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The influence of genetic polymorphisms in drug metabolism enzymes and transporters on the pharmacokinetics of different fluvastatin formulations

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ABSTRACT

The purpose of the present study was to investigate the impact of genetic polymorphism on fluvastatin pharmacokinetics. In addition, we compared the fluvastatin pharmacokinetics differences between extended-release (ER) 80 mg tablet and immediate-release (IR) 40 mg capsule in terms of drug metabolism enzyme and transporter genetic polymorphisms. In this open-label, randomized, two-period, two-treatment, crossover study ($n = 24$), effects of ABCG2, SLCO1B1, ABCB1, CYP2C9 and CYP3A5 polymorphisms on the pharmacokinetics of fluvastatin were analyzed. The administration dosage for IR 40 mg and ER 80 mg were twice and once daily, respectively, for total 7 d. Blood samples for pharmacokinetic evaluation were taken on the 1st and 7th d. The lower exposure following ER was observed. For ER tablets, SLCO1B1 T521C genotype correlated with AUC_{0-24} of repeat doses ($P = 0.010$). SLCO1B1 T521C genotype had no statistically significant effect on AUC_{0-24} of IR capsule of fluvastatin after single or repeated doses. *In vitro* study demonstrated that when the concentration of fluvastatin was low ($< 1 \mu\text{mol/l}$), the uptake of fluvastatin in the HEK293-OATP1B1 with SLCO1B1 521TT ($K_m = 0.18 \mu\text{mol/l}$) was faster than that with SLCO1B1 521CC ($K_m = 0.49 \mu\text{mol/l}$). On the other hand, when concentration reached to higher level ($> 1 \mu\text{mol/l}$), transport velocity of fluvastatin by HEK293-OATP1B1 with SLCO1B1 521TT ($K_m = 11.4 \mu\text{mol/l}$) and with SLCO1B1 521TCC ($K_m = 15.1 \mu\text{mol/l}$) tend to be the same. It suggests that the increased effect of SLCO1B1 T521C genotype on ER formulation of fluvastatin was mainly caused by lower blood concentrations. We recommend that formulation should be incorporated into future pharmacogenomics studies.

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1. Introduction

Fluvastatin sodium is a potent synthetic competitive reversible inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, which is the rate limiting enzyme for cholesterol biosynthesis in the liver [1]. It is widely used to improve lipid profiles in patients with primary hypercholesterolemia and mixed dyslipidemia. Fluvastatin treatment has been shown to reduce concentrations of total cholesterol, low-density lipoprotein (LDL) and triglycerides, and to increase high-density lipoprotein (HDL) levels [2]. Therefore, it exerts antiatherogenic effects and reduces the incidence of coronary heart disease [3,4]. A widely applied fluvastatin regimen includes a 40 mg immediate-release (IR) capsule twice daily or an 80 mg extended-release (ER) tablet once daily. Both formulations have been reported to be effective in patients with primary hypercholesterolemia and mixed dyslipidemia; however, the ER formulation is more potent than the IR formulation [5,6].

The treatment effects of statins could be influenced by pharmacogenetics. Genetic variations could alter the activity of cytochrome P450 (CYP) isoenzymes and transporter proteins [7,8], therefore result in pharmacokinetic differences. Significant Statistical differences have been shown in the pharmacokinetics of fluvastatin in subjects with different numbers of CYP2C9*3 alleles ($P < 0.0001$, F -test) [9]. Although it has been shown that the pharmacokinetics of fluvastatin was not altered by SLCO1B1 (encoding OATP1B1) T521C polymorphism [10], a tendency of attenuated reduction in total cholesterol and LDL in subjects carrying one or more variant allele(s) of SLCO1B1 T521C polymorphism has been observed [11,12]. In addition, it has been shown that SLCO1B1 contributed to inter-individual variability of fluvastatin pharmacodynamics [13]. Therefore, the association of SLCO1B1 single nucleotide polymorphism (SNP) with pharmacokinetics and pharmacodynamics of fluvastatin needs to be further clarified.

ER formulations enable the less frequent administration of drugs and reduce peak serum concentrations, thereby lowering the risk of adverse events. Since the genetic variations influencing pharmacokinetics are usually related to proteins involved in drug metabolism, which would be expected to be affected by peak concentrations of serum drugs, the influence of genotype on pharmacokinetics might be different in different formulations that modify drug exposure. However, there have been very few studies addressing this. Clinical Pharmacogenetics Implementation Consortium posted a series of gene/drug clinical practice guidelines, which emphasized the pharmacokinetics-genetic polymorphism relationship [14]. These guidelines have a great influence on global clinical application. However, none of them mentioned the potential effects of the dosage form on the pharmacokinetics-genetic polymorphism relationship.

After reviewing all pharmacogenetic studies concerning fluvastatin [9,10,15], we found that these studies were usually performed in signal dose settings with IR 40 mg fluvastatin formulation, whereas in the studies investigating the effect of genetic polymorphisms on fluvastatin efficacy, the treatment regimen included repeated doses of fluvastatin

with either IR 40 mg or ER 80 mg formulation. There has been no study investigating the difference in the relationships of genetic polymorphisms with pharmacokinetics parameters under different treatment settings. With the extensive application of sustained-release dosage forms, it is important to clarify the effect of formulation on the pharmacokinetics/pharmacodynamic-gene relationship. Therefore, the present study aimed to examine the pharmacokinetics of fluvastatin in two treatment settings under different formulations with the same total daily dose [16]. The effects of SLCO1B1 T521C, G388A, CYP2C9 *3, CYP3A5*3, ABCG2 C421A, ABCB1 G2677T/A and C3435T genetic polymorphisms on the pharmacokinetics of fluvastatin, for both initial and repeated doses, were examined.

