Effects of Grapefruit Juice and SLCO1B1 388A>G Polymorphism on the Pharmacokinetics of Pitavastatin

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Summary: Pitavastatin undergoes little hepatic metabolism but it is a substrate for uptake and efflux transporters, particularly OATP1B1 (gene SLCO1B1). A previous study in 8 Japanese healthy subjects showed that co-administration with grapefruit juice (GFJ) resulted in a small increase in systemic exposure to pitavastatin. We examined whether common polymorphisms in SLCO1B1 might influence the pharmacokinetics of pitavastatin or the interaction with GFJ. Twelve Chinese healthy male volunteers took pitavastatin 2 mg orally with water or with GFJ on separate occasions and plasma concentrations of pitavastatin acid and lactone were measured over 48 h. GFJ increased the mean area under the plasma concentration-time curve (AUC0–48h) for both pitavastatin acid and lactone by 14% (p < 0.05). Subjects with SLCO1B1 *1b/*1b haplotype (388GG-521TT) had 47% and 44% higher systemic exposure for pitavastatin acid and lactone than the SLCO1B1 *1a carriers (388AA/AG-521TT, p < 0.05 and p = 0.005, respectively). The SLCO1B1 388A>G polymorphism, which increases transporter activity for some statins, was associated with higher plasma levels of pitavastatin acid and lactone in subjects with the homozygous variant indicating decreased hepatic uptake. Co-administration of pitavastatin with GFJ resulted in a small but significant increase in plasma levels in healthy Chinese subjects.

Keywords: Chinese; grapefruit juice; herb-drug interaction; pharmacogenetics; pitavastatin; SLCO1B1

Introduction

Pitavastatin, a newly developed potent hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, is administered as an active acid and undergoes reversible conversion into an inactive lactone. Pitavastatin is minimally metabolized in the liver and is mainly excreted unchanged in the feces via the bile.1-2 P-glycoprotein (P-gp, gene ABCB1) mediated transport may not play a major role in its disposition in man but organic anion transporting polypeptide (OATP) 1B1 (gene SLCO1B1) is the most important transporter for its hepatic uptake and the efflux transporter breast cancer resistance protein (BCRP, gene ABCG2) contributes to its biliary excretion.3-5 Polymorphisms in SLCO1B1 leading to reduced transport activity were found to affect cellular uptake of pitavastatin6) and influence its pharmacokinetics,7-9) but the common ABCG2 421C>A polymorphism did not have a significant effect on the pharmacokinetics of pitavastatin in vivo.10) Grapefruit juice (GFJ) contains the furanocoumarin 6,7-dihydroxybergamottin and the flavonoids naringenin and naringin, which have been found to be inhibitors of the cytochrome P450 (CYP) 3A4 enzymes and certain drug transporters, particularly P-gp.11-15) Previous studies showed that administration with GFJ increased the plasma levels of the acid and lactone of lovastatin, simvastatin and atorvastatin, probably by decreasing CYP3A4-mediated metabolism in the small intestine, but it had no significant effect on the pharmacokinetics of pravastatin.16-18) Repeated doses of GFJ for 4 days had a small effect on the pharmacokinetics of pitavastatin (4 mg oral dose), increasing the mean area under the...
plasma concentration-time curve (AUC₀–₂₉₈) of pitavastatin acid and lactone by 13% (95% CI = 3 to 29%) and by 30% (95% CI = 2 to 62%), respectively, in 8 healthy Japanese male subjects. This effect was much less than for atorvastatin acid for which the AUC₀–₂₉₈ was increased by 83% (23–144%).¹⁹ This interaction study and other data suggest that pitavastatin undergoes very limited CYP3A4-mediated metabolism.²⁰

In the present study, we examined the effect of repeated doses of GFJ on the plasma concentrations of pitavastatin acid and lactone in healthy Chinese subjects and retrospectively examined the influence of common polymorphisms in SLCO1B on the pharmacokinetics of pitavastatin.

Methods

Study design: This study was an open-label, single-dose, randomized, two-phase, crossover clinical pharmacokinetic study design with a wash-out interval of at least 3 weeks. The study followed the protocol which was approved by the local clinical research Ethics Committee and all participants gave written informed consent before any study procedures were undertaken.

