Nonlinear Mixed Effects Model Analysis of the Pharmacokinetics of Routinely Administered Bepridil in Japanese Patients with Arrhythmias

Masato Taguchi, Akira Fujiki, Jotaro Iwamoto, Hiroshi Inoue, Katsutoshi Tahara, Katsuya Saigusa, Isao Horiuchi, Yukari Oshima, and Yukiya Hashimoto

*Graduate School of Pharmaceutical Sciences, University of Toyama; and **Second Department of Internal Medicine, Faculty of Medicine, University of Toyama; 2630 Sugitani, Toyama 930–0194, Japan.

Received September 29, 2005; accepted December 2, 2005

This study was performed to evaluate variability in the pharmacokinetics of bepridil in 38 Japanese patients with arrhythmias, and to investigate the effects of aprindine as well as CYP2D6 and CYP3A5 polymorphisms on the oral clearance of bepridil. We determined the polymorphic alleles of CYP2D6 and CYP3A5 in each subject. The plasma concentration of bepridil at steady-state following repetitive oral administration was measured with an HPLC-based method, and the oral clearance was estimated using the nonlinear mixed effects model (NONMEM) program. Mean oral clearance was significantly greater in the patients with the CYP2D6*10 allele than in those without it. On the other hand, no significant effect of the CYP3A5 polymorphism was observed on the pharmacokinetics of bepridil. In addition, aprindine seemed to reduce the oral clearance of bepridil in the patients with the CYP2D6*10 allele.

Key words aprindine; bepridil; CYP2D6*10; nonlinear mixed effects model; pharmacokinetics

Bepridil, a diarylaminopropylamine derivative, was originally developed as an anti-anginal drug with the properties of a calcium antagonist. The drug also blocks sodium and potassium channels, and prolongs action potentials and refractory periods of normal ventricular and arterial myocardium. These multiple ion channel-blocking properties similar to amiodarone suggest clinical potential in the management of patients with arrhythmias. Indeed, several groups in Japan have reported a beneficial effect of bepridil in the treatment of patients with atrial fibrillation (AF), though there seemed to be large interindividual variability in the clinical efficacy. That is, Yoshida et al. reported that bepridil effectively prevented paroxysmal AF in 15 of 23 patients, and Nakazato et al. reported that bepridil restored sinus rhythm in 65 of 112 patients with persistent AF with a mean conversion time of 2.1 months. In addition, Fujiki et al. reported that the oral administration of bepridil alone or in combination with aprindine restored sinus rhythm in 22 of 32 patients with long-lasting persistent AF, and that the rate of termination of AF was enhanced by the coadministration of aprindine. They also found that pharmacological conversion with bepridil alone or in combination with aprindine recovered atrial mechanical function better and maintained sinus rhythms longer than electrical conversion in patients with long-lasting persistent AF.

There is limited information available regarding the clinical pharmacokinetics of bepridil and the variability therein. Benet reviewed several studies, and reported that bepridil is completely metabolized presumably by hepatic oxidative processes. Wu et al. also reported that oxidative reactions at seven sites are involved in metabolic pathways for bepridil, but that the formation of metabolites is adequately described by four interrelated pathways, namely, aromatic hydroxylation, followed by N-dealkylation, N-debenzylation, and N-acetylation. In addition, unpublished data obtained by Sankyo Co., Ltd. (Tokyo, Japan) suggested that bepridil is mainly metabolized by hepatic cytochrome P450 (CYP) 2D6 and to a lesser extent by CYP3A4 (personal communication). The genetic polymorphism of CYP2D6 has been studied extensively, and more than 50 variants have been documented. Among Asian extensive/intermediate metabolizers, the three most common alleles of the CYP2D6 gene are CYP2D6*1, *2, and *10. The mutant allele of CYP2D6 (CYP2D6*2) does not affect the enzymatic activity, whereas the CYP2D6*10 allele reduces the affinity of CYP2D6 for several drugs. On the other hand, more than 30 single nucleotide polymorphisms have been identified in the CYP3A4 gene. For the most common variant, CYP3A4*1B, increased transcription was demonstrated in vitro, which may theoretically result in greater enzymatic activity in vivo. However, the allele frequency was 0% in Japanese. On the other hand, CYP3A5 is another important CYP3A protein in the liver, and exhibits significant overlap with CYP3A4 in substrate specificity. The defective allelic variant, CYP3A5*3, is frequently observed among Japanese. In addition, precisely why the use of aprindine together with bepridil increases the rate of AF termination has not been clarified. It is possible that coadministration of aprindine prevents the metabolism of bepridil, because aprindine is a competitive inhibitor of CYP2D6.

