Effect of *SLCO1B1* genetic polymorphism on the pharmacokinetics of nateglinide

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Aims

Nateglinide is a meglitinide analogue with antidiabetic action. A recent study showed that *SLCO1B1* (which codes the *OATP1B1* gene, also known as *OATP-C*, *OATP2*) is a major determinant which markedly affects the pharmacokinetics of repaglinide. Our objective was to assess the association between single nucleotide polymorphisms (SNPs) of *SLCO1B1* and the pharmacokinetics of nateglinide.

Methods

Seventeen healthy volunteers with different *SLCO1B1* genotypes (11 with 521TT, four with 521TC and two with 521CC) were enrolled in this study. Each was given a single oral dose of 90 mg nateglinide. Plasma concentrations of nateglinide were measured up to 8 h by HPLC.

Results

The $C_{\max}$ and $AUC(0,\infty)$ of nateglinide were 83% ($P = 0.002$) and 82% ($P = 0.001$) higher in the *SLCO1B1*521TC subjects ($n = 4$), and 76% ($P = 0.016$) and 108% ($P = 0.001$) higher in the *SLCO1B1*521CC subjects ($n = 2$) than in the *SLCO1B1*521TT subjects ($n = 11$), respectively. The $t_{1/2}$ of nateglinide in *SLCO1B1*521CC subjects was 78% longer than that in 521TT subjects ($P = 0.036$). The difference in $t_{\max}$ values among the three genotypic groups was not statistically significant.

Conclusions

Our results suggest that *OATP1B1*-mediated hepatic uptake of nateglinide may be the prior step for its metabolism and elimination. *SLCO1B1*521T > C SNP might play an important role in the pharmacokinetics of nateglinide.

Introduction

The worldwide prevalence of diabetes is predicted to increase from 124 million to more than 221 million by the year 2010. However, of these, approximately 97% will have type 2 diabetes [1]. Nateglinide is a novel phenylalanine derivative and is administered prior to meals and stimulates rapid, short-term insulin secretion in a dose-dependent manner, decreasing mealtime plasma glucose concentrations [2]. As with other meglitinides, nateglinide stimulates pancreatic β -cell secre-

tion by acting on ATP-sensitive K^+ channels and on voltage-sensitive Ca^{2+} channels [3, 4].

The oral bioavailability of nateglinide is about 73%, and it is rapidly absorbed and extensively metabolized primarily by cytochrome P450 2C9 (CYP2C9) in the liver and a smaller fraction by CYP3A4 and CYP2D6 [5]. A previous report showed that the *CYP2C9**3 allele was associated with significantly reduced oral nateglinide clearance and pharmacokinetic parameters, which seemed to be unaffected by *CYP2C9**2 and *CYP2D6**4

or *5 carriers. However, the interindividual variability in the pharmacokinetics of nateglinide could not be fully explained by *CYP2C9* genotype [6]. A recent study carried out by Niemi *et al.* identified that *SLCO1B1* genetic polymorphism is a major determinant of repaglinide disposition *in vivo*, which explained most of the interindividual variability in the pharmacokinetics of repaglinide and elucidated an actively transporting process in its disposition prior to metabolism by CYP450s in hepatocytes [7].

Genetic polymorphisms of *SLCO1B1*, causing reduced transport activity both *in vitro* and *in vivo*, have recently gained increasing interest. Several studies in Japanese subjects, white German and Finnish subjects showed that *SLCO1B1**5, *SLCO1B1**15 (a haplotype that consists of *SLCO1B1**1b and *5 SNPs) and *17 (a haplotype that consists of *SLCO1B1**15 and -11187G > A SNPs) were associated with markedly increased pravastatin plasma concentrations compared with those homozygous for the wild-type *1a or *1b alleles [8–10]. Because the allelic frequency of the 521T > C (Val174Ala) SNP, which defines the *5, *15 or *17 haplotypes, is relatively high (about 14%) in Chinese subjects, these findings would suggest that a large number of substrates of OATP1B1 may have plasma concentrations of the drug higher than expected.

Nateglinide is structurally very similar to repaglinide, and both are meglitinide analogues. Our study aimed to determine the effect of the most common single nucleotide polymorphism (521T > C) in the *SLCO1B1* gene on the pharmacokinetics of nateglinide.

Methods

Subjects

After genotyping a total of one hundred and eleven Chinese healthy male volunteers aged from 19 to 22 years, 17 subjects were recruited for nateglinide pharmacokinetic analysis including 11 homozygotes for 521TT and six carriers with at least one 521C mutant allele (four subjects were 521TC heterozygotes and two were 521CC homozygotes). The study protocol was approved by the Ethics Committee of Central South University, Xiangya School of Medicine, and written informed consent was obtained from each participant. Each of them was healthy as determined by medical history, physical examination, routine blood tests, and electrocardiography. All the subjects were nonsmokers, abstained from drugs for at least 2 weeks before entry into the study, and did not take any coffee or alcohol for 1 week before the study.

Study design

After an overnight fast, each subject received a single oral dose of 90 mg nateglinide (three 30 mg tablets, Hunan Medicine Co. Ltd, Dongting Pharmaceutical Factory, China) with 100 ml water. All subjects received a standardized breakfast of 250 ml milk and 90 g cake 1 h after administration of nateglinide. Four hours later, warm meals were allowed. Venous blood samples (5 ml) were collected into EDTA tubes before and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, and 8 h after nateglinide intake. During the whole trial, a group of physicians and nurses stood by for emergency medical treatment. The plasma was separated by centrifugation and immediately stored in polypropylene tubes at -20°C.

ARMS-PCR for 521T > C genotyping

The four primers used in amplification refractory mutation system (ARMS-PCR) were as follows. P1: 5'-AAGTAGTTAAATTTGTAATAGAAATGC-3', P2: 5'-GGGTCATACATGTGGATATAAGT-3', P3: 5'-AAGCATATTACCCATGAAC G-3', and P4: 5'-GTAGACAAAGGGAAAGTGATCATA-3'. PCR amplification was performed in a Perkin-Elmer DNA Model PJ2000 Thermal Cycler (Foster city, CA) in a total volume of 25 µl containing 2.5 µl 10× buffer (with MgCl₂), 0.2 mmol l⁻¹ of each dNTP, 40 pmol l⁻¹ of each primer, 2 U LA Taq enzyme and approximately 200 ng genomic DNA as a template. The PCR conditions involved an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 48°C for 30 s and extension at 72°C for 30 s with a final extension at 72°C for 7 min. The PCR products were detected by means of 2.5% agarose gel electrophoresis and were detected by ethidium bromide staining. Wild-type 521TT yielded two fragments of 260 bp and 179 bp in length, while the variant 521CC homozygotes gave two fragments of 260 bp and 123 bp and the heterozygotes were three fragments of 260 bp, 123 bp and 179 bp. Two samples of each different genotype were sequenced to confirm the expected sequences of each genotype. (Figure 1)

Assay of nateglinide

The detection of nateglinide was performed on an Agilent 1100 HPLC system (California, USA) consisting of a HP1100 pump, an automatic sampler, and a Variable Wavelength Detector (VWD). The Chem Station software (Agilent) was used for data analysis and processing. Compounds was separated on a Hypersil BDS C18 column (4.6 mm × 200 mm, 5 µm particle size) with a Phenomenex SecurityGuard™ guard column (Phenomenex, US) and quantified by UV detection at 214 nm.

Figure 1

(a) ARMS-PCR analysis result for the *SLCO1B1* 521T > C polymorphism. Lane 1, genotype with T/C at 521; Lanes 2 and 3, genotype with T/T at 521; Lane 4, genotype with C/C at 521; Lane 5, DNA marker. (b) Direct sequencing results for the *SLCO1B1* 521T > C SNP. Panel I represents a trace for a sample with a *SLCO1B1* 521TT genotype. Panel II represents a trace for a sample from subject with a *SLCO1B1* 521TC genotype. Panel III represents a trace for a homozygous *SLCO1B1* 521CC individual

The mobile phase was composed of 0.02 M ammonium acetate solution : acetonitrile (60 : 40 v : v), and was delivered at a flow rate of 1.0 ml min⁻¹. The mobile phase was prepared daily and was filtered through a 0.45 µm cellulose acetate membrane. Repaglinide was used as an internal standard. Each sample was prepared by the following procedure: In a 1.5 ml plastic tube, 200 µl of plasma were mixed with 400 µl of internal standard (10 µg ml⁻¹), and the mixture was vortexed for 2 min. After centrifugation for 10 min at 13000 rev min⁻¹, the supernatants were transferred into an injection vial, and 20 µl was injected into the HPLC. The limit of quantification was 93.75 ng ml⁻¹ for nateglinide. The interday coefficients of variation (CV) were 5.03% at 750 ng ml⁻¹, 6.49% at 1.5 µg ml⁻¹ and 3.75% at 12 µg ml⁻¹ (*n* = 5) replicates.

Pharmacokinetics analysis

The pharmacokinetics of nateglinide were analyzed with a noncompartmental method using the WinNonlin 4.1 (Pharsight Corp., Mountain View, CA, USA). The maximum concentration (*C*_{max}) and the time to *C*_{max} (*t*_{max}) were directly observed from the concentration-time curve. The plasma nateglinide half-life (*t*_{1/2}) and the area under the plasma concentration-time curve (AUC) were calculated with the following equations: $t_{1/2} = \ln 2 / \lambda_z$, $AUC(0, \infty) = AUC(0, 8 \text{ h}) + C_p(8 \text{ h}) / \lambda_z$, where λ_z represents the slope of the linear terminal part of the plasma concentration vs. time curve after semilogarithmic transformation, *C*_p(8 h) is the plasma concentration at 8 h and AUC(0, 8 h) the AUC for the 8 h period after nateglinide intake by the linear trapezoidal rule.

Statistical analysis

Statistical analyses were performed by the SPSS software for Windows (Ver 10.0, SPSS Inc., 202 Chicago, IL). Data are expressed as mean ± SE. The differences in pharmacokinetic data were analysed by ANOVA test. A *P* value of less than 0.05 was considered significant.

Results

According to *SLCO1B1* genotype, all the subjects were divided into three groups, 521TT homozygotes (*n* = 11), 521TC heterozygotes (*n* = 4) and 521CC homozygotes (*n* = 2). *C*_{max}, AUC(0, 8 h) and AUC(0, ∞) of nateglinide were statistically different among all three genotypic groups (Figures 2 and 3 and Table 1). The *C*_{max} was 83% and 76% higher in *SLCO1B1* 521TC and 521CC subjects than in *SLCO1B1* 521TT subjects (*P* = 0.002 and *P* = 0.016, respectively). The AUC(0, ∞) was 82% and 108% in *SLCO1B1* 521TC and 521CC subjects than in *SLCO1B1* 521TT subjects (*P* = 0.001 and *P* = 0.001, respectively). The *t*_{1/2} of nateglinide in *SLCO1B1* 521CC subjects was 78% longer than that in 521TT subjects (*P* = 0.036). The differences of *t*_{max} values among the three genotypic groups were not statistically significant.

Discussion

In this study, we have provided evidence for the first time that *SLCO1B1* polymorphism may have a considerable impact on the pharmacokinetics of nateglinide. The plasma concentration of nateglinide was significantly higher in subjects carrying the *SLCO1B1* 521C allele than in subjects with the reference genotype.

Figure 2

The plasma concentration-time curve of nateglinide in 17 healthy volunteers with different *SLCO1B1* genotypes after a single oral dose of 90 mg nateglinide. Solid circles represent *SLCO1B1*521TT genotype group ($n = 11$) (●), triangles represent *SLCO1B1*521TC genotype group ($n = 4$) (▲), open circles represent *SLCO1B1*521CC genotype group ($n = 2$) (○). Data are expressed as mean $\pm$ SEM

Figure 3

Individual AUC of nateglinide from time 0 to infinity values in subjects with different *SLCO1B1* genotypes after a single oral dose of 90 mg nateglinide. Horizontal rules show mean values for all genotype groups

Table 1

Pharmacokinetic changes after a single 90 mg oral dose of nateglinide in relation to *SLCO1B1*521T > C single nucleotide polymorphism

<i>SLCO1B1</i> genotypes	C_{max} (ng ml ⁻¹) (95% CI)	$t_{1/2}$ (h) (95% CI)	AUC(0,8 h) (ng ml ⁻¹ h) (95% CI)	AUC(0,∞) (ng ml ⁻¹ h) (95% CI)
521TT ($n = 11$)	2708 $\pm$ 244 (2164, 3253)	1.24 $\pm$ 0.12 (0.96, 1.51)	5350 $\pm$ 530 (4170, 6530)	5705 $\pm$ 523 (4539, 6871)
521TC ($n = 4$)	4944 $\pm$ 626 (2950, 6938)	1.65 $\pm$ 0.46 (0.19, 3.10)	9748 $\pm$ 896 (6895, 12601)	10322 $\pm$ 1108 (6795, 13849)
521CC ($n = 2$)	4772 $\pm$ 1007 (-8025, 17568)	2.21 $\pm$ 0.06* (1.7, 2.7)	10974 $\pm$ 928 (-819, 22768)	11842 $\pm$ 1132 (-2251, 26236)
<i>P</i> value	0.002	0.080	0.000	0.000

Data are expressed as mean $\pm$ SEM; CI confidence interval; C_{max} observed peak plasma concentration; $t_{1/2}$ elimination half-life; AUC(0,8 h) area under the concentration vs. time curve to 8 h; AUC(0,∞) area under the concentration vs. time curve to infinity.

* $P = 0.036$ compared with *SLCO1B1*521TT subjects.

The AUC(0,∞) values of nateglinide varied five-fold among all the individuals and the mean AUC(0,∞) of this drug was about 82% and 108% higher in the *SLCO1B1*521TC and 521CC groups than that in the *SLCO1B1*521TT group. Our data strongly suggest that the OATP1B1-mediated transportation is at least partly involved in the hepatic uptake of nateglinide.

Nateglinide is metabolized by oxidative biotransformation mainly via CYP2C9 (70% of metabolism), and to a minor extent by CYP3A4 and CYP2D6 (30% of metabolism) [5, 11]. Kirchheiner *et al.* [6] found that the area under the concentration-time curve from 0 to 12 h and the peak plasma concentration of nateglinide were almost doubled in *CYP2C9**3/*3 homozygotes com-

pared with *CYP2C9**1/*1 wild type homozygotes, while the total oral clearance of this drug decreased by about 50%. We ignored the genotype of *CYP2C9* among subjects in the present study, which is a shortcoming. However, the allelic frequencies of *CYP2C9**2 and *CYP2C9**3 in Chinese subjects are about 0 ($n = 1016$) and 3.3% ($n = 866$), respectively [12], which indicates that there should be no more than one *CYP2C9**1/*3 heterozygote among our 17 subjects.

They observed no association between the *CYP2D6* genetic polymorphisms and the pharmacokinetics of nateglinide [6]. However, the effect of *CYP3A4* gene mutation on the kinetics of nateglinide still remains unknown, which is possibly because of the rare functional variation of this isoenzyme and its extremely low frequencies. In the present study, we observed a five-fold variation in AUC(0,∞) of nateglinide among individuals with different genotypes. Since the frequency of *CYP2C9**3/*3 homozygotes is very low (less than 0.2%) in Chinese healthy volunteers, metabolic phase seems not to be the major determinant of the interindividual variability in the exposure to nateglinide.

OATP1B1, one of the key transporters located on the basolateral membrane of human hepatocytes, transports a large number of structurally divergent compounds and has been implicated as a determinant factor in hepatic drug uptake and elimination [13]. Thus, impaired OATP1B1 transport activity caused by functional polymorphism may alter the pharmacokinetic features of OATP1B1 substrate drugs. *SLCO1B1*521T>C (Val174Ala), which defines either the *5 or *15 haplotypes, is relatively common (10% to 16%) in both Caucasians and Asians, which resulted in increased plasma concentration and decreased nonrenal clearance of pravastatin [8–10]. 521T>C mutation was also found to be associated with a markedly higher concentration of repaglinide in a recent study [7].

Gemfibrozil, a fibric acid derivative, is a potent inhibitor for *CYP2C8*, *CYP2C9* and OATP1B1 [14, 15]. Co-administration of gemfibrozil with repaglinide, the substrate for *CYP2C8* and *CYP3A4*, greatly increases the AUC by 8.1-fold [16]. *In vitro*, gemfibrozil is a more potent inhibitor of *CYP2C9* than *CYP2C8*. However, concomitant use of gemfibrozil and itraconazole only raised the AUC of nateglinide, which is a substrate for *CYP2C9* and *CYP3A4*, by less than 47% [17]. The discrepancy in the inhibitory effects of these two drug–drug interactions could be explained by the fact that both repaglinide and nateglinide could be substrates for OATP1B1, and OATP1B1 is (maybe) the only basolateral membrane transporter involved in the uptake of repaglinide (but not for nateglinide). If this transporter

was inhibited by gemfibrozil, repaglinide would accumulate in the blood circulation and could not be metabolized and eliminated. Another plausible explanation is that the glucuronide metabolite of gemfibrozil is a much more potent inhibitor of *CYP2C8* than *CYP2C9* *in vivo* [18].

In conclusion, the *SLCO1B1*521T>C polymorphism plays an important role in the interindividual variability of plasma concentrations of nateglinide. Further studies are needed to confirm whether these differences in nateglinide kinetics caused by *SLCO1B1* genotypes could lead to a clinical blood glucose-lowering effect. Genotype assay of patients with respect to the *SLCO1B1*521T>C SNP may be helpful for patients who require treatment with nateglinide.

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