2. Materials and methods

2.1. Material

The extended-release (ER) 80 mg tablets and immediate-release (IR) 40 mg capsules were provided by Novartis Pharmaceutical Co., Ltd. Phosphate buffer solution (PBS) was purchased from Beijing solarbio science & technology co., ltd. Fluvastatin was bought from Sigma-Aldrich® (Hongkong, China). The dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), the 100 × mixture of penicillin and streptomycin (PS), 0.25% trypan were all obtained from Gibco (USA). The cell lines HEK 293-Mock (carrying the blank plasmid), HEK293-OATP1B1 (with SLCO1B1 521TT) and HEK293-OATP1B1(with SLCO1B1 521CC) were friendly offered by State key laboratory of drug release technology and pharmacokinetics in Tianjin Pharmaceutical Research Institute New Drug Evaluation Company.

2.2. Study population

A total of 24 healthy Chinese subjects were recruited to participate in the present crossover study. The health status of participants was confirmed based on medical history, physical examination, vital signs (blood pressure, pulse rate and temperature), safety laboratory tests (blood chemistry, hematology and urine analysis), and 12-lead electrocardiogram. The subjects had not been taking any medicine for at least 4 weeks prior to initiation of the study. All subjects were briefed about the purpose, duration, and potential risks of the study. Written informed consent was obtained from each subject. The study was conducted in compliance with the Declaration of Helsinki and the study protocol was approved by the Ethical Review Board of Peking University First Hospital.

2.3. Study design

Subjects were randomly assigned in a 1:1 ratio into 2 groups to receive fluvastatin as either a 40 mg IR capsule twice daily (8:00 a.m. and 8:00 p.m.) or an 80 mg ER tablet once daily (8:00 a.m.) for 7 days. Following a washout period for a week, subjects were switched to the other treatment. During the

study period, subjects were served the same meals. At day 1 and day 7 of each treatment period, all subjects fasted overnight (10h) before administration of the first dose and were not allowed to eat until 2h after the first dose. Each formulation was administered with 240 ml of water. During day 2 to day 6, breakfast was provided half an hour after administration of fluvastatin. Lunch and dinner were provided at 12:00 a.m. and 6:00 p.m., respectively.

On day 1 and day 7, whole blood samples (3.5 ml) were collected by intravenous puncture or forearm catheter at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16 and 24 h after administration in the ER group, and at 0.25, 0.5, 1, 2, 4, 6, 8, 12 (before the second dose), 12.5, 13, 14, 16, 20 and 24 h in the IR group. At day 5 and day 6, blood samples of 3.5 ml were taken just before drug administration. Blood samples were collected in serum separation tubes at room temperature, stored away from direct sunlight and ultraviolet radiation, and then centrifuged at 4 °C and $1500 \times g$ for 15 min. The separated serum samples were collected in 4-ml centrifuge tubes and stored at -70 °C until assay.

2.4. Determination of serum fluvastatin concentration

The high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was employed to detect serum fluvastatin. The analytical column was a Waters Xterra MS C₁₈ (50 mm \times 2.1 mm; 3.5 μ m; Waters Technologies, Inc.). The mobile phase A was 0.1% acetic acid aqueous solution, and mobile phase B was 100% methanol. The injection volume was 10.0 μ L. A linear gradient was ramped up from 35% to 100% of mobile phase A in 5 min at the flow rate was 320 μ L/min. The column temperature was maintained at 40 °C, and the mass spectrometer was used in the negative scan mode. Quantitation was accomplished by API4000 (AB Sciex Pte. Ltd.) and monitoring of the precursor-to-product pairs m/z 410.2–348.2 for fluvastatin and m/z 418.2–356.2 for the internal standard. The linear calibration range was 0.5–500 ng/ml. The five QC sample concentrations were 0.500, 1.50, 15.0, 125 and 400 ng/ml. The inter- and intra-day precision (RSD) were < 3.6% (less than 19.1% for lower limit of quantification), and accuracy was within 97.6%–112.8%. Absolute recovery rates were $90.3\% \pm 13.5\%$, $94.3\% \pm 13.2\%$, and $106.5\% \pm 14.5\%$ for 1.50, 125 and 400 ng/ml, respectively. No significant matrix effect was found. All samples were analyzed within established storage stability periods.

2.5. Analysis of CYP2C9, SLC01B1, ABCB1, and ABCG2 genotypes

DNA was extracted from peripheral whole blood samples of each subject using a DNA purification kit (Wizard, Promega, USA). Further, SLC01B1 G388A (rs4363657), ABCB1 C3435T (rs1045642), CYP2C9*3 (rs1057910) and CYP3A5*3 (rs776746) alleles were detected using polymerase chain reaction (PCR)-restriction fragment length polymorphism, and SLC01B1 T521C (rs4149056), ABCG2 C421A (rs2231137) and ABCB1 G2677T/A (rs2032582) alleles were evaluated using PCR sequencing. The primers and restriction enzymes that were used are listed in Table S1. All methods were verified

by sequencing and all sequences were compared with the reference sequences in GenBank.

2.6. Pharmacokinetic evaluation

Pharmacokinetic analyses and simulations were conducted using WinNonlin Professional Edition, Version 3.1 (Pharsight Corp., Mountain View, CA, USA). C_{max} and T_{max} were directly estimated from the observed serum concentration-time data. AUC_{0-t} and AUC_{t1-t2} were calculated using the linear trapezoidal rule. Pharmacokinetic parameters determined after first dosing included AUC_{0-t} , AUC_{t1-t2} , C_{max} , T_{max} and $T_{1/2}$, and after multiple dosing included AUC_{0-t} , AUC_{t1-t2} , C_{max} , T_{max} , $T_{1/2}$, C_{min} and average concentration (C_{av}).

