Effect of pitavastatin in different SLC01B1 backgrounds on repaglinide pharmacokinetics and pharmacodynamics in healthy Chinese males

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Abstract: The effect of pitavastatin and SLC01B1 genetic background on the pharmacokinetic and pharmacodynamic properties of repaglinide was investigated. In this randomized, placebo-controlled, crossover study, twelve healthy Chinese males were administered with pitavastatin 4 mg/d or the placebo for 5 d followed by repaglinide 4 mg given orally on d 5. Plasma repaglinide and glucose levels were measured by liquid chromatography-tandem mass spectrometry (LC/MS/MS) and the glucose oxidase method, respectively. Treatment with pitavastatin significantly increased the peak plasma concentration (Cmax) of repaglinide (P=0.003) in SLC01B1*1b homozygotes (P=0.015) and SLC01B1*15 carriers (P=0.031). Treatment with pitavastatin led to a marginal increase in the area under plasma concentration-time curve from 0 h to infinity (AUC 0 →∞) of repaglinide (P=0.091). There was no significant difference in pharmacokinetic parameters or hypoglycemic effects of repaglinide among SLC01B1 genotypes in either the pitavastatin or control group. Pitavastatin increased the Cmax of the plasma concentration of repaglinide in an SLC01B1 genotype dependent manner, but had no apparent effect on the pharmacodynamics of repaglinide in healthy volunteers. The p values for this statement were not reported.

Keywords: Pitavastatin, repaglinide, SLC01B1, pharmacokinetics, pharmacodynamics.

INTRODUCTION

Repaglinide is a non-sulfonylurea insulin secreting agent that has proven efficacious in treating type 2 diabetes mellitus (T2DM) patients. The drug, which has an oral bioavailability of approximately 60% (Kalliokoski et al., 2008), is primarily metabolized by cytochrome P450 (CYP) isoenzymes CYP3A4 and CYP2C8 into several non-active metabolites (Bidstrup et al., 2003). Hepatic uptake of repaglinide, an important step in metabolism, occurs through the organic anion transporting polypeptide 1B1 (OATP1B1), which is expressed on the sinusoidal membrane of hepatocytes. Genetic polymorphisms in SLC01B1, the gene encoding OATP1B1, contribute to interindividual variability in repaglinide pharmacokinetics (Niemi et al., 2005). Following hepatic uptake, repaglinide is metabolized and eliminated by CYP3A4 and CYP2C8. Thus, there are likely interactions between repaglinide and CYP3A4 or CYP2C8 substrates, such as cyclosporine A, rifampicin, itraconazole, gemfibrozil, and cyclosporin (Scheen, 2007). However, little is known about whether there are drug-drug interactions between epaglinide and co-medications that are also transported through OATP1B1-mediated drug uptake.

The common lipid-lowering drugs statins are inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and are transported by OATP1B1 (Sabia et al., 2004; Kyrklund et al., 2000). Although co-administration of atorvastatin and repaglinide increases plasma concentration of repaglinide (Kalliokoski et al., 2008), the interaction between repaglinide and other statins is largely unstudied. The hypercholesterolemia drug pitavastatin is a candidate statin for interaction with repaglinide through OAT1B1. In humans, pitavastatin is minimally metabolized by CYP2C9 (Fujino et al., 2002) and primarily metabolized through lactonization (Yamada et al., 2003), In vitro, pitavastatin is a known substrate of OATP1B1 (Km = 3.0 mM) (Hirano et al., 2004). Genetic polymorphisms in SLC01B1 can partially account for individual differences in pharmacokinetic parameters of pitavastatin (Deng et al., 2008; Chung et al., 2005). These studies suggest that pitavastatin is likely an OATP1B1 substrate in vivo.

OATP1B1 is an influx transporter that is expressed on the sinusoidal membrane of human hepatocytes (Hagenbuch et al., 2004) and plays an important role in the hepatic
uptake of many endogenous and exogenous compounds, including common drugs repaglinide, statins, and angiotensin II receptor antagonists (Niemi et al., 2007). Several genetic polymorphisms in SLCO1B1 gene have been identified (Nozawa et al., 2002). Two common SLCO1B1 single nucleotide polymorphisms (SNPs) c.A388G (Asn130Asp, rs2306283) and c.T521C (Val174Ala, rs4149056) are found in Asians populations for 5 d (d 1 to d 5). On d 5, after an overnight fast, a single 4 mg oral dose of repaglinide (Novonorm, NovoNordisk, Bagsvaerd, Denmark) was given 1 h the after pitavastatin calcium (or placebo) administration. The pretreatment medications (pitavastatin and the placebo) were supplied, packed, and labeled by the investigators according to a randomization list for each subject. All subjects remained seated for 3 h after repaglinide administration and were under direct medical supervision for 8 h. Each participant received standardized breakfast (eaten over 10 min) 15 min after repaglinide administration, snacks at 1 h and 2 h (eaten over 5 min), a warm meal at 4 h, and a snack at 6 h after drug administration(Bidstrup et al., 2003). Glucose for intravenous administration and glucagon for intramuscular administration were available in cases of severe hypoglycemia. Blood samples (~7 mL) were drawn in ethylenediaminetetraacetic acid (EDTA) containing tubes before repaglinide administration and at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, and 8.0 h after repaglinide ingestion. Blood glucose concentrations were measured immediately using the glucose oxidase method on a Precision G Blood Glucose Testing System (Medisense, MA, US). The between-day coefficient of variation (CV) was 7.6% at 2.7 mMol/L and 5.1% at 18.4 mMol/L (n=12). Plasma was separated within 30 min of the blood draw and stored at -40°C until analysis.

**SUBJECTS, METERIALS AND METHODS**

**Participants**

To ensure that a range of genotypes were present in the study population, we determined the SLCO1B1 genotype of 36 healthy males by reaction-restriction fragment length polymorphism (PCR-RFLP) analysis and direct sequencing. SLCO1B1 haplotypes were inferred using PHASE 2.0 (http://en.bio-soft.net/tree/PHASE.html). Written informed consent was obtained from 12 randomly selected males who were found to be healthy by medical history, physical examination, and routine laboratory tests before participation. No subject smoked tobacco or took any continuous medication two weeks before or during the study. They were not allowed to use other drugs and grapefruit products one week before the administration of pitavastatin or the placebo and were told to avoid vigorous physical exercise and alcohol consumption during the study.

**Trial design**

The trial has been successfully registered in the Australian New Zealand Clinical Trials Registry (ANZCTR) and allocated the ACTRN: ACTRN12611001254987. The Coordinating Ethics Committee of the Xiang Ya Third Hospital of Central South University approved this randomized, placebo-controlled, crossover study with two phases and a washout period of at least two weeks. The 12 participants were randomly divided into two treatment groups using statistical software. In each phase, participants received either 4 mg pitavastatin calcium (Livalo, Kowa, Japan) or placebo once daily at 7:00 am for 5 d (d 1 to d 5). On d 5, after an overnight fast, a single 4 mg oral dose of repaglinide (Novonorm, NovoNordisk, Bagsvaerd, Denmark) was given 1 h after pitavastatin calcium (or placebo) administration.
Pharmacokinetics and pharmacodynamics
The pharmacokinetics of repaglinide were characterized by peak plasma concentration (C\text{max}), time to C\text{max} (t\text{max}), elimination half-life (t\text{1/2}), area under plasma concentration-time curve from 0 to 8 h (AUC\text{0-8 h}), and area under the plasma concentration-time curve from 0 to infinity (AUC\text{0→∞}). The elimination rate constant (k\text{e}) was determined by linear regression analysis of the log-linear part of the concentration-time curve. The t\text{1/2} was calculated according to the equation t\text{1/2}=\ln2/k\text{e}. AUC was calculated using a combination of linear (for increasing concentrations) and log-linear (for decreasing concentrations) trapezoidal rules, with extrapolation to infinity, when appropriate, by dividing the last measured concentration by k\text{e}. All pharmacokinetic calculations were conducted in DAS, version 2.0 (Innaphase, Bucks, UK).

Blood glucose response to repaglinide was measured as the maximum decrease from baseline and the difference in mean glucose concentrations from 0 to 3 h and 0 to 8 h after drug administration.

STATISTICAL ANALYSIS
Data were expressed as mean ± SD and mean ± standard errors of the mean (SEM). C\text{max} and AUC were logarithmically transformed before statistical analysis. One-way analysis of variance (ANOVA) was used to compare the differences in pharmacokinetic (other than t\text{max}) and pharmacodynamic variables among genotypes in the two treatment phases. Multi-factorial ANOVA was conducted to analyse the interaction between pitavastatin and the SLCO1B1 genotype on pharmacokinetics of repaglinide. Differences between treatment phases within each genotype group were analysed by paired-sample t-test. Differences in t\text{max} among genotypes during each treatment phase and between phases were compared with the Mann-Whitney U-test and Wilcoxon signed rank test, respectively. All analyses were conducted in SPSS 13.0 (SPSS, Chicago, IL). Differences were considered significant at P < 0.05.

RESULTS
SLCO1B1 SNPs, haplotypes, and genotypes in healthy Chinese males
The genetic frequencies among 36 healthy Chinese males at SLCO1B1 c.A388G were 47.2% GG, 50.0% GA, and 2.8% AA. At SLCO1B1 c.T521C the genotypic frequencies were 88.9% TT and 11.1% TC. No CC homozygotes were observed. Based on these SNPs, three SLCO1B1 haplotypes (*1a, *1b, and *15) were inferred by PHASE. SLCO1B1*1b (68.1%) was the most common haplotype followed by SLCO1B1*1a (26.4%) and SLCO1B1*15 (5.6%). SLCO1B1*5 haplotype was not observed.

Participants were assigned to one of three groups based on their SLCO1B1 genotype: SLCO1B1*1b homozygotes (n = 4), SLCO1B1*1a carriers in the absence of SLCO1B1*15 (n = 5), and SLCO1B1*15 carriers (n = 3).

Group 1 included four SLCO1B1*1b homozygotes (table 1). Group 2 included two SLCO1B1*1a/*1a individuals and three SLCO1B1*1a/*1b individuals, and group 3 included one SLCO1B1*1a/*15 individual and two SLCO1B1*1b/*15 individuals (table 1).

Effects of pitavastatin pretreatment on repaglinide pharmacokinetics
Pharmacokinetic parameters of repaglinide in healthy volunteers with placebo or pitavastatin pretreatment are shown in Table 2. Compared with those in the placebo pretreatment phase, individuals in the pitavastatin pretreatment phase had a significantly increased C\text{max} (86.50 ± 16.22 µg/L vs. 99.42 ± 22.44 µg/L, P = 0.003) and marginally increased AUC\text{0→∞} of repaglinide (94.00 ± 40.37 µg·h/L vs. 101.51 ± 34.09 µg·h/L, P = 0.091). Pitavastatin pretreatment resulted in a 15% (95% confidence interval (CI): 6%-23%) increase in C\text{max}.

Neither t\text{1/2} nor oral clearance of repaglinide was significantly different between the two treatment phases (table 2).

Effect of pitavastatin pretreatment on repaglinide pharmacokinetics and association with SLCO1B1 genetic polymorphisms
The plasma concentration-time profile of repaglinide after a 5-d pretreatment with placebo or pitavastatin for different SLCO1B1 genotypes is shown in fig. 1. No significant difference in pharmacokinetic parameters of repaglinide was observed among SLCO1B1 genotype groups in the placebo-treated phase, although C\text{max}, AUC\text{0→∞}, and AUC\text{0→∞} were increased in SLCO1B1*15 carriers (table 2). Following pitavastatin treatment, the mean C\text{max} of repaglinide increased significantly in group 1 (SLCO1B1*1b/*1b) and group 3 (SLCO1B1*15 carriers) (P < 0.05, table 2 and fig. 1) but not in group 2 (carriers of SLCO1B1*1a without SLCO1B1*15 haptotype, P = 0.291). The mean increase in repaglinide C\text{max} was 13% (95% CI: 4%-22%) and 24% (3%-45%) for group 1 and group 3, respectively. No significant change in AUC\text{0→∞} or t\text{1/2} of repaglinide was observed in any genotype group after pitavastatin pretreatment (table 2). No significant interaction between pitavastatin and SLCO1B1 genotype on the AUC\text{0→∞} of repaglinide was observed (table 2).

Effects of pitavastatin pretreatment on the glucose-lowering response of repaglinide and association with SLCO1B1 genetic polymorphisms
All subjects tolerated the glucose conditions in the clinical trial during the study. Only one subject in group 3 with an SLCO1B1*1b/*15 genotype required oral administration.
of glucose solution because of hypoglycemia. Overall, no marked difference in the blood glucose levels was observed between placebo phase and pitavastatin phase after repaglinide administration (Table 3). Compared to those in the placebo phase, pitavastatin-treated \textit{SLCO1B1}*15 carriers had marginally decreased mean blood glucose levels within 0-3 h and 0-8 h after repaglinide administration (Table 3). No significant difference in changes in blood glucose relative to baseline was observed between the two phases for all subjects or for subjects separated by genotype group (Fig. 2).

\textbf{DISCUSSION}

In the present study, we investigated the effect of pitavastatin on the pharmacokinetics and pharmacodynamics of repaglinide in healthy Chinese males of different \textit{SLCO1B1} (OATP1B1) genotypes. We observed that a 5-day pitavastatin pretreatment significantly increased the $C_{\text{max}}$ of repaglinide in *1b homozygotes and *15 carriers. We also observed a marginal increase (not statistically-significant) in the AUC$_{0\rightarrow\infty}$ and marginal decrease in oral clearance of repaglinide in all pitavastatin-treated subjects, but this effect disappeared when analysed by genotype group. We also found that pitavastatin-treated \textit{SLCO1B1}*15 carriers had the lowest mean blood glucose levels after repaglinide administration. A marginal decrease in mean blood glucose for \textit{SLCO1B1}*15 carriers was also observed in the pitavastatin treatment phase.

To our knowledge, this study is the first to describe the effects of pitavastatin on the pharmacokinetics and pharmacodynamics of repaglinide in humans. As both repaglinide and pitavastatin are substrates of the OATP1B1 transporter, competition between these compounds during hepatic uptake may occur during co-administration. In that case, we expected that the inhibitory effect of pitavastatin on pharmacokinetics of repaglinide would be greater in \textit{SLCO1B1}*15 carriers, who have a lower pitavastatin clearance (Deng \textit{et al.}, 2008) and higher pitavastatin plasma concentration (Chung \textit{et al.}, 2005). In support of this hypothesis, pretreatment with pitavastatin increased the $C_{\text{max}}$ of repaglinide significantly in \textit{SLCO1B1}*1b/*1b individuals and \textit{SLCO1B1}*15 carriers. Our results indicated that the effect of pitavastatin on plasma concentration of repaglinide is OATP1B1-mediated and \textit{SLCO1B1} genotype dependent, although the overall effect is very modest. By contrast, pitavastatin pretreatment had no effect on the $t_{1/2}$ of repaglinide, suggesting that it decreases the hepatic uptake of repaglinide but does not affect metabolism in the liver.

Although pitavastatin did not affect the blood glucose lowering effects of repaglinide in healthy Chinese male subjects, a significant increase in the $C_{\text{max}}$ of repaglinide was observed (15%). In the pitavastatin-treated subjects, mean blood glucose decreased after repaglinide administration in \textit{SLCO1B1}*15 carriers, suggesting a part for \textit{SLCO1B1} genotype in the pitavastatin-repaglinide interaction.
interaction. These results suggest that when pitavastatin and repaglinide are used concomitantly, dose adjustment for repaglinide may be necessary in \(SLCO1B1*15\) carriers to prevent severe hypoglycemia.

In CYP2C19-mediated drug interactions, CYP2C19 inhibitors have significant effects on the pharmacokinetics of its substrates in individuals with at least one extensive metabolizer (EM) allele, whereas only a negligible effect was found in individuals with only poor metabolizer (PM) alleles (Uno et al., 2006; Yasui-Furukori et al., 2004; Yasui-Furukori et al., 2004; Miura et al., 2005). In CYP2D6-mediated drug interactions, the extent of drug interaction between CYP2D6 substrates and CYP2D6 inhibitors is influenced by CYP2D6 genotype (Lim et al., 2010; Lim et al., 2008). Similarly, individuals with genotypes associated with reduced activity of a drug uptake transporter are less susceptible to inhibition or competition with other substrates of the transporter. For example, the effect of cyclosporine on the AUC\(_{0-\infty}\) of repaglinide was less extensive in subjects with an \(SLCO1B1\) genotype of TC at c.521 than those with a TT genotype (Kajosaari et al., 2005). Kalliokoski et al. reported that individuals with a CC genotype at \(SLCO1B1\) c.521 had a significantly higher mean repaglinide AUC than those with TC or TT genotypes (Kalliokoski et al., 2008). His team observed that among healthy Chinese subjects, individuals with an \(SLCO1B1*1a/*1b, *1a/*1a, *15/*1a, or *5/*1a\) genotype (similar to group 2 and group 3 in our study) had significantly higher repaglinide AUC than those with the *1b/*1b genotype (He et al., 2011). Along these lines, we also observed that among individuals not pretreated with pitavastatin, \(SLCO1B1*15\) carriers had 20% higher \(C_{max}\) of repaglinide and 40%
higher AUC$_{0→∞}$ compared to *1b/*1b homozygotes. In contrast to He et al. (2011), we observed no significant differences in the pharmacokinetic parameters of repaglinide in individuals with different SLCO1B1 genotypes (the result was not reported in the table). Neither study observed a difference in pharmacodynamics.
among SLCO1B1 genotypes (He et al., 2011). Our study only included three SLCO1B1*15 carriers, limiting the power of our analyses.

In summary, we observed a genotype-dependent increase in the peak plasma concentration (C_{max}) of repaglinide in healthy Chinese male subjects. The effect on repaglinide by pretreatment of pitavastatin likely depends on the genetic background at SLCO1B1, and future studies of the role of genetic background on the pharmacodynamics of repaglinide require a larger sample size.

REFERENCES


