*p*=0.075; *p*=0.000; *p*=0.000) in trial 2. The mean CL of carriers with 521C allele was 34.4% lower than 521TT carriers correspondingly (*p*=0.000).

2.3. The effect of SLCO1B1 polymorphism on the pharmacokinetics of 2-hydroxyatorvastatin acid

The pharmacokinetic parameters of 2-hydroxyatorvastatin acid are presented in Table 3 and the mean plasma concentration-time profiles are shown in Fig. 1.

521TC carriers showed 53.8%, 39.4%, and 34.4% more than subjects with 521TT genotype in the mean C$_{max}$, AUC$_{0-24h}$ and AUC$_{0-\infty}$ of 2-hydroxyatorvastatin acid in trial 1 (*p*=0.002; *p*=0.009; *p*=0.018). The mean CL of subjects with 521TC genotype was 27.1% lower than 521TT carriers (*p*=0.005). As with pharmacokinetic variation trends of 2-hydroxyatorvastatinacid in trial 1, the 521T carriers had more than 32.4%, 30.7% and 27.1% times in the mean C$_{max}$, AUC$_{0-24h}$ and AUC$_{0-\infty}$ of 2-hydroxyatorvastatin acid in trial 2 compared with 521TT carriers (*p*=0.033; *p*=0.024; *p*=0.025). Meanwhile, the mean CL of subjects with 521TC genotype was 22.6% lower than 521TT carriers (*p*=0.044).

3. Discussion

Our study suggested that the polymorphism of *SLCO1B1* had an effect on the pharmacokinetics of atorvastatin and its metabolite, 2-hydroxyatorvastatin in Chinese Han population. Subjects with 521C allele showed higher plasma concentrations systemic exposure and lower clearance of atorvastatin and 2-hydroxyatorvastatin.

Atorvastatin is administered as the calcium salt of the active hydroxyl acid form, but is partly converted in vivo into an inactive lactone metabolite (Kearney et al. 1993). The lactone form of atorvastatin penetrates passively across cell membranes into peripheral tissues due to its high lipophilicity (Ulvestadet et al. 2013), which would mean that the acid form is more dependent on uptake transporters to be taken up into the liver than the lactone form. Many studies have reported that the polymorphism of *SLCO1B1* has little effect on atorvastatin lactone and its metabolites (Ulvestad et al. 2013; Lee et al. 2010; Pasanen et al. 2007). So we just focus on the acid form of atorvastatin and its metabolite in our study. 2-Hydroxyatorvastatin was reported to be similar in activity with atorvastatin. Lau et al. (2006) showed that rifampin, an inhibitor of OATP1B1, significantly increased the AUC of 2-hydroxyatorvastatin acid indicating that 2-hydroxyatorvastatin is a substrate of OATP1B1. The *SLCO1B1* 521T>C variant (rs4149056) leads to reduced activity of OATP1B1, resulting in decreased hepatic stain uptake. Consistent with previously clinical findings (Daka et al. 2015; Birmingham et al. 2015; Lee et al. 2010), our results indicated that C carriers of *SLCO1B1* 521T>C showed higher serum concentration and systemic exposure (AUC) of atorvastatin acid and 2-hydroxyatorvastatin acid and lower clearance compared with

Table 2: Effect of *SLCO1B1* c.521T>C on the pharmacokinetics of atorvastatin acid

SLCO1B1 genotype	T$_{1/2}$ (h)	T$_{max}$ (h)	C$_{max}$ (ng/mL)	AUC$_{0–24h}$ (ng/mL·h)	AUC$_{0–\infty}$ (ng/mL·h)	CL (L/h)
Trial 1						
TT(60)	7.89±3.13	1.50(0.30-5.00)	3.20±1.76	20.95±6.74	23.92±8.07	450.59±141.24
TC (12)	6.94±1.61	1.00(0.50-6.00)	4.30±1.75	30.70±9.40	33.85±9.51	296.31±79.30
p	0.311	0.370	0.024 [a]	0.000 [a]	0.000 [a]	0.000 [a]
Trial 2						
TT(44)	8.50±2.99	0.50(0.25-4.00)	3.81±1.83	19.96±8.65	21.66±8.70	517.09±168.42
TC+CC(16)	8.69±1.45	0.50(0.25-3.00)	5.68±3.69	30.45±10.05	32.14±9.75	339.28±105.65
p	0.805	0.697	0.075	0.000 [b]	0.000 [b]	0.000 [b]

[a] *p*<0.05, was a comparison of TT genotype with TC genotype.
[b] *p*<0.05, was a comparison of TT genotype with TC + CC genotypes.