2.7. Statistical analyses

Allele and genotype frequencies for the variant SNPs were assessed for deviation from Hardy–Weinberg equilibrium using the χ^2 test. The Mann–Whitney U-test was applied to evaluate significant differences in pharmacokinetic parameters between genotypic group pairs. Data from three or more different genotypic groups were compared using the Kruskal–Wallis H-test. Correlations between variables were examined using Pearson's correlation analysis. Statistical analyses were performed using SPSS for Windows, version 22.0. A P-value ≤ 0.05 was considered statistically significant.

2.8. In vitro study

The lines cryopreserved in -80 °C ultra-cold storage freezer were revived and after two time passages, the confluent cells were treated by 0.25% trypsin and the cell suspensions were diluted with culture medium (DMEM+10%FBS+PS) to the concentration of 2.0×10^5 (cells/ml). The diluted suspension were injected into 24-well cell culture plates by 1 ml per well and thereafter, the plates were removed to the incubator with 5% CO₂ and 95% air humidity at the temperature of 37 °C. After 48 h culture, when cells were completely confluent, the plates with different cell lines were removed to water bath of 37 °C. The old medium was replaced by 1 ml PBS. After incubation for 10 min, the PBS was replaced by 0.5 ml PBS with different concentrations of fluvastatin. After incubated for 5 min, the fluvastatin solution was removed and all the cells were washed by cold PBS for three times to stop the uptake action. Thereafter, the cells were lysed by multigelation for three times, and then added 0.4 ml methyl alcohol with 50 mg/l simvastatin taken as interior label. After ultrasonic treatment for 10 min, the samples were centrifuged at $10000 \times g$ for 5 min and 0.1 ml supernatant was carried to analyze by LC-MS.

3. Results and discussion

3.1. In vivo pharmacokinetic study

A total of 24 healthy subjects were recruited and completed the study, which included 14 males and 10 females. The mean

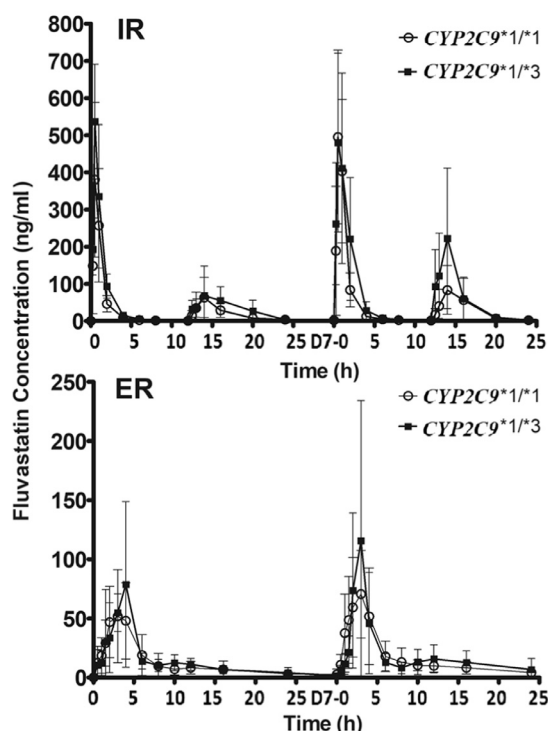


Fig. 1 – Mean serum concentrations of fluvastatin in different CYP2C9*3 genotypes after the administration of the first single dose and repeated doses. Values are represented as mean \pm SD.

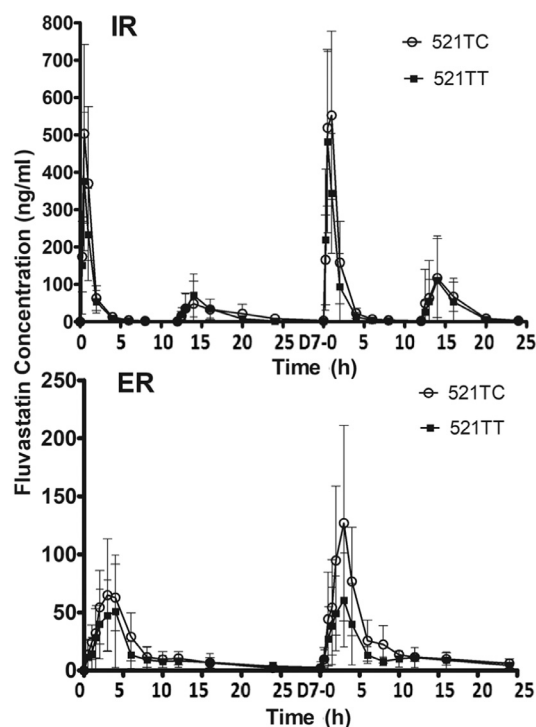


Fig. 2 – Mean serum concentrations of fluvastatin in different SLCO1B1 T521C genotypes after the administration of a first dose and repeated doses. Values are represented as mean \pm SD.

age was 27.5 years (range: 21–42 years), the average height was 166.6 \pm 6.95 cm (range: 155–177 cm), the mean weight was 60.8 \pm 5.15 kg (range: 51.0–71.0 kg), and the mean body mass index was 21.9 \pm 1.25 kg/m² (range: 19.5–23.9 kg/m²). For CYP2C9*3 SNP, there were 19 subjects with *1/*1 and 5 subjects with *1/*3 genotype. For SLCO1B1 T521C SNP, there were 17 individuals with TT and 7 with TC genotype. Genotypes of CYP2C9*3/*3 and SLCO1B1 521CC were not observed in the subjects of the present study. Serum concentrations on day1 and day7 are illustrated for subjects with different CYP2C9*3 and SLCO1B1 T521C genotypes, respectively (Figs. 1 and 2). Following single and multiple doses of IR formulation, the serum concentrations of fluvastatin after the second dosing were lower than that after the first time. The most likely explanation for this phenomenon is a reduced exposure due to the effect of food as the evening meal was provided to the subjects 2 h before the second daily dose. This is consistent with previous studies [17,18].