The present study was designed to evaluate the variability in the pharmacokinetics of bepridil in Japanese patients with arrhythmias, and to investigate the effects of aprindine as well as the CYP2D6 and CYP3A5 genotypes on the oral clearance of bepridil. A pharmacokinetic analysis was performed using a nonlinear mixed effects model (NONMEM) program, because population pharmacokinetics based on a NONMEM analysis can simultaneously evaluate the mean pharmacokinetic parameters, the covariates affecting the pharmacokinetics of a drug, and also unknown inter- and intra-individual pharmacokinetic variability.

Materials and Methods

Subjects and Study Protocol Thirty-eight Japanese patients with arrhythmias (29 males and 9 females) participated

© 2006 Pharmaceutical Society of Japan
in this study. The patients ranged in age from 41 to 79 years old (mean ± S.D.: 59.9 ± 10.1), and in body weight from 36 to 92 kg (mean ± S.D.: 64.7 ± 13.0 kg). In this study, 29 patients had AF, 34 had ventricular premature contraction, 5 had ventricular tachycardia, 4 had supraventricular tachycardia, and 2 had Brugada syndrome. No patients had severe hepatic or renal failure. The patients were routinely treated with an oral administration of bepridil hydrochloride (Bepricor® Tablet, Sankyo, Tokyo, Japan) was concomitantly administered to sixteen of these patients at doses of between 20 mg and 80 mg per day. 

Ampicillin and metronidazole were also concomitantly administered to 17 and 30 patients, respectively. No patients received any potent inhibitor of CYP2D6 (e.g. amiodarone or quinidine). One to three blood samples were withdrawn from each patient at steady-state following repetitive dosing for more than two weeks, and the total number of blood samples was 65. Fifty-two samples were obtained at 2—7 h after dosing, and two weeks, and the total number of blood samples was 65. One to three blood samples were withdrawn from each patient at steady-state following repetitive dosing for more than two weeks, and the total number of blood samples was 65. Fifty-two samples were obtained at 2—7 h after dosing, and 13 samples were obtained at 10—24 h after dosing. All patients gave written informed consent to participate in this study, which was approved by the ethics committee of Toyama Medical and Pharmaceutical University.

**Genotyping of CYP2D6 and CYP3A5** Genomic DNA was extracted from the peripheral blood with a QiAmp® Blood Kit (QIAGEN, Hilden, Germany). CYP2D6 genotypes were determined as described previously. Briefly, CYP2D6*1, *10 and *14 were determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, and CYP2D6*2 was detected by the allele-specific PCR method. In addition, the detection of CYP2D6*5 was carried out using two different long-PCR methods. The CYP3A5*1 (wild-type) allele and the defective allelic variant CYP3A5*3 were determined by direct sequencing.

**Assay of Bepridil** The concentration of bepridil in plasma was measured using the reversed-phase HPLC method as described by Ng et al. with some modifications. Briefly, 0.5 ml of 100 mM phosphate buffer (pH 7.4) was added to plasma samples (0.25 ml), and bepridil was mildly extracted with 5 ml of hexane for 20 min. The hexane layer was transferred to another tube, and was evaporated dry with a SpeedVac® system (Savant, Famingdale, NY, U.S.A.). The residue was dissolved in 0.8 ml of mobile phase, and 50 μl was injected onto the HPLC column. The HPLC system consisted of a Shimadzu LC-10ADvp (Kyoto, Japan), and a column of COSMOSIL 5C18-AQ-II (15 cm×4.6 mm i.d.; 4.5 μm particle size; Nacalai Tesque, Kyoto, Japan). The mobile phase comprised 10 mM KH₂PO₄ (pH 3.3) containing 2% (w/v) triethylamine/acetoniitrile (60/40). The flow rate was kept at 1.0 ml/min, and the temperature of the column oven was maintained at 40°C. The peaks were monitored at 254 nm (Shimazu SPD-10Avp, Kyoto, Japan), and the retention time was 16 min for bepridil. The coefficient of intra-day variation of this assay was 2.7% at a plasma bepridil concentration of 0.4 μg/ml. The detection limit for bepridil was 0.02 μg/ml for its plasma concentration.

