Effect of *CYP2D6*10* on the Pharmacokinetics of *R*- and *S*-Carvedilol in Healthy Japanese Volunteers

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This study was performed to investigate the effect of *CYP2D6*10* on the pharmacokinetics of *R*- and *S*-carvedilol in healthy Japanese volunteers. Five or 10 mg of carvedilol was orally administered to 23 subjects (22—44 years old), and blood samples were taken at 2 and 6 h after dosing. We determined the polymorphic alleles of CYP2D6 in each subject. The whole blood concentration of *R*- and *S*-carvedilol was measured by an HPLC method. The pharmacokinetic parameters in individual subjects were estimated by the Bayesian method using the nonlinear mixed effects model (NONMEM) program. The mean values of oral clearance for *R*- and *S*-carvedilol were estimated to be 1.01 and 2.15 l/h/kg, respectively. The oral clearance was highly correlated with the apparent volume of distribution among the subjects, suggesting that the interindividual difference in bioavailability was largely responsible for the pharmacokinetic variability of carvedilol. The oral clearance and also volume of distribution of both enantiomers were significantly lower in the subjects with the *CYP2D6*10* allele than with the *CYP2D6*1/*1* or **1/*2* genotype. These results suggested that the systemic and/or pre-systemic metabolism of *R*- and *S*-carvedilol in the liver is significantly decreased in Japanese with the *CYP2D6*10* allele.

Key words carvedilol; *CYP2D6*10*; pharmacokinetics; Bayesian analysis

Carvedilol is a β-adrenoceptor antagonist, and has been clinically used to treat chronic heart failure as well as hypertension, angina pectoris, and cardiac arrhythmias.[1—4] Carvedilol is highly lipophilic and eliminated predominantly by hepatic metabolism, with renal excretion accounting for only 0.3% of the administered dose.[5] The drug is absorbed rapidly from the gastrointestinal tract after oral administration; however, the amount of unchanged drug excreted in the feces was 23% of the administered dose probably because of incomplete intestinal absorption.[6] In addition, orally administered carvedilol undergoes stereoselective first-pass metabolism, and the maximal plasma concentration of *R*-enantiomer with low β-blocking activity is approximately 2-fold higher than that of *S*-enantiomer with high β-blocking activity.[6] The mean absolute bioavailability of *R*- and *S*-enantiomer in humans is 31% and 15%, respectively.[7]

It was reported that CYP2D6 in microsomes derived from lymphoblastoid cells with human cDNA shows strong enzyme activity for the metabolism of *R*- and *S*-carvedilol.[8] Poor metabolism through CYP2D6 was found in 7% of Caucasian subjects, and two common defective alleles responsible for the poor metabolism are *CYP2D6*4* and **5*.[9] The gene frequency of individual variants of CYP2D6 shows a marked interethnic difference, and poor metabolism is found in less than 1% of Asian subjects.[9] 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 enzyme activity, whereas the *CYP2D6*10* allele causes the low expression and affinity of CYP2D6.[10,11]

In the present study, we estimated the pharmacokinetic parameters of *R*- and *S*-carvedilol in 23 healthy Japanese volunteers by the Bayesian method using a nonlinear mixed effects model (NONMEM) program. We then examined the effect of the CYP2D6 polymorphisms on the stereoselective pharmacokinetics of carvedilol.

MATERIALS AND METHODS

Subjects and Study Protocols Twenty-three healthy Japanese volunteers (19 men and 4 women) participated in this study. The age was between 22 and 44 years old (mean: 29.1), and the body weight was between 47 and 86 kg (mean: 64.7). They were given 5 mg (two 2.5 mg-tablets: 7 men and 2 women) or 10 mg (one 10 mg-tablet: 12 men and 2 women) of carvedilol (Artist® tablet; Daiichi Pharmaceutical Co. Ltd., Tokyo, Japan) at least 2 h before a meal, because the peak blood drug concentrations are attained at 0.5—1 h after oral administration following an over night fast.[12] Carvedilol was taken with a glass of water, and 5 ml of blood was taken at 2 and 6 h after dosing. All the subjects were physicians or pharmacists, and they chose the dose of carvedilol by themselves. The mean ($\pm$S.D.) body weight in the subjects taking 5 mg and 10 mg carvedilol was 57.9 ± 6.7 kg and 69.1 ± 9.9 kg, respectively. They all gave written consent to participate in this study, which was approved by the ethics committee of Toyama Medical and Pharmaceutical University.

Genotyping of CYP2D6 Genomic DNA was isolated from the peripheral blood with an extraction kit (Qiagen, Hilden, Germany), and was stored at -80 °C. *CYP2D6*1*, **10*, and **14* were determined by the PCR-RFLP method, whereas the *CYP2D6*2* allele was detected by the allele-specific PCR method.[13] In addition, the detection of *CYP2D6*5* was carried out using the two long-PCR methods reported by Steen *et al.*[14] and Johansson *et al.*[15]

Assay of Carvedilol The blood concentration of carvedilol was measured using chiral high performance liquid chromatography (HPLC) as described by Mihara *et al.* with minor modification.[16] Briefly, whole blood samples (0.2 ml) were mixed with 0.5 ml of distilled water to hemolyze the blood cells. After alkalinization with 3 ml of 0.1 M Britton–Robinson buffer (pH 8), the samples were extracted with 5 ml of diethylether and back-extracted from the organic

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phase with 0.3 ml of 50 mM H_2SO_4. The organic layer was discarded, and the remaining aqueous phase was alkalinized with 3 ml of 0.1 M Britton–Robinson buffer (pH 8). This mixture was then re-extracted with 5 ml of diethylether. The organic phase was evaporated to dryness in a water bath at 45 °C. The residue was dissolved in 500 μl of mobile phase, and 50 μl was injected onto the column. The HPLC system consisted of a Shimadzu LC-10AS (Kyoto, Japan). The separation was achieved with a chiral stationary phase column (CHIRALPAC AD-H: 5 μm particle size, 2 mm i.d.×25 cm.; Daicel Chemical Industries, Tokyo, Japan). The temperature of the column oven was set at 40 °C. The mobile phase consisted of 75% hexane, 25% isopropanol, and 0.1% (v/v) diethylamine, and the flow rate was 0.3 ml/min. The peaks were monitored at an excitation wavelength of 284 nm and an emission wavelength of 343 nm (Shimadzu RF-10A; Kyoto, Japan). The whole blood carvedilol concentration in each sample was estimated to be the mean value of duplicate measurements. The coefficient of variation for the assay of *R*-carvedilol was 2.7% and 4.1% at whole blood concentrations of 0.5 ng/ml and 3 ng/ml, respectively. The coefficient of variation for the assay of *S*-carvedilol was 2.3% and 3.0% at whole blood concentrations of 0.5 ng/ml and 3 ng/ml, respectively. The detection limit for each enantiomer was 0.2 ng/ml for its blood concentration.

Estimation of Pharmacokinetic Parameters of Carvedilol Mean pharmacokinetic parameters and their interindividual variations were estimated using the NONMEM analysis.[11,17] The pharmacokinetic parameters in individual subjects were obtained from the population estimates according to Bayes' theorem using the NONMEM *post-hoc* option. The one-compartment model with very rapid absorption was parameterized in terms of the oral clearance (CL/F) and the apparent volume of distribution (V/F), with NONMEM-PREDPP library subroutines, ADVAN1 and TRANS2.[11,17] The oral clearance in the ith individual (CL/F_i) was modeled using the following equation:

$$CL/F_i = \theta_1 \cdot \theta_3^S \cdot WT_i \cdot (1 + \eta_{CL/F_i}) \quad (1)$$

where θ_1 is the predicted population mean of the oral clearance, and θ_3 is the S/R ratio for CL/F. The S value is fixed to one for *S*-carvedilol, and to zero for *R*-carvedilol. WT_i is the individual body weight, and η_{CL/F_i} is a random variable distributed with a mean of zero and variance of $\omega^2_{CL/F}$. The apparent volume of distribution in the ith individual (V/F_i) was modeled using the following equation:

$$V/F_i = \theta_2 \cdot \theta_4^S \cdot WT_i \cdot (1 + \eta_{V/F_i}) \quad (2)$$

where θ_2 is the predicted population mean of the apparent volume of distribution, θ_4 is the S/R ratio for V/F, and η_{V/F_i} is a random variable distributed with a mean of zero and variance of $\omega^2_{V/F}$. In the present study, we assumed that η_{CL/F_i} is correlated with η_{V/F_i}, and that the covariance is $\omega_{CL/F,V/F}$. Finally, the jth observed blood concentration in the ith subject (Cb_{ij}) was assumed to be randomly and normally distributed from the predicted value (Cb^*_{ij}):

$$Cb_{ij} = Cb^*_{ij} + Cb^{*1/2}_{ij} \cdot \varepsilon_{ij} \quad (3)$$

where ε_{ij} is a random variable that describes intraindividual variability with a mean of zero and variance of σ^2.