The blood concentration time curve of 521TC was always above 521TT after repeated dosing of ER fluvastatin. Pharmacokinetic parameters of fluvastatin in the study are summarized in Tables 1 and 2. The CYP2C9*3 genotypes were significantly associated with the AUC₀₋₂₄ after the first dose of IR ($P=0.043$). AUC₀₋₂₄ values after repeated doses for 7 d also showed an increase in CYP2C9*3 carriers; however, the differences were not statistically significant (Table 1). SLCO1B1 T521C genotype significantly influenced the AUC₀₋₂₄ for repeated doses in the ER regimen ($P=0.010$), whereas no

significant correlation was observed for the first and repeated doses in the IR regimen (Table 2). To further clarify the effect of CYP2C9*3 and SLCO1B1 T521C, we performed a combined analysis of the two genes. Fig. 3 shows the comparison of the fluvastatin AUC₀₋₂₄ among four different CYP2C9/SLCO1B1 genotype groups. The results did not show any significant correlations between pharmacokinetic parameters with other genotypes, including SLCO1B1 G388A, ABCG2 A421C, ABCB1 G2677T/A, ABCB1 C3435T, and CYP3A5*3 (Table S2). Both these treatments were generally well tolerated by the healthy subjects in the present study.

To the best of our knowledge, this is the first study to investigate the relationship between genetic polymorphism and pharmacokinetics of ER fluvastatin and repeated doses of IR fluvastatin. The findings of the present study indicated that with ER formulation, the effect of CYP2C9*3 on pharmacokinetics was less significant and the influence of SLCO1B1 T521C was more significant than those with IR formulation. The SLCO1B1 T521C was associated with AUC₀₋₂₄, C_{max}, and C_{av} after the repeated doses of ER formulation. In addition, C_{min} after the repeated doses of IR formulation were influenced by SLCO1B1 T521C. Conversely, the CYP2C9*3 genotype was significantly correlated with the AUC₀₋₂₄ and AUC₀₋₁₂ after the first dose of the IR formulation, but not with those in the ER formulation settings.

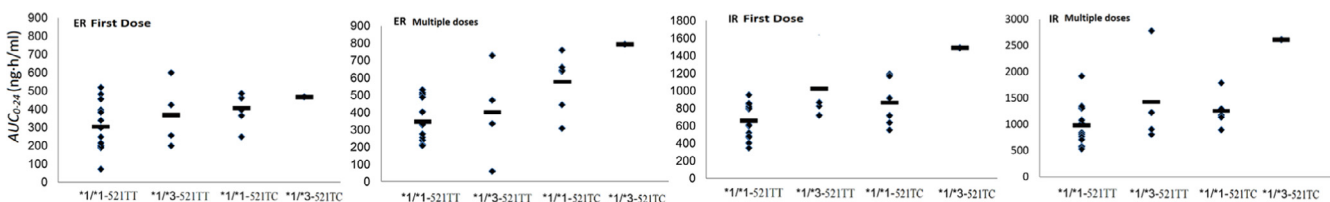
To date, there are only three studies that have reported the effects of genetic polymorphisms on fluvastatin pharmacokinetics, and all of these studies administering fluvastatin IR 40 mg in a single dose [10,15]. Niemi et al.

Table 1 – Summary of pharmacokinetic parameters of fluvastatin after the first and subsequent dosing subjects with CYP2C9*1/*1 and CYP2C9*1/*3 genotypes.

CYP2C9		ER 80 mg once every day			IR 40 mg twice every day		
		*1/*1 (n = 19)	*1/*3 (n = 5)	P	*1/*1 (n = 19)	*1/*3 (n = 5)	P
First dose	AUC ₀₋₂₄ (ng h/ml)	336.7 ± 129.2	387.2 ± 162.5	0.499	721.9 ± 239.8	1116.1 ± 434.3	0.043
	AUC ₀₋₁₂ (ng h/ml)				469.3 ± 184.3	691.1 ± 210.0	0.030
	AUC ₄₋₁₂ (ng h/ml)				31.6 ± 12.6	59.5 ± 44.0	0.271
	C _{max} (ng/ml)	73.1 ± 29.3	101.6 ± 56.0	0.374	408.3 ± 200.2	559.4 ± 159.6	0.110
	T _{max} (h)	2.95 ± 1.13	3.10 ± 1.02	0.630	0.70 ± 0.40	0.60 ± 0.22	0.732
	T _{1/2} (h)	8.22 ± 6.68	10.1 ± 8.43	0.804	2.27 ± 1.23	2.39 ± 1.07	0.823
Repeated dose	AUC ₀₋₂₄ (ng h/ml)	420.1 ± 173.9	478.8 ± 298.6	0.499	1069.4 ± 388.5	1665.5 ± 957.8	0.271
	AUC ₀₋₁₂ (ng h/ml)				704.9 ± 266.0	972.5 ± 555.0	0.455
	AUC ₄₋₁₂ (ng h/ml)				20.6 ± 6.67	38.8 ± 26.0	0.241
	C _{max} (ng/ml)	89.1 ± 38.7	126.1 ± 110.3	0.644	533.2 ± 231.4	501.4 ± 239.2	0.972
	T _{max} (h)	2.84 ± 0.94	2.60 ± 0.55	0.521	0.66 ± 0.24	1.00 ± 0.61	0.167
	T _{1/2} (h)	10.7 ± 11.2	14.7 ± 18.2	0.859	1.76 ± 0.27	1.69 ± 0.23	0.569
	C _{min} (ng/ml)	1.15 ± 1.43	0.91 ± 0.65	0.971	1.31 ± 0.53	1.90 ± 1.40	0.915
	C _{av} (ng/ml)	17.5 ± 7.22	19.9 ± 12.4	0.499	58.7 ± 22.2	81.0 ± 46.2	0.455

Table 2 – Summary of pharmacokinetic parameters of fluvastatin after the first and subsequent dosing subjects with SLCO1B1 T521C TT and TC genotypes.