Table 3: Effect of *SLCO1B1* c.521T>C on the pharmacokinetics of 2-hydroxyatorvastatinin acid

SLCO1B1 genotype	T$_{1/2}$ (h)	T$_{max}$ (h)	C$_{max}$ (ng/mL)	AUC$_{0–24h}$ (ng/mL·h)	AUC$_{0–\infty}$ (ng/mL·h)	CL (L/h)
Trial 1						
TT(60)	11.42±4.10	4.50 (0.50-12.00)	2.21±0.85	35.97±13.14	39.76±713.83	280.18±95.91
TC (12)	9.79±2.88	3.00(1.00-10.00)	3.40±1.41	50.14±20.37	53.44±22.08	204.29±71.26
p	0.196	0.512	0.002 [a]	0.009 [a]	0.018 [a]	0.005 [a]
Trial 2						
TT(44)	8.96±2.88	1.75(0.50-8.00)	1.48±0.85	17.96±7.23	19.97±7.54	575.16±229.46
TC+CC(16)	8.86±2.03	0.50(0.25-3.00)	1.96±0.84	23.47±8.79	25.39±8.62	444.96±176.42
p	0.899	0.498	0.033 [b]	0.024 [b]	0.025 [b]	0.044 [b]

[a] *p*<0.05, a comparison of TT genotype with TC genotype.
[b] *p*<0.05, was a comparison of TT genotype with TC + CC genotypes.

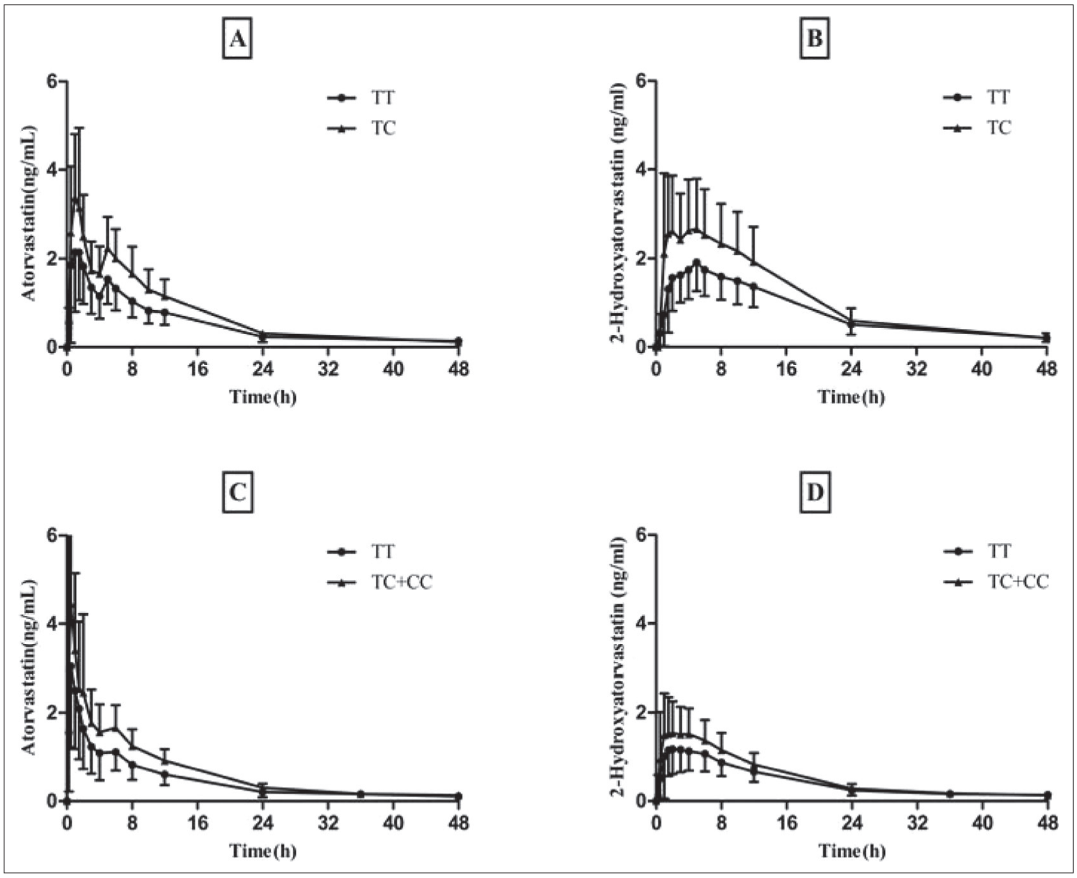

Fig. 1: The mean (±SD) plasma concentrations of (A) atorvastatin acid in trial 1, (B) 2-hydroxyatorvastatin acid in trial 1, (C) atorvastatin acid in trial 2, (D) 2-hydroxyatorvastatin acid in trial 2 in relation to the *SLCO1B1* c.521T>C.