Twelve healthy male Chinese volunteers (age range 21–25 years; weight range 53.4–74.3 kg) were randomized to receive either double-strength GFJ or water 200 ml 3 times a day for 2 days before taking pitavastatin as a 2 mg tablet. Subjects were required to take the morning dose of GFJ in the study center and they were given 2 bottles of 200 ml double-strength GFJ to take away for the afternoon and evening doses. Subjects were asked to return these bottles the next morning and they were required not to take any drink or food 1 h before and after the consumption of GFJ and compliance was carefully monitored. On the morning of the dosing day, after an overnight fast, a single dose of 2 mg pitavastatin tablet was administered with 200 ml double-strength GFJ or water at 9 am. The same volumes of GFJ or water were given at 0.5, 1.5, 22, 27 and 35 h after dosing with no drink and food allowed 1 h before and after the administration of GFJ. Plasma concentrations of pitavastatin acid and pitavastatin lactone were measured over 48 h after the dose. After a wash-out period of at least 3 weeks, the volunteers were given the alternative regimen with GFJ or water according to the same timetable. Blood samples were taken to determine the pharmacokinetics after a second single dose of 2 mg pitavastatin. During each sampling time, 10 ml of blood was collected into light-protected tubes containing sodium heparin. There were 10 time points: pre dose (0 h), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 32 and 48 h after oral dosing. After sample collection, the tubes were kept on ice and centrifuged at 3,000 rpm for 10 min at 4°C within 15 min after collection. Plasma was aliquoted, protected from light and placed in tubes, which were packed and stored at –20°C until analysis.

Assay of pitavastatin acid and pitavastatin lactone: Plasma samples were extracted with methyl tert-butyl ether and then the extract was subjected to methylation with diazomethane to prevent the mutual conversion between pitavastatin acid and lactone. The analytical methods used for the assay of pitavastatin acid and lactone were previously developed by Kowa Company and further validated by SRL Inc. of Japan as described previously.²¹ The extract was injected into a column-switching HPLC system. The limits of quantification for both pitavastatin acid and lactone were 0.5 ng/ml. The coefficient of variation (CV) of intra- and inter-day assay was ≤2.3% and ≤5.3% for the analytes at relevant concentrations in plasma, respectively.

Pharmacokinetic analysis: The pharmacokinetic parameters of pitavastatin acid and lactone were calculated with non-compartmental methods using the computer program WinNonLin (version 2.1, Pharsight Corporation). Cmax and tmax were obtained directly from the observed concentration-time data. The terminal elimination rate constant (λz) was estimated by linear regression of the terminal portion of the concentration-time curve, and the t½ was calculated as 0.693/λz. The area under the plasma concentration-time curve (AUC₀–∞) was calculated using the trapezoidal rule, and extrapolated to infinity. The CL/F was calculated as Dose/AUC₀–∞.

Genotyping: A 10 ml blood sample was drawn from each subject and DNA was extracted from peripheral blood leucocytes using the traditional phenol chloroform method or High Pure PCR Template Preparation Kits (Roche Applied Science, Indianapolis, IN, USA). Genotyping of SLCO1B1 388A>G and 521T>C were performed in the Genome Research Centre, University of Hong Kong using the mass-spectroscopy based, high-throughput MassARRAY iPLEX™ platform (Sequenom, San Diego, CA, USA). The SNPs examined were in Hardy-Weinberg equilibrium (χ² > 0.05).

Statistical analysis: All continuous variables were expressed as mean ± SD unless otherwise indicated. The pharmacokinetic parameters of pitavastatin acid and pitavastatin lactone after consumption of GFJ were compared with those when the drug was taken with water by paired two-tailed t-tests, except for tmax values, for which the Wilcoxon signed rank test was used. Comparisons of the pharmacokinetic parameters among genotype groups were determined using the t-test or analysis of variance (ANOVA) for normally distributed data or Mann-Whitney U tests or the Kruskal-Wallis test for skewed data. A p value of < 0.05 was considered statistically significant. The data were analyzed using SPSS Version 17.0 software (SPSS Inc., Chicago, IL, USA).

Results

Effect of GFJ on the pharmacokinetics of pitavastatin: Twelve subjects entered and completed the study with no adverse events reported. Compared with the values when pitavastatin was taken with water, after consumption of GFJ the mean AUC₀–₂₉₈ values were both increased by 14%, and the apparent oral clearances (CL/F) were lower by 10% and 15% for pitavastatin acid and lactone, respectively (p < 0.05) (Table 1), whereas, the mean Cmax values of pitavastatin acid and lactone decreased by 12% (p = 0.117) and 13% (p < 0.05), respectively. The times to reach Cmax (tmax) were increased after administration of GFJ, but the elimination half-lives (t½) were unchanged. Similar results were observed after adjustment for body weight (data not shown).

Effect of SLCO1B1 polymorphisms on the pharmacokinetics of pitavastatin: The genotype distributions of the single nucleotide polymorphisms (SNPs) examined for the 12 subjects were similar to those reported for Chinese. There were no homozygous 521CC subjects and only two heterozygous SLCO1B1 521TC subjects identified, who appeared to have increased levels of Cmax and AUC₀–₂₉₈ values for both pitavastatin acid and lactone compared to those with the 521TT genotype (Table 2). After excluding those two subjects with the 521TC genotype, homozygous 388GG (*1b/*1b) subjects showed higher plasma concentrations of pitavastatin acid and lactone when pitavastatin was taken with water than those carriers with one or two copies of wild-type allele (388AG and 388AA) (Table 2, Fig. 1). Mean Cmax
Pharmacokinetic values are given as mean ± SD; tmax are median and range; ratio of pharmacokinetic values during grapefruit phase vs. water phase are means (90% CI).