SLCO1B1 T521C		ER 80 mg once every day			IR 40 mg twice every day		
		TT (n = 17)	TC (n = 7)	P	TT (n = 17)	TC (n = 7)	P
First dose	AUC ₀₋₂₄ (ng h/ml)	319.9 ± 143.0	413.6 ± 87.3	0.092	742.1 ± 303.8	954.2 ± 343.0	0.153
	AUC ₀₋₁₂ (ng h/ml)				462.0 ± 163.3	645.2 ± 254.9	0.105
	AUC ₄₋₁₂ (ng h/ml)				31.3 ± 21.3	52.2 ± 26.6	0.012
	C _{max} (ng/ml)	73.8 ± 38.2	91.6 ± 32.3	0.081	406.3 ± 176.3	521.1 ± 242.0	0.216
	T _{max} (h)	2.97 ± 1.21	3.00 ± 0.82	0.816	0.69 ± 0.41	0.64 ± 0.24	0.939
	T _{1/2} (h)	10.1 ± 7.7	4.99 ± 1.86	0.172	2.15 ± 1.26	2.70 ± 0.85	0.059
Repeated dose	AUC ₀₋₂₄ (ng h/ml)	360.0 ± 164.2	608.0 ± 172.5	0.010	1086.6 ± 565.1	1453.6 ± 579.6	0.105
	AUC ₀₋₁₂ (ng h/ml)				677.2 ± 298.6	963.3 ± 399.4	0.081
	AUC ₄₋₁₂ (ng h/ml)				21.3 ± 10.7	31.9 ± 20.0	0.070
	C _{max} (ng/ml)	78.3 ± 38.8	141.8 ± 78.5	0.017	488.5 ± 233.4	618.9 ± 200.2	0.295
	T _{max} (h)	2.74 ± 0.87	2.93 ± 0.93	0.637	0.71 ± 0.40	0.79 ± 0.27	0.278
	T _{1/2} (h)	10.3 ± 11.1	14.4 ± 16.3	0.634	1.72 ± 0.24	1.79 ± 0.33	0.975
	C _{min} (ng/ml)	0.95 ± 1.29	1.44 ± 1.34	0.300	1.22 ± 0.61	1.95 ± 0.97	0.024
	C _{av} (ng/ml)	15.0 ± 6.82	25.3 ± 7.19	0.010	56.4 ± 24.9	80.3 ± 33.3	0.081

**Fig. 3 – Comparison of AUC₀₋₂₄ of fluvastatin among four different CYP2C9*3/SLCO1B1T521C genotype groups. —, mean value of AUC₀₋₂₄.**

[10] recruited specific SLCO1B1 T521C genotype volunteers to investigate the effect of the genotype on fluvastatin pharmacokinetics after a single dose and found no difference between the genotypes. Specific CYP2C9 genotype volunteers were investigated in another study, in a treatment setting with fluvastatin at IR 40 mg for 15 days, where CYP2C9*3 genotypes showed significant influence on AUC [9]. However, in the study, the blood samples for pharmacokinetic evaluation

were only taken after the first dose on day 1. Based on our results and those from previous studies, we concluded that fluvastatin pharmacokinetics after the first dose of IR 40 mg were correlated with CYP2C9*3 genotypes, but not with SLCO1B1 T521C.

In conflict with previous pharmacokinetic studies [9,10,15], *in vitro* studies have demonstrated the affinity of fluvastatin to OATP1B1 transporter as being 1.4–3.5 μmol/l [17,18].

What is more, the influence of *SLCO1B1* genotype on pharmacodynamics after repeated doses of IR or ER fluvastatin has been reported previously. Meyer et al. [11], conducted a cohort study that evaluated the influence of *SLCO1B1* on the therapeutic efficacy of fluvastatin in dyslipidemia patients during the 5-year follow-up. They pointed out that subject carrier 521TT received a higher dose (65.1 ± 5.6 mg) than *SLCO1B1* 521TC and CC carriers (40.9 ± 7.6 mg), without demonstrating any other significant effects. In this study, the authors did not specify the fluvastatin formulation. But according to the dose they presented, it seems they used IR. Another cohort study provided evidence that *SLCO1B1* 521T was not associated with total cholesterol and LDL in patients treated with fluvastatin IR 20mg per day [12]. On the other hand, Couvert et al. [13] found that there was a tendency of attenuated reduction in total cholesterol ($P=0.06$) and LDL ($P=0.17$) that lowered the response in subjects carrying one or more variant allele(s) of *SLCO1B1* T521C polymorphism in elderly hypercholesterolemic patients receiving ER 80mg fluvastatin therapy after 2 months follow-up. These authors also demonstrated that C463A (Pro155Thr) of *SLCO1B1* was significantly associated with a superior response to fluvastatin therapy ($P < 0.001$). Table 3 lists all previous studies that have reported the influence of *CYP2C9* or *SLCO1B1* genetic polymorphisms on fluvastatin pharmacokinetics and pharmacodynamics [9–11,13,15,19–21]. Our study provided evidence that *SLCO1B1* 521C was associated with an increased in fluvastatin exposure. As shown in Fig. 2, the blood concentration time curve of 521TC is always above 521TT after repeated doses of ER fluvastatin. Therefore, the conflict of previous pharmacokinetic and pharmacodynamic studies could be explained by attributed to the fluvastatin formulation.