**Estimation of Population Pharmacokinetic Parameters of Bepridil** Mean pharmacokinetic parameters and their interindividual variations were estimated from 65 measurements of the plasma bepridil concentration using the NONMEM method. Since the mean (± S.D.) half-life of bepridil at steady-state is reported to be considerably long (42 ± 12 h), the intra-day variation in the plasma concentration of bepridil was speculated to be relatively small following long-term administration. In this study, therefore, the plasma bepridil concentration at steady-state (Css) was modeled using the following equation:

\[ \text{Css}_i = \frac{D_{Di}}{CL/F_i} \cdot (1 + \epsilon_i) \] (1)

where \( D_{Di} \) is the daily dose for the \( i \)th patient (Css), \( \epsilon_i \) is a random variable that describes intraindividual variability with a mean of zero and variance of \( \sigma^2 \). CL/F is the oral clearance in the \( i \)th individual. For the simplest basic model (Model 1), CL/F was modeled using the following equation:

\[ \text{CL/F}_i = \theta_i \cdot W_T_i \cdot (1 + \eta_i) \] (2)

where \( \theta_i \) is the predicted population mean of oral clearance, \( W_T \) is the individual body weight, and \( \eta_i \) is a random variable distributed with a mean of zero and variance of \( \omega^2 \).

Table 1 summarizes the 8 models used in this study. The effect of the CYP2D6*10 genotype on CL/F was evaluated using Models 2 and 3; where \( G2 = 0 \) and \( G3 = 0 \) for Group 1 patients with CYP2D6*1/*1, *1/*2, and *2/*2; \( G2 = 1 \) and \( G3 = 0 \) for Group 2 patients with CYP2D6*1/*10 and *2/*10; and \( G2 = 0 \) and \( G3 = 1 \) for Group 3 patients with CYP2D6*10/*10. The effect of the CYP3A5*3 genotype on CL/F was evaluated with Model 4; where \( G2 = 0 \) and \( G3 = 0 \) for patients with CYP3A5*3/*3; \( G2 = 1 \) and \( G3 = 0 \) for patients with CYP3A5*1/*3; and \( G2 = 0 \) and \( G3 = 1 \) for patients with CYP3A5*1/*1. The effect of coadministration of aprindine on CL/F was evaluated using Models 5, 6, and 7; where \( APR \) is one for the patients taking both bepridil and aprindine, and zero for the patients taking bepridil alone. In addition, the effect of age (AGE) on CL/F was evaluated with

<table>
<thead>
<tr>
<th>Model</th>
<th>Formula</th>
<th>No. of bs</th>
<th>LLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CL/F = θ₁·W_T·(1+η₁)</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>CL/F = [(θ₁ + θ₂·G2 + θ₃·G3)·W_T·(1+η)]</td>
<td>3</td>
<td>213.67 vs. Model 1</td>
</tr>
<tr>
<td>3</td>
<td>CL/F = [(θ₁ + θ₂·G2 + θ₃·G3)·W_T·(1+η)]</td>
<td>3</td>
<td>0.29 vs. Model 2</td>
</tr>
<tr>
<td>4</td>
<td>CL/F = [(θ₁ + θ₂·G2 + θ₃·G3)·(θ₁ + θ₂·G2 + θ₃·G3)·W_T·(1+η)]</td>
<td>3</td>
<td>1.02 vs. Model 2</td>
</tr>
<tr>
<td>5</td>
<td>CL/F = (θ₁ + θ₂·G2 + θ₃·G3)·W_T·(1+η)</td>
<td>3</td>
<td>7.52 vs. Model 2</td>
</tr>
<tr>
<td>6</td>
<td>CL/F = (θ₁ + θ₂·G2 + θ₃·G3)·(θ₁ + θ₂·APR·G2 + θ₃·APR·G3)·W_T·(1+η)</td>
<td>3</td>
<td>11.12 vs. Model 2</td>
</tr>
<tr>
<td>7</td>
<td>CL/F = (θ₁ + θ₂·G2 + θ₃·G3)·(θ₁ + θ₂·APR·G2 - θ₁·APR·G3)·W_T·(1+η)</td>
<td>4</td>
<td>0.59 vs. Model 6</td>
</tr>
<tr>
<td>8</td>
<td>CL/F = (θ₁ + θ₂·G2 + θ₃·G3)·(θ₁ + θ₂·APR·G2 + θ₃·APR·G3)·W_T·(1+η)</td>
<td>4</td>
<td>0.96 vs. Model 6</td>
</tr>
</tbody>
</table>
Model 8.

**Data Analysis** Data analysis was performed with NONMEM software (double precision NONMEM Version V Level 1.1) running on a mainframe UNIX computer at the Kyoto University Data Processing Center. In the present study, we used a first-order conditional estimation method with η–e interaction. The statistical significance of the parameters was evaluated with the likelihood ratio test using the minimum value of the objective function (–2 log likelihood) produced by NONMEM. In this study, when the –2 log likelihood difference (LLD) between two models allowing a parameter of interest freely estimated versus a fixed hypothetical value was greater than 3.84, the parameter value was considered to be statistically significant (p<0.05). In addition, NONMEM provides estimates of the standard error (S.E.) for all parameters, and S.E. can be used to define 95% confidence intervals (CI) for true parameter values: 95% CI=(the estimated parameter value)±1.96·S.E.

**RESULTS**

Table 2 shows the genotypes of CYP2D6 and CYP3A5 in 38 Japanese patients with arrhythmias. Nine patients homozygous for the CYP2D6*1 allele, six heterozygous for the CYP2D6*1/*2 alleles, and three homozygous for the CYP2D6*2 allele were classified into Group 1. Eight patients heterozygous for the CYP2D6*1/*10 alleles and six heterozygous for the CYP2D6*2/*10 alleles were classified into Group 2. Six patients homozygous for the CYP2D6*10 allele were classified into Group 3. No subject had the null alleles of CYP2D6 (CYP2D6*5 and CYP2D6*14). In addition, nineteen patients homozygous for the CYP3A5*3 allele were classified into Group 1’. Fourteen patients heterozygous for the CYP3A5*1/*3 alleles were classified into Group 2’. Five patients homozygous for the CYP3A5*1 allele were classified into Group 3’.

Figure 1 shows the plasma concentration of bepridil in individual patients taking bepridil alone or in combination with aprindine. The plasma concentrations of bepridil seemed to be slightly higher in the patients taking aprindine, though large interindividual variability was observed. In addition, the regression line for patients taking bepridil alone differed from that for patients taking bepridil and aprindine (Fig. 1). The population mean pharmacokinetic parameter, θq, for the simplest basic model (Model 1) was estimated to be 0.226 l/h/kg, and the ω value for Model 1 was estimated to be 38.2%, indicating large interindividual variability in the disposition kinetics of bepridil.

Table 1 summarizes 8 models for analyzing the oral clearance of bepridil and the LLD values between two models. The effect of the CYP2D6*10 genotype on the CL/F of bepridil was evaluated using Models 2 and 3. The θq, θω, and ω values for Model 2 were estimated to be 0.173 l/h/kg, 0.0859 l/h/kg, and 35.6%, respectively, and the LLD value for Model 2 was 13.67 (Table 1). The effect of CYP2D6 genotype on the variability in the pharmacokinetics of bepridil was significant, but no further significant improvement on the introduction of θq was observed in the LLD value for Model 3 (0.29). When the effects of CYP3A5 genotype on the CL/F of bepridil were evaluated with Model 4, the θq and ω values were estimated to be 0.110 and 35.4%, respectively. However, the LLD value for Model 4 (1.02) did not reach a statistically significant level (Table 1).

The effect of coadministered aprindine on the CL/F value of bepridil was evaluated using Models 5, 6, and 7. The θq and ω values for Model 5 were estimated to be 0.316 and 33.5%, respectively, whereas the θq and ω values for Model 6 were estimated to be 0.467 and 31.6%, respectively. The LLD value for Model 6 (11.12) was greater than that for Model 5 (7.52). Therefore, in the subsequent analysis, the effect of aprindine on the CL/F of bepridil was assumed only for the patients with at least one CYP2D6*10 allele. However, no further significant improvement on the introduction of θq was observed in the LLD value for Model 7 (0.59). The effect of age on CL/F was evaluated with Model 8, because the activity of CYP2D6 may be decreased in older patients. The θq value for Model 8 was estimated to be 6.21×10⁻³, but the LLD value for Model 8 (0.96) did not reach a statistically significant level (Table 1).

In the present study, Model 6 was selected to describe the pharmacokinetics of bepridil in Japanese patients. Table 3 shows the final estimates of population pharmacokinetic parameters of bepridil and their 95% CI for Model 6. The final ω value for Model 6 was estimated to be 31.6%, which was less than that for the simplest basic model, Model 1 (38.2%).

The oral clearance in individual patients was obtained from population estimates according to Bayes’ theorem using
DISCUSSION

We evaluated the pharmacokinetic variability of routinely administered bepridil in 38 Japanese patients with arrhythmias in the present study, and made two major findings. That is, mean oral clearance was significantly higher in the patients with the $\text{CYP2D6*10}$ allele than in those without it. In addition, coadministered aprindine decreased the oral clearance of bepridil in the patients with the $\text{CYP2D6*10}$ allele.

$\text{CYP2D6*10}$ produces an enzyme with Pro$^{34}\text{Ser}$ and Ser$^{486}\text{Thr}$ amino acid substitutions.$^{22,23}$ Pro$^{34}\text{Ser}$ occurs in a proline-rich region, which follows the signal-anchor sequence in the amino-terminal portion of CYP2D6.$^{24}$ Yamazaki et al. proposed that the proline residues in the proline-rich region are crucial for the correct conformation of microsomal P450 molecules.$^{24}$ It is also suggested that the expression of CYP2D6 containing Ser$^{34}$ is weaker than that of wild-type CYP2D6.$^{11,23}$ In addition, Nakamura et al. reported that the thermal stability of CYP2D6*10 in human liver microsomes is lower than that of CYP2D6*1.$^{25}$ On the other hand, Fukuda et al. found that the apparent $K_m$ values of CYP2D6*10 for bufuralol and venlafaxine were significantly higher than those of CYP2D6*1, though the $V_{\text{max}}$ values of CYP2D6*10 were slightly larger than those of CYP2D6*1.$^{11,23}$ Previously, we reported that oral clearance values of metoprolol and carvedilol were significantly lower in patients with the $\text{CYP2D6*10}$ allele than those with the $\text{CYP2D6*1}$ or $\text{CYP2D6*1/*2}$ genotype.$^{10,26}$ At present, therefore, the molecular and biochemical mechanisms responsible for the increased oral clearance of bepridil in patients with the $\text{CYP2D6*10}$ allele are not clear (Fig. 3).

Ebner et al. reported that hydroxylation of aprindine is mediated by CYP2D6 since no hydroxylated metabolites of aprindine were detected in liver microsomes isolated from a poor metabolizer of sparteine.$^{16}$ They also reported that aprindine competitively inhibits the metabolism of sparteine and propafenone, and suppresses the formation of their metabolites by human liver microsomes.$^{16}$ Therefore, we concluded that aprindine may inhibit the metabolism of bepridil competitively and reduce the oral clearance of bepridil in patients with arrhythmias (Fig. 3). In addition, the increased plasma bepridil concentration caused by aprindine may be at least partly responsible for the antiarrhythmic effects of bepridil (Fig. 1). In fact, Fujiki et al. reported that oral administration of bepridil alone restored sinus rhythm in only 11 of 32 patients whose persistent AF was resistant to intravenous aprindine, but that this rate was increased 2-fold by the coadministration of aprindine.$^5$ On the other hand, it is still unclear whether and/or why the inhibitory effect of aprindine on CYP2D6*10 is greater than that on CYP2D6*1 (Fig. 3). Further systematic studies will be needed in order to clarify the drug metabolizing activities of CYP2D6*10 in the absence and presence of substrates/inhibitors of CYP2D6.

In conclusion, the present study suggested that the large variability in the pharmacokinetics of bepridil in Japanese patients with arrhythmias is partly due to polymorphisms of CYP2D6 and to drug interaction with a substrate/inhibitor of CYP2D6. These findings provide new insights into understanding the interindividual variability in the pharmacokinetics and antiarrhythmic effects of bepridil.
Acknowledgements This work was supported in part by a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science (JSPS).

REFERENCES