AUC, area under the plasma concentration-time curve; CL/F, apparent oral clearance; Cmax, peak plasma drug concentration; t1/2, elimination half-life.

Table 2. Effect of SLC01B1 polymorphisms on the pharmacokinetics of pitavastatin

<table>
<thead>
<tr>
<th>SLC01B1 polymorphisms</th>
<th>Pitavastatin acid</th>
<th>Pitavastatin lactone</th>
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<tbody>
<tr>
<td></td>
<td>Cmax (ng/ml)</td>
<td>AUC0–48h (ng⋅h/ml)</td>
</tr>
<tr>
<td>521T&gt;C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>521TT (n = 10)</td>
<td>36.3 ± 14.5</td>
<td>84.1 ± 24.6</td>
</tr>
<tr>
<td>521TC (n = 2)</td>
<td>49.2</td>
<td>102.7</td>
</tr>
<tr>
<td>p</td>
<td>0.254</td>
<td>0.331</td>
</tr>
<tr>
<td>388A&gt;G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>388AA/AG (n = 6)</td>
<td>30.5 ± 10.1</td>
<td>74.7 ± 21.8</td>
</tr>
<tr>
<td>388GG (n = 6)</td>
<td>46.4 ± 13.4</td>
<td>99.7 ± 19.0</td>
</tr>
<tr>
<td>p</td>
<td>0.043</td>
<td>0.060</td>
</tr>
<tr>
<td>Haplotypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>388A/G-521TT</td>
<td>27.1 ± 6.3</td>
<td>68.1 ± 16.3</td>
</tr>
<tr>
<td><em>1a</em>1a or <em>1a</em>1b (n = 5)</td>
<td>45.5 ± 14.8</td>
<td>100.1 ± 21.3</td>
</tr>
<tr>
<td>p</td>
<td>0.033</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Pharmacokinetic values are given as mean ± SD or mean in subgroup with n < 3.

AUC0–48h, area under the plasma concentration-time curve from 0 to 48 h; CL/F, apparent oral clearance; Cmax, peak plasma drug concentration; t1/2, elimination half-life.

Fig. 1. Effect of the SLC01B1 388A>G-521TT polymorphism on the pharmacokinetics of pitavastatin acid (a) and pitavastatin lactone (b) in 10 subjects after taking a single 2 mg dose of pitavastatin orally with water or with GFJ

values for pitavastatin acid were higher by 68% (p < 0.05), AUC0–48h by 47% (p = 0.06) and CL/F values were lower by 34% (p < 0.05). Similar significant differences in pharmacokinetic parameters of pitavastatin lactone between the 388A>G-521TT haplotype groups were observed with mean Cmax higher by 63% (p < 0.005) and AUC0–48h by 44% (p = 0.005) in the homozygous 388GG-521 TT haplotype group compared to the wild-type 388A allele carriers after adjusting for multiple testing (Table 2).
The 388A>G-521TT haplotype had similar effects on the pharmacokinetic parameters when pitavastatin was taken with GFJ (Fig. 1) and the changes in pharmacokinetic parameters associated with administration of GFJ did not differ significantly between the SLCO1B1 haplotype groups.

Discussion

GFJ produces a mechanism-based irreversible inactivation of intestinal CYP3A4 resulting in reduced presystemic metabolism and increased oral bioavailability of drugs that are highly dependent on this pathway, such as lovastatin, simvastatin and atorvastatin.15-18 In this study we found a small increase in the AUC<sub>0-48h</sub> for pitavastatin acid and lactone but no change in the elimination half-life when the drug was taken with GFJ, similar to that reported in a previous study.18 This may be due to an effect of GFJ on the drug transporters expressed in the intestine as pitavastatin is not thought to be metabolized by intestinal CYP3A.22 The study also showed a small but significant decrease in C<sub>max</sub> for pitavastatin lactone with administration of GFJ and this result may suggest that GFJ influences the disposition of pitavastatin by altering its absorption and elimination through its effect on both intestinal uptake and efflux transporters.