To further clarify the effect of *CYP2C9**3 and *SLCO1B1* T521C, we undertook a combined analysis of the two genes. After the first and repeated doses of ER (Fig. 3), the mean value of AUC_{0-24} in the four groups were $*1/*1-521TT < *1/*3-521TT < *1/*1-521TC < *1/*3-521TC$, and after repeated doses the subjects harboring both *CYP2C9**1/*3 and *SLCO1B1* 521TC had the highest AUC_{0-24} . However, after the first and repeated doses of IR the mean values of AUC_{0-24} were $*1/*3-521TT > *1/*1-521TC$. This reversal suggests that the weight of the two genes might have changed. *CYP2C9**3 might has greater influence on the IR fluvastatin exposure, whereas *SLCO1B1* T521C was more important for ER.

3.3. Hypothesis

The difference of pharmacokinetics between fluvastatin IR 40mg twice every day and ER 80mg once every day were reported in detail in previous studies [22,23]. The pharmacokinetics profile of IR and ER fluvastatin in previous studies was consistent with the present study. The systemic exposure to fluvastatin following the same total dose over 24 h period was about 2–3 fold lower when subjects received ER compared with IR following single and multiple doses. The lower exposure following the ER could be due to the efficient first pass uptake of the drug as a result of slower and sustained drug release [23]. The average concentration

was much lower in ER than IR at each time point. Therefore, we speculated that the influence of genotype on different formulation of fluvastatin was mainly caused by different blood concentrations.

If this hypothesis is correct, then when the serum concentration was decreased at 4h after the first IR dose, AUC_{4-12} should be influenced by *SLCO1B1* T521C. We found that after administration of the first dose of IR, the mean values of AUC_{4-12} of *SLCO1B1* 521TT carriers and *SLCO1B1* 521TC carriers were 31.3 ± 21.3 and 52.2 ± 26.6 ng h/ml, respectively ($P=0.012$). Therefore, the significant association between AUC_{4-12} and *SLCO1B1* T521C supported our hypothesis. Base on the hypothesis we designed in vitro study to detect the characteristics of OATP1B1 with *SLCO1B1* 521TT and OATP1B1 with *SLCO1B1* 521CC at high and low concentrations.

3.4. In vitro study

The uptake-concentration curve of fluvastatin in HEK293-OATP1B1 (carrying *SLCO1B1* 521TT; the following is presented as OATP1B1-521TT) and HEK293-OATP1B1 (carring *SLCO1B1* 521CC; The following is presented as OATP1B1-521CC) were shown in Fig. S1. Results showed that the transport velocity in OATP1B1-521TT was significantly faster than that in OATP1B1-521CC. Results of diagraph analysis through Eadie-Hofstee method (Figs. S2A and S2B) suggested that the uptake proceed of fluvastatin in OATP1B1-521TT and OATP1B1-521CC were biphasic kinetics, in which the kinetic profile does not follow saturation kinetics and has two distinct phases. At lower substrate concentrations ($< 0.3 \mu\text{mol/l}$) the kinetic profile exhibits curvature similar to a hyperbolic profile, however, as the substrate reaches higher concentrations ($< 0.3 \mu\text{mol/l}$), the profile increases linearly (instead of becoming asymptotic) with no evidence of saturation as shown in Fig. S2.

It was easy to speculate that fluvastatin may either binding in multiple productive orientations within the enzyme active site, or there are two substrate molecules bound within the enzyme active site. In this case, one of the binding orientations results in a low K_m and V_{max} component (OATP1B1-521TT, $K_m=0.18 \mu\text{mol/l}$, $V_{max}=4.01 \text{ pmol/mg/min}$; OATP1B1-521CC, $K_m=0.49 \mu\text{mol/l}$, $V_{max}=5.28 \text{ pmol/mg/min}$) as shown in Figs. S3A and S4A, while the other binding orientation produces the high K_m and V_{max} component (OATP1B1-521TT, $K_m=11.4 \mu\text{mol/l}$, $V_{max}=95.3 \text{ pmol/mg/min}$; OATP1B1-521CC, $K_m=15.1 \mu\text{mol/l}$, $V_{max}=100.9 \text{ pmol/mg/min}$) as shown in Figs. S3B and S4B.

Results also suggested that for OATP1B1-521TT the concentration of V_{max} inward was about $0.36 \mu\text{mol/l}$ (148.0 ng/ml , 2 km), while for OATP1B1-521CC concentration of V_{max} inward was about $0.98 \mu\text{mol/l}$ (402.8 ng/ml , 2 km), when the transport of fluvastatin under the first kinetic phase (low concentrations). As the uptake of fluvastatin in OATP1B1-521TT was faster than OATP1B1-521CC, accumulation and elimination of fluvastatin in the liver of *SLCO1B1* 521TT carriers would be higher compared to *SLCO1B1* 521CC carriers, which may lead to significantly lower blood concentration in *SLCO1B1* 521TT compared to *SLCO1B1* 521CC carriers. On the other hand, when concentration reached to a higher level ($> 1 \mu\text{mol/l}$), the transport velocity of fluvastatin by

Table 3 – The previous studies investigated the influence of CYP2C9 or SLC01B1 genetic polymorphisms on fluvastatin pharmacokinetics and pharmacodynamics.

Study	Meyer [14]	Thompson [15]	Pasanen [19]	Niemi [12]	Couvert [13]	Mirošević Skvrce [20]	Kirchheiner [11]	Buzková [21]	Zhou [16]
Gene	SLCO1B1	SLCO1B1	SLCO1B1	SLCO1B1	SLCO1B1	CYP2C9	CYP2C9	CYP2C9	SLCO1B1 CYP2C9
Formulation and Dose	IR	IR 20 mg	IR 40 mg single dose	IR 40 mg single dose	ER 80 mg/d 15d	IR or ER 80 mg/d	IR 40mg	IR 40mg	IR 40 mg single dose
Ethnicity	West Pomerania	Caucasian	Caucasian	Caucasian	Caucasian	Croatian	German	Czechs	Chinese
Study Design	Cohort	Cohort	Cohort	Cohort	Large-scale randomized double-blind placebo controlled multicenter	Case-control	Cohort	Case-control study	Cohort
Sample size	214	477	32	32	420(experiment) 304(control)	52(ADR) 52(control)	26	135	12
Subject	Patients	Patients	Healthy volunteers	Healthy volunteers	Elderly hypercholesterolemic subjects	Patients	Healthy volunteers	Patients	Healthy volunteers
Outcome	Statistically significant association of OATP1B1 genotype and fluvastatin on lipid parameters at the 5-year follow-up.	SLCO1B1 T521C was not associated with total cholesterol and LDL	The SLC01B1 genotype have no significant on the fluvastatin short-term pharmacodynamics.	The SLC01B1 c.521 T > C have no significant effects on the pharmacokinetics of fluvastatin	There were a tendency for an attenuated reduction in total cholesterol and LDL lowering response in subjects carrying one or more variant allele(s) of SLC01B1 T521C polymorphism	CYP2C9 homozygous and heterozygous mutant allele (*2 or *3) carriers had 2.5-times greater odds of developing adverse effects.	Pharmacokinetics showed statistically significant differences according to the number of CYP2C9*3 alleles (P < 0.0001).	In hypercholesterolemic patients, LDL serum concentration was decreased more significantly in fluvastatin-treated subjects bearing the CYP2C9*1/*3 compared to CYP2C9*1/*1	Pharmacokinetics of fluvastatin was not related with SLC01B1SNPs. Effects of CYP2C9*3 on AUC were statistically significant (P < 0.01).

OATP1B1-521TT and OATP1B1-521CC tend to be the same (2Km-OATP1B1TT < 1 μmol/l < 2Km-OATP1B1CC). It means that there was probably no significant difference on the accumulation and elimination of fluvastatin in liver between SLCO1B1 521TT and SLCO1B1 521CC carriers. The results of *in vitro* study were consistent with the *in vivo* pharmacokinetic study.

OATP1B1 is important not only in the elimination of statins but also in their entry to the intracellular site of action in hepatocytes. Accordingly, low uptake capacity of OATP1B1 at high fluvastatin concentration might decrease the cholesterol-lowering effect of statins, resulting in increased plasma statin concentrations and the risk of muscle toxicity [7]. Interestingly, it can explain the phenomenon that the ER formulation is more potent than the IR formulation [5,6]. Further research should explore the influence of SLCO1B1 genotype on fluvastatin-induced muscle toxicity and hepatotoxicity. At the same time, it is very interesting to investigate the different performance of OATP1B1 at high and low concentrations of other statins.

The limited sample size and absence of rare genotypes *in vivo* study such as CYP2C9*3/*3 homozygous and SLCO1B1 521CC subjects make our results preliminary in nature. According to the data of 1000 Genomes Project (<http://www.1000genomes.org/>), the frequencies of CYP2C9*3/*3 and SLCO1B1 521CC in East Asian population are 2% and 0.2%. It means if we want include one CYP2C9*3/*3 or SLCO1B1 521CC carriers, we need to expand our sample size to ≥ 50 or ≥ 500. It is hard to carry out in the present study. On the other hand, it means the result of our study could represent >98% of EAS population. In further *in vivo* study, researchers could include subjects with specific genotype, such as 521CC, 521TC and 521TT to verify the results of this study.

4. Conclusion

In conclusion, the present study provides evidence to challenge previous opinion that the T521C (p.Val174Ala) genetic polymorphism of SLCO1B1 (encoding OATP1B1) has no significant effect on fluvastatin pharmacokinetics [7,16,20]. The conflict of previous pharmacokinetic and pharmacodynamic studies can be attributed to the fluvastatin formulation, and the main reason is probably the different profile of concentration time. Further studies should explore the influence of the SLCO1B1 genotype on fluvastatin-induced muscle toxicity and hepatotoxicity, especially for ER formulation. In addition, we recommend that formulation should be incorporated into future pharmacogenomics studies.

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Declarations Competing of Interest

The authors declare that there is no conflicts of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ajps.2019.06.002.

REFERENCES

- [1] Suzumura K, Yasuhara M, Tanaka K, Suzuki T. Protective effect of fluvastatin sodium (XU-62-320), 3-hydroxy-3-methylglutaryl coenzyme a (HMG-CoA) reductase inhibitor, on oxidative modification of human low-density lipoprotein *in vitro*. *Biochem Pharmacol* 1999;57(6):697–703.
- [2] Jacotot B, Banga JD, Pfister P, Mehra M. Efficacy of a low dose-range of fluvastatin (XU 62-320) in the treatment of primary hypercholesterolaemia. A dose-response study in 431 patients. The French–Dutch fluvastatin study group. *Br J Clin Pharmacol* 1994;38(3):257–63.
- [3] Mathew LE, Rajagopal VA H. Betulinic acid and fluvastatin exhibits synergistic effect on toll-like receptor-4 mediated anti-atherogenic mechanism in type II collagen induced arthritis. *Biomed Pharmacother* 2017;93:681–94.
- [4] Kowalski J, Barylski M, Banach M, Grycewicz J, Irzmanski R, Pawlicki L. Neutrophil superoxide anion generation during atorvastatin and fluvastatin therapy used in coronary heart disease primary prevention. *J Cardiovasc Pharmacol* 2006;48(4):143–7.
- [5] Isaacsohn JL, LaSalle J, Chao G, Gonasun L. Comparison of treatment with fluvastatin extended-release 80-mg tablets and immediate-release 40-mg capsules in patients with primary hypercholesterolemia. *Clin Ther* 2003;25(3):904–18.
- [6] Olsson AG, Pauciullo P, Soska V, Luley C, Pieters RE, Broda G, et al. Comparison of the efficacy and tolerability of fluvastatin extended-release and immediate-release formulations in the treatment of primary hypercholesterolemia: a randomized trial. *Clin Ther* 2001;23(1):45–61.
- [7] Ramsey LB, Johnson SG, Caudle KE, Haidar CE, Voora D, Wilke RA, et al. The clinical pharmacogenetics implementation consortium guideline for SLCO1B1 and simvastatin-induced myopathy: 2014 update. *Clin Pharmacol Ther* 2014;96(4):423–8.
- [8] Messas N, Dube MP, Tardif JC. Pharmacogenetics of lipid-lowering agents: an update review on genotype-dependent effects of HDL-targeting and statin therapies. *Curr Atheroscler Rep* 2017;19(11):43.
- [9] Kirchheiner J, Kudlicz D, Meisel C, Bauer S, Meineke I, Roots I, et al. Influence of CYP2C9 polymorphisms on the pharmacokinetics and cholesterol-lowering activity of (-)-3S,5R-fluvastatin and (+)-3R,5S-fluvastatin in healthy volunteers. *Clin Pharmacol Ther* 2003;74(2):186–94.
- [10] Niemi M, Pasanen MK, Neuvonen PJ. SLCO1B1 polymorphism and sex affect the pharmacokinetics of pravastatin but not fluvastatin. *Clin Pharmacol Ther* 2006;80(4):356–66.
- [11] Meyer zu Schwabedissen HE, Albers M, Baumeister SE, Rimbach C, Nauck M, Wallaschofski H,

- et al. Function-impairing polymorphisms of the hepatic uptake transporter *SLCO1B1* modify the therapeutic efficacy of statins in a population-based cohort. *Pharmacogenet Genomics* 2015;25(1):8–18.
- [12] Thompson JF, Man M, Johnson KJ, Wood LS, Lira ME, Lloyd DB, et al. An association study of 43 SNPs in 16 candidate genes with atorvastatin response. *Pharmacogenom J* 2005;5(6):352–8.
- [13] Couvert P, Giral P, Dejager S, Gu J, Huby T, Chapman MJ, et al. Association between a frequent allele of the gene encoding *OATP1B1* and enhanced LDL-lowering response to fluvastatin therapy. *Pharmacogenomics* 2008;9(9):1217–27.
- [14] Clinical Pharmacogenetics Implementation Consortium. CPIC guidelines. Available at: <https://cpicpgx.org/guidelines/>.
- [15] Zhou Q, Ruan ZR, Yuan H, Zeng S. *CYP2C9*3*(1075A>C), *MDR1 G2677T/A* and *MDR1 C3435T* are determinants of inter-subject variability in fluvastatin pharmacokinetics in healthy Chinese volunteers. *Arzneimittelforschung* 2012;62(11):519–24.
- [16] Neuvonen PJ, Backman JT, Niemi M. Pharmacokinetic comparison of the potential over-the-counter statins simvastatin, lovastatin, fluvastatin and pravastatin. *Clin Pharmacokinet* 2008;47(7):463–74.
- [17] Kopplow K, Letschert K, Konig J, Walter B, Keppler D. Human hepatobiliary transport of organic anions analyzed by quadruple-transfected cells. *Mol Pharmacol* 2005;68(4):1031–8.
- [18] Noe J, Portmann R, Brun ME, Funk C. Substrate-dependent drug-drug interactions between gemfibrozil, fluvastatin and other organic anion-transporting peptide (OATP) substrates on *OATP1B1*, *OATP2B1* and *OATP1B3*. *Drug Metab Dispos* 2007;35(8):1308–14.
- [19] Pasanen MK, Miettinen TA, Gylling H, Neuvonen PJ, Niemi M. Polymorphism of the hepatic influx transporter organic anion transporting polypeptide 1B1 is associated with increased cholesterol synthesis rate. *Pharmacogenet Genom* 2008;18(10):921–6.
- [20] Mirosevic Skvrce N, Bozina N, Zibar L, Barisic I, Pejnovic L, Macolic Sarinic V. *CYP2C9* and *ABCG2* polymorphisms as risk factors for developing adverse drug reactions in renal transplant patients taking fluvastatin: a case-control study. *Pharmacogenomics* 2013;14(12):1419–31.
- [21] Buzkova H, Pechandova K, Danzig V, Vareka T, Perlik F, Zak A, et al. Lipid-lowering effect of fluvastatin in relation to cytochrome P450 2C9 variant alleles frequently distributed in the Czech population. *Med Sci Monit* 2012;18(5):CR512–17.
- [22] Barilla D, Prasad P, Hubert M, Gumbhir-Shah K. Steady-state pharmacokinetics of fluvastatin in healthy subjects following a new extended release fluvastatin tablet, lescol xL. *Biopharm Drug Dispos* 2004;25(2):51–9.
- [23] Xu HR, Chen WL, Chu NN, Li XN, Zhu JR. The difference in pharmacokinetics and pharmacodynamics between extended-release fluvastatin and immediate-release fluvastatin in healthy Chinese subjects. *J Biomed Biotechnol*. 2012;2012:386230.