TT carriers. The clearance of 521C carriers was lower than TT carriers that may caused by less atorvastatin pumped into the liver. We reported two clinic trials and analyzed the results in our study. That was because the difference of C_{max} was statistically significant between trial 1 and trial 2 (3.38±1.79 ng/mL vs 4.31±2.57 ng/mL, p=0.011) as AUC_{0-24h} and $AUC_{0-\infty}$ were similar (22.57±8.05 ng/mL˙h vs 22.76± 10.10 ng/mL˙h, p=0.867; 24.46±10.06 ng/mL˙h vs 25.58±9.06 ng/mL˙h, p=0.353). We inferred that the difference might be caused by the impact of amlodipine.

There are some limitations to this study. Firstly, A388>G (rs2306283) is another variant of *SLCO1B1.* c.521T>C and c.388A>G are in linkage disequilibrium and form four haplotypes together: *SLCO1B1**1A, *1B, *5 and *15 (Giacomini et al. 2013). But our study failed to detect the variant of c.388A>G. Secondly, two trials cannot be combined resulting in a small number of subjects with CC genotype. Thirdly, 4-hydroxyatorvastatin has similar activity with atorvastatin and 2-hydroxyatorvastatin. But our detection method is not suitable for its quantification.

In conclusion, our results suggested that *SLCO1B1* c.521T>C had an effect on the pharmacokinetics of atorvastatin and 2-hydroxyatorvastatin in Chinese Han population. Subjects with 521C allele have an increased risk of toxic effect caused by atorvastatin.

4. Experimental

4.1. Study design

Two pharmacokinetic studies were conducted in Chinese Han volunteers. In trial 1, 60 volunteers were requested to take a pill of compound preparation containing 5 mg amlodipine and 10 mg atorvastatin calcium and in trial 2, a pill of 10 mg atorvastatin calcium only was given to 72 volunteers. The study protocols were approved by the Independent Ethics Committee Institute of Clinical Pharmacology, Central South University (Hunan, China) and the Chinese Clinical Trial Register (registration number: ChiCTR-TTRCC-12002952 and ChiCTR-IPR-15006175).

4.2. Subjects

All volunteers (age range, 18-28 years; BMI range, 19.0-24.0 kg/m^2) gave their written informed consent. They were determined to be healthy by electrocardiogram, and laboratory tests including blood routine and biochemistry testing and urine alysis before the study. Participants were excluded for the following reasons: any significant medical history, taking prescription or over-the counter medication or alcohol within two weeks before enrollment, history of smoking, alcohol or drug abuse, donation of blood within the past three months. After an overnight fast, subjects were required to take a tablet containing 5 mg amlodipine and 10 mg atorvastatin calcium or 10 mg atorvastatin alone. Blood samples (5 mL) were drawn into heparin lithium-anticoagulant tubes before drug intake and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 h after dosing and were centrifuged at 4000 rpm for 5 min. Plasma was separated and stored at -70 °C until assay. For genetic analysis, 2 mL blood sample was drawn from each subject and stored at -70 °C until DNA extraction.

4.3. SNP genotyping

Genomic DNA was extracted from peripheral whole blood using a Qiagen DNA Isolation Kit (Valencia, CA, USA). *SLCO1B1* c.521T>C (rs4149056) was identified by the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and Sequenom MassARRAY system (Sequenom, San Diego, CA, USA).

4.4. Pharmacokinetics

A sensitive and specific liquid chromatography-tandem mass spectrometer (LC-MS/MS) technology was developed and applied to the identification and quantification of atorvastatin acid, 2-hydroxyatorvastatin acid and 4-hydroxyatorvastatinin acid in human plasma. Pharmacokinetic parameters were calculated by the software DAS 2.1.1. Peak concentration in plasma (C_{max}), time to C_{max} (T_{max}), elimination half-life ($T_{1/2}$), plasma clearance (CL) and areas under the plasma concentration-time curve from 0 to 24 h (AUC_{0-24h}), and 0 h to infinity ($AUC_{0-\infty}$) were calculated for atorvastatin acid and 2-hydroxyatorvastatin acid (since more than two-thirds of 4-hydroxyatorvastatin acid concentration points was below the lower limit of quantitation, the pharmacokinetic parameters were not calculated).

4.5. Statistical analysis

The data were analysed by the statistical program SPSS software (version 19.0, SPSS, Chicago, IL, USA). Deviations from the Hardy-Weinberg equilibrium were calculated by Chi-square test. Continuous variables are presented as mean±standard deviation (SD), except for T_{max}, which is given as median (range). The C_{max} and AUC values were logarithmically transformed before analysis. The pharmacokinetic variables of atorvastatin acid and 2-hydroxyatorvastatin acid were compared between the genotypes by a two-tailed Student's t test. The T_{max} data was analysed by the Mann-Whitney test. The drug concentration-time curve was conducted by GraphPad Prism software (version 5.0, GraphPad Software, Inc. San Diego, California). A *p*-value of less than 0.05 was considered statistically significant.

Acknowledgements: This work was supported by National Development of Key Novel Drugs for Special Projects of China (2012ZX09303014001) and Postgraduate Innovation Project of Central South University (2016zzts127).

Conflict of interest: The authors declare that there is no conflict of interest.

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