An in vitro study found that GFJ might enhance oral drug bioavailability through inhibition of P-gp in a substrate-dependent manner reducing intestinal efflux transport,23 but GFJ had little effect on the pharmacokinetics of the P-gp substrate digoxin in a human study.24 It has been suggested that GFJ might interact with and modulate the activity of intestinal uptake transporters such as SLCO1A2 and SLCO2B1.13 A recent in vitro study showed that the main constituent flavonoid of GFJ, naringin, inhibits Oatp1a5- and Mdr1a-mediated transport of pitavastatin in a concentration-dependent manner in rats with IC<sub>50</sub> values of 18.5 µM and 541 µM, respectively, leading to a decrease or increase in pitavastatin absorption according to the GFJ concentration.22 In the present study, GFJ increased the drug exposure to pitavastatin acid and lactone but retarded the absorption of pitavastatin, possibly via the inhibitory effect of naringin on OATP-mediated uptake (e.g. via SLCO1A2 and SLCO2B1) and perhaps more predominantly by inhibiting ABCB1-mediated efflux as the naringin concentration was reported to be about 1,000 µM in the commercial GFJ product.22,25

This study showed that the SLCO1B1 388A>G polymorphism was associated with pharmacokinetics of pitavastatin acid and, more significantly, the lactone. The uptake transporter SLCO1B1 was thought to be expressed exclusively on the basolateral membrane of hepatocytes26 but a limited degree of expression in the small intestine has also been described.13,27 The hepatic uptake of pitavastatin is almost completely accounted for by SLCO1B1, SLCO1B3 and SLCO2B1, with 90% of the total hepatic clearance mediated by SLCO1B1.4 Sodium taurocholate co-transporting polypeptide (NTCP) may also contribute to a minor extent.9 The two common polymorphisms in SLCO1B1, 388A>G and 521T>C, result in four distinct haplotypes: *1a (388A-521T); *1b (388G-521T); *5 (388A-521C) and *15 (388G-521C) with differences in transporter activity.28

The 521T>C variant in SLCO1B1 usually results in reduced transporter activity and has been associated with increased systemic exposure to most statins.9,29 A significant effect on the pharmacokinetics of pitavastatin acid but not lactone has been described with the SLCO1B1 521T>C variant in previous studies in Japanese and Korean subjects,7,9,10 but the effects of the 388A>G in SLCO1B1 were not directly evaluated in these studies. The 521C variant is uncommon in most populations and there were only two subjects with the 521TC genotype among our study subjects. These two individuals had numerically higher values for C<sub>max</sub> and AUC<sub>0-48h</sub> than those with the 521TT genotype, and this is consistent with previous observations.

The 388A>G polymorphism in SLCO1B1 appears to result in increased transporter activity and lower systemic exposure with some substrates including pravastatin.30 However, the effects of the 388G allele have not always been consistent and in vitro studies with cell lines transfected with the SLCO1B1 variants showed different results with different substrates.31 Likewise, the effect of this polymorphism on statin pharmacokinetics and responses appears to vary with different statins.29,32 In the present study, 388GG (*1b/*1b) homozygous subjects showed higher plasma concentrations of pitavastatin acid and lactone compared to 388AG and 388AA genotypes combined, with increases of C<sub>max</sub> and AUC<sub>0-48h</sub> by 68% and 47%, respectively, suggesting this variant is less active in the hepatic uptake of pitavastatin. Similar results have previously been reported in 18 healthy Chinese volunteers, where the mean C<sub>max</sub> and AUC<sub>0-48h</sub> values were 71% and 85% (p < 0.05) higher in *1b carriers (n = 6) than those in *1a/*1a subjects (n = 9) but that study did not report the influence of the 521T>C polymorphism.33 The 388A>G polymorphism is more common in East Asians (G allele frequency 60–90%) than Caucasians,13,34 and thus the plasma levels of pitavastatin may be somewhat higher in East Asians in relation to the frequency of this polymorphism.

Neither polymorphism in SLCO1B1 examined in the study had any effect on the interaction between pitavastatin and GFJ. The OATP1B1 uptake transporter is thought to be exclusively expressed in the liver, whereas the interaction between pitavastatin and GFJ occurs predominantly in the small intestine. Therefore it may be not surprising to see a lack of interaction between the SLCO1B1 polymorphisms and GFJ on the pharmacokinetics of pitavastatin although we were also interested to examine whether a higher plasma exposure to pitavastatin in subjects with the SLCO1B1 *1b/*1b genotype would result in a greater interaction between GFJ and pitavastatin. A recent in vivo study showed that GFJ markedly reduced the systemic exposure to the renin-inhibiting antihypertensive drug aliskiren (a substrate of SLCO1A2, SLCO2B1, P-gp, and CYP3A4)36 and in vitro studies with cell lines transfected with the SLCO1B1*1a (388A-521T) and naringin showed different results with different substrates.31 The 388A>G polymorphism is more common in East Asians (G allele frequency 60–90%) than Caucasians,13,34 and thus the plasma levels of pitavastatin may be somewhat higher in East Asians in relation to the frequency of this polymorphism.

Acknowledgments: We thank Ms. Evelyn Chau for her assistance with the clinical conduct of this study.

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