Statistical Analysis Values were expressed as the mean ±S.D. The statistical significance of the difference between mean values was calculated using an unpaired t-test. A p value of less than 0.05 was considered to be significantly different.

RESULTS

The whole blood concentrations of *R*- and *S*-carvedilol enantiomers in 23 healthy volunteers are shown in Fig. 1. The blood concentration of *R*-carvedilol was higher than that of *S*-carvedilol in all 23 subjects. Table 1 shows the pharmacokinetic parameters of carvedilol estimated by the NONMEM analysis. The θ_1 and θ_2 values of *R*-carvedilol were estimated to be 1.01 l/h/kg and 2.53 l/kg, respectively. The θ_3 and θ_4 values that indicate the S/R ratio for CL/F and V/F were estimated to be 2.13 and 2.94, respectively. 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. The obtained 95% CI values for θ_3 and θ_4 indicated that the CL/F and V/F values for *S*-carvedilol were significantly greater than those for *R*-carvedilol (Table 1). The $\omega^2_{CL/F}$ value was estimated to be 0.130, which indicated that the coefficient of variation of CL/F was 36.1%. Similarly, the estimated $\omega^2_{V/F}$ value (0.161) indicated that the coefficient of variation of V/F was 40.1%. The correlation coefficient value between CL/F and V/F ($\omega_{CL/F,V/F}/\omega_{CL/F}/\omega_{V/F}$) was estimated to be very high (0.899). Moreover, Fig. 2 shows the relation between the individual

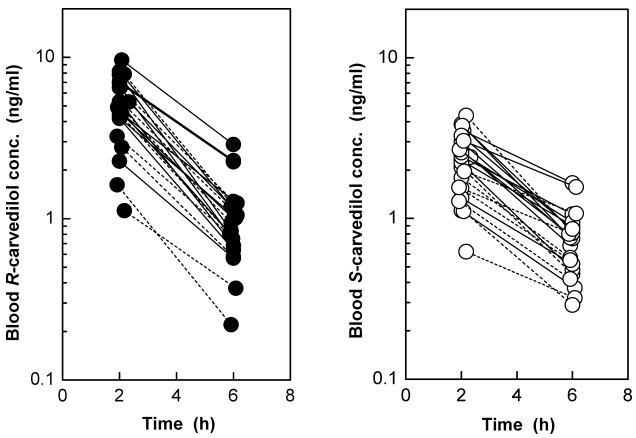

Fig. 1. Individual Blood Concentrations of *R*- and *S*-Carvedilol after Oral Administration to 23 Healthy Volunteers at a Dose of 5 mg (Dotted Line) or 10 mg (Solid Line)

Table 1. Pharmacokinetic Parameters of *R*- and *S*-Carvedilol in 23 Healthy Japanese

Parameters	Estimates	95% CI
θ_1 (l/h/kg)	1.01	0.84—1.18
θ_2 (l/kg)	2.53	2.04—3.02
θ_3	2.13	1.64—2.62
θ_4	2.94	1.98—3.90
$\omega^2_{CL/F}$	0.130	0.073—0.187
$\omega^2_{V/F}$	0.161	0.054—0.268
$\omega_{CL/F,V/F}$	0.130	0.058—0.202
σ^2	0.0584	0.0074—0.1094

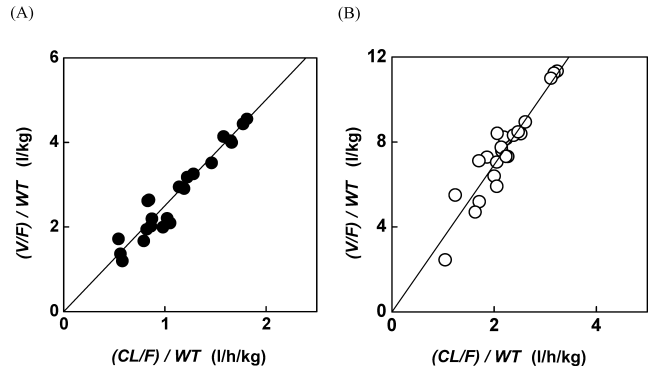

Fig. 2. Correlation between (*CL*/*F*)/*WT* and (*V*/*F*)/*WT* of *R*-Carvedilol (A) and *S*-Carvedilol (B)

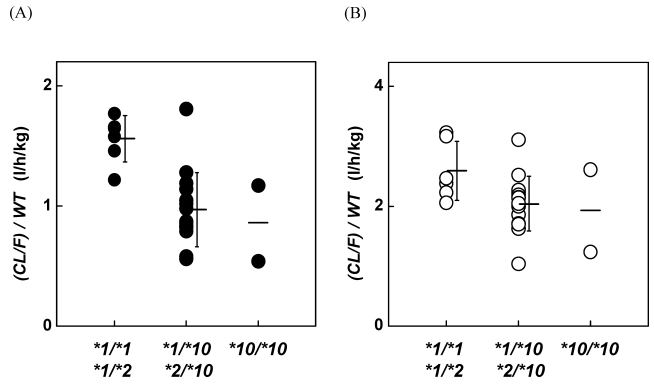

Fig. 3. Effect of *CYP2D6*10* on the (*CL*/*F*)/*WT* Values of *R*-Carvedilol (A) and *S*-Carvedilol (B)

Horizontal and vertical bars represent the mean and S.D., respectively.

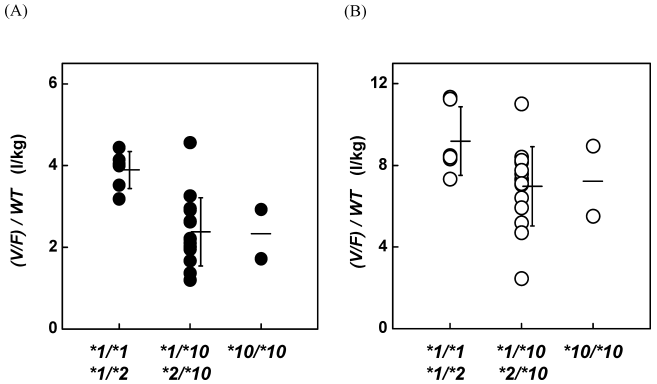

Fig. 4. Effect of *CYP2D6*10* on the (*V*/*F*)/*WT* Values of *R*-Carvedilol (A) and *S*-Carvedilol (B)

Horizontal and vertical bars represent the mean and S.D., respectively.

weight-corrected oral clearance ((*CL*/*F*)/*WT*) values and the individual weight-corrected apparent distribution volume ((*V*/*F*)/*WT*) values, which were obtained from the population estimates according to Bayes' theorem using the NONMEM *post-hoc* option. (*V*/*F*)/*WT* of both enantiomers was highly correlated with (*CL*/*F*)/*WT* (Fig. 2). These findings suggested that the interindividual difference of bioavailability (*F*) was largely responsible for the pharmacokinetic variability of carvedilol.

Figure 3 shows the effect of CYP2D6 genotypes on the individual (*CL*/*F*)/*WT* values. No subject had the null alleles of CYP2D6 (*CYP2D6*5* and **14*). The five subjects were homozygous for the *CYP2D6*1* allele, one subject was heterozygous for the *CYP2D6*1/*2* alleles, 12 subjects were heterozygous for the *CYP2D6*1/*10* alleles, three subjects were heterozygous for the *CYP2D6*2/*10* alleles, and two subjects were homozygous for the *CYP2D6*10* allele. The mean (*CL*/*F*)/*WT* value of *R*-carvedilol in the subjects with at least one *CYP2D6*10* allele (1.0±0.3 l/h/kg) was significantly ($p<0.001$) lower than that in the subjects with *CYP2D6*1/*1* or **1/*2* (1.6±0.2 l/h/kg). The mean (*CL*/*F*)/*WT* value of *S*-carvedilol in subjects with at least one *CYP2D6*10* allele (2.0±0.5 l/h/kg) was also significantly ($p<0.05$) lower than that in the subjects with *CYP2D6*1/*1* or **1/*2* (2.6±0.5 l/h/kg). In addition, Fig. 4 shows the effect of CYP2D6 genotypes on the individual (*V*/*F*)/*WT* values. The (*V*/*F*)/*WT* value of *R*- and *S*-carvedilol in the subjects with at least one *CYP2D6*10* allele was also significantly ($p<0.05$) lower than that in the subjects with *CYP2D6*1/*1* or **1/*2*. That is, the (*V*/*F*)/*WT* value of *R*-carvedilol in the subjects with *CYP2D6*1/*1* or **1/*2* was 3.9±0.5 l/kg, while that in the subjects with *CYP2D6