PHARMACOKINETICS OF CITALOPRAM IN RELATION TO GENETIC POLYMORPHISM OF CYP2C19

BANG-NING YU, GUO-LIN CHEN, NAN HE, DONG-SHENG OUYANG, XIAO-PING CHEN, ZHAO-QIAN LIU, AND HONG-HAO ZHOU

Pharmacogenetics Research Institute, Institute of Clinical Pharmacology, Central South University, Changsha, Hunan, Republic of China

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ABSTRACT:

The study was designed to define the contribution of cytochrome P450 2C19 (CYP2C19) and cytochrome P450 3A4 (CYP3A4) to citalopram N-demethylation and to evaluate the relationship between the disposition of citalopram and CYP2C19 genotype. A single oral 40-mg dose of citalopram was administered to eight extensive metabolizers and five poor metabolizers recruited from 77 healthy Chinese volunteers whose genotypes and phenotypes were predetermined. The plasma concentrations of citalopram and desmethylcitalopram were determined by high-performance liquid chromatography. It was found that the genotype of CYP2C19 had a significant effect on the N-demethylation of citalopram. Poor metabolizers with m1 mutation had higher area under the plasma concentration versus time curve (AUC0-∞) values than did extensive metabolizers. Terminal elimination half-life (t1/2) values of citalopram in poor metabolizers were significantly higher than the values in extensive metabolizers who were either homozygous or heterozygous with CYP2C19*1. The oral clearance (CLoral) of citalopram in poor metabolizers was significantly lower than that of extensive metabolizers. The AUC0-∞ and maximum plasma concentration (Cmax) of desmethylcitalopram in poor metabolizers were significantly lower than the values of extensive metabolizers. The results show that CYP3A4 is not the major enzyme in the N-demethylation of citalopram among extensive metabolizers.

Citalopram (CIT1) is a potent and selective serotonin reuptake inhibitor in the central nervous system and is widely used to treat depression (Hyttel, 1982). It is metabolized mainly through N-demethylation in the liver to desmethylcitalopram (DCT) and desmethylcitalopram (Milne and Goa, 1991). Major problems in the clinical use of this drug are the large interpatient variations in plasma and the difference in clinical response and toxicity. The major source of this variation is considered to be the interindividual difference in the activities of the cytochrome P450 enzymes that catalyze citalopram metabolism.

Some in vitro studies have shown that the formation of desmethylocitalopram was catalyzed by multiple isoforms of P450s in human liver microsomes, including CYP2C19, CYP2D6, and CYP3A4 (Kobayashi et al., 1997; Rochat et al., 1997; Olesen and Linnet, 1999). Nevertheless, there is some confusion as to which P450 isoforms are responsible for citalopram N-demethylation in vivo. Recently, Kobayashi et al. (1997) reported that at least three P450 isoforms are involved in the liver microsomal N-demethylation of citalopram and that the contribution of CYP3A4 is the most important among them.

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Address correspondence to: Professor Hong-Hao Zhou, Pharmacogenetics Research Institute, Institute of Clinical Pharmacology, Central South University, Changsha, Hunan 410078, Republic of China. E-mail: hhzhou@public.cs.hn.cn

Kobayashi et al. noticed that the results of their study were not consistent with Sindrup et al. (1993), whose in vivo studies revealed that N-demethylation of citalopram was significantly related to CYP2C19 genotype.

Interindividual and ethnic differences in drug metabolism and response are a clinically important issue in the use of many drugs. One of the major causes is the variability of activities of drug-metabolizing enzymes in the liver. CYP2C19 is a clinically important enzyme because of its genetic polymorphism that separates people into two different phenotypes: extensive metabolizers (EMs) and poor metabolizers (PMs) of S-mephentoin. CYP2C19 activity is genetically determined, and its genetic polymorphism shows marked inter racial difference. The incidence of the poor metabolizer phenotype is markedly higher in Asian populations (13–23%) than in white populations (2–5%) (de Morais et al., 1994b). The normal allele and seven defective alleles of CYP2C19 have been designated as CYP2C19*1 (wild-type), CYP2C19*2 (m1), CYP2C19*3 (m2), CYP2C19*4 (m3), CYP2C19*5 (m4), CYP2C19*6 (m5), CYP2C19*7 (m6), and CYP2C19*8 (m7). The combinations of CYP2C19*2 and CYP2C19*3 can account for 100% of Asian poor metabolizers, whereas all the genetic defects are found in white populations (Gonzalez et al., 1990; de Morais et al., 1994a,b; Ferguson et al., 1998; Ibeanu et al., 1998).

Troleandomycin is a macrolide antibiotic that has been shown to effectively inhibit in vitro CYP3A4 and 3A5 in human liver microsomes. It is a selective inhibitor, in that the activity of seven other P450 forms, including CYP1A2 and CYP2C9, was unaffected (Watkins et al., 1989). In addition, Watkins et al. showed that, using the erythromycin breath test, CYP3A activity in vivo was inhibited by
80% by treatment with 250 mg of oral troleandomycin in healthy volunteers (Watkins et al., 1989, 1992; Wang et al., 1997). Our study was designed to determine the effect of CYP2C19 and CYP3A4 in citalopram N-demethylation by testing the effect of troleandomycin on the pharmacokinetics of citalopram in CYP2C19 genotyped subjects.

### Materials and Methods

#### Subjects

Before the pharmacokinetic study of citalopram, 77 unrelated healthy Chinese volunteers were screened for their CYP2C19 genotype. Their phenotypes were identified by omeprazole, a probe drug of CYP2C19. Eight extensive metabolizers (genotyped as CYP2C19*1/*1, CYP2C19*1/*2, or CYP2C19*1/*3) and five poor metabolizers (genotyped as CYP2C19*2/*2 or CYP2C19*2/*3) were randomly selected for participation in the study. The mean age and body weight were 21 ± 1 years (range, 20–22 years) and 64 ± 8 kg (range, 56–81 kg). All subjects were male nonsmokers and healthy as determined by medical history, physical examination, and laboratory screening tests. No medication or ethanol consumption was allowed for at least 2 weeks before and through the clinical study. The protocol of the study was approved by Ethics Committees of Xiang-Ya School of Medicine, Central South University, Hunan, China. Informed written consent was obtained from all subjects.

#### Determination of CYP2C19 Genotypes and Phenotypes

All subjects belonged to a pool of volunteers with predetermined CYP2C19 genotypes. CYP2C19*2 (m1) and CYP2C19*3 (m2) mutations were determined by conventional restriction fragment length polymorphism and polymerase chain reaction (de Morais et al., 1994b; Ferguson et al., 1998). CYP2C19 phenotypes were determined with the use of omeprazole as a probe drug (Balian et al., 1995). In brief, 13 subjects whose genotypes were known were given a single oral 20-mg dose of omeprazole (Astra, Minneapolis, Mo) and 8 kg (range, 56–81 kg). The subjects were separated into two groups randomly receiving a single oral dose of 40 mg citalopram (H. Lundbeck A/S, Copenhagen, Denmark) on two occasions: 1) 24 h after oral administration phase, troleandomycin, and genotype of CYP2C19 were then extracted with 4 ml of hexane. The organic phase was transferred to 1 ml of the plasma samples. The samples were then extracted with 4 ml of hexane. The organic phase was transferred to another glass tube and evaporated to dryness at 40°C. The residues were dissolved in 50 μl of mobile phase, 20 μl of which was injected into the high-performance liquid chromatography system equipped with an ultraviolet detector. Chromatography was performed on a reversed-phase Hypersil OD5 C18 (Thermo Hypersil, Keystone Scientific Operations, Bellefonte, PA) column (250 × 4 mm i.d., 5-μm particle size). Detection limits were 1 μg/l for citalopram and desmethycitalopram. Calibration curves ranged from 2.5 to 500 μg/l for citalopram and desmethycitalopram. The coefficients of variation for intraday and interday reproducibility ranged from 3.2 to 4.7% and 6.2 to 8.5% for citalopram and desmethycitalopram, respectively.

#### Data Analysis

Pharmacokinetic analysis was performed by a noncompartmental approach. Area under the concentration versus time curve (AUC_{0→∞}) was calculated by the trapezoidal rule from zero to the last measured concentration point. The elimination rate constant (k_e) of citalopram was estimated by least regression analysis. The AUC from zero hour to infinity (AUC_{0→∞}) and the oral clearance of citalopram (Cl_{oral}) were calculated with the following equations:

\[
AUC_{0→∞} = C_{max} \times t_{1/2} / k_e
\]

\[
CL = dose / AUC_{0→∞}
\]

where C is the drug concentration in plasma and CL is the apparent oral clearance of the drug from plasma.

Data were analyzed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL). Variance analysis was used to investigate the effect of administration phase, troleandomycin, and genotype of CYP2C19 on the N-demethylation of citalopram. Then, Student’s t test was used to compare the pharmacokinetic parameters of citalopram and desmethycitalopram between the extensive metabolizers and poor metabolizers. The minimal level of significance accepted was P < 0.05.

#### Results

Genotype analysis of 77 healthy Chinese young volunteers indicated that the incidence of poor metabolizers and extensive metabolizers was 14.3 and 85.7% (data not shown), respectively, which is consistent with previous reports (Bertilsson et al., 1992; Xiao et al., 1997). Eight extensive metabolizers and five poor metabolizers chosen by the stratified random selection from 66 healthy extensive metabolizers and 11 poor metabolizers, respectively, were enrolled for the pharmacokinetic study of citalopram. Subsequently, CYP2C19 phenotypes of 13 subjects were determined by hydroxylation indices of omeprazole [(log_{10} (omeprazole/[5-hydroxymeprazole])]. The results were consistent with their previous genotypes.

As shown in Table 1, there was no statistically significant difference in citalopram pharmacokinetic data in EMs, whether they had TAO or not. However, TAO treatment did have a significant effect on citalopram and desmethycitalopram AUC_{0→∞} in PMs.

The genotype of CYP2C19 had a significant effect on the N-demethylation of citalopram. The extensive metabolizers and poor metabolizers had distinct plasma concentration-time profiles for cita-
The poor metabolizers had a distinct increase in citalopram pharmacokinetic parameters of citalopram and desmethylcitalopram than those in extensive metabolizers (516.7 g/L versus 166.2 g/L, \( P < 0.001 \)). Pharmacokinetic parameters of citalopram in heterozygous metabolizers was almost three times higher than that of poor metabolizers. The oral clearance (CL oral) of citalopram in poor metabolizers was significantly lower than that in extensive metabolizers. Our previous study also indicated that two defects in the CYP2C19 gene (CYP2C19*2 and CYP2C19*3) may account for 100% of the Chinese poor metabolizers. The subsequent phenotype analysis of 13 subjects in the study showed concordance with their genotypes.

Citalopram is mainly metabolized to N-desmethyldesmethylcitalopram and further demethylated to didesmethylcitalopram (Milne and Goa, 1991). In vitro studies have shown that the N-demethylation of citalopram is catalyzed by multiple P450 isomorphs in human liver microsomes, including CYP2C19, CYP2D6, and CYP3A4 (Kobayashi et al., 1991). However, in vitro study found that CYP2C19 and CYP3A4 might be the major enzymes contributing to the citalopram N-demethylation because of their high affinity for citalopram (Olesen and Linnet, 1999). In addition, Sindrup et al. (1993) reported that the in vivo disposition of citalopram is associated with the polymorphism of CYP2C19. Our results also demonstrate that the CYP2C19 genotype has a significant impact on the metabolism of citalopram. The extensive metabolizers and poor metabolizers exhibited significantly different pharmacokinetic parameters of citalopram and desmethylcitalopram. The poor metabolizers had moderately higher AUC\(_{0-\infty}\) and \( t_{1/2} \) values of citalopram than the extensive metabolizers. The CL\(_{oral}\) of citalopram in poor metabolizers (17.1 ± 2.0 l/h) was significantly lower than that of extensive metabolizers (24.1 ± 1.6 l/h). In addition, the AUC\(_{0-\infty}\) and C\(_{max}\) of desmethylcitalopram in poor metabolizers were significantly lower than those of extensive metabolizers. The \( t_{1/2} \) values of desmethylcitalopram were significantly longer in poor metabolizers than in extensive metabolizers.

The fact that gene dose has an effect on drug metabolism has been reported previously. Broly et al. (1991) have shown that the metabolic ratio in heterozygous extensive metabolizers of CYP2D6 is significantly higher than that in homozygous extensive metabolizers. Our previous studies also find that gene dose affects the metabolism of \( \beta \)-mephenytoin,
TABLE 2
Pharmacokinetic data (mean ± S.D.) of citalopram and desmethylcitalopram in CYP2C19 genotyped subjects

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Citalopram</th>
<th>Desmethylcitalopram</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AUC_{0–24h}</td>
<td>C_{max}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>µg · h/l</td>
<td>nM</td>
</tr>
<tr>
<td>PM</td>
<td>5</td>
<td>2132.5 ± 242.3</td>
<td>68.3 ± 16.6</td>
</tr>
<tr>
<td>CYP2C19*2/*2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C19*2/*3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM</td>
<td>8</td>
<td>1738.0 ± 166.2**</td>
<td>59.9 ± 5.9</td>
</tr>
<tr>
<td>CYP2C19*1/*2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C19*1/*3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C19*1/*1</td>
<td>4</td>
<td>1774.2 ± 184.0*</td>
<td>63.9 ± 23.9*</td>
</tr>
<tr>
<td>CYP2C19*1/*3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1677.5 ± 159.3**</td>
<td>46.4 ± 31.3*</td>
</tr>
</tbody>
</table>

* P < 0.05 and ** P < 0.01 compared with CYP2C19*2/*2 and CYP2C19*2/*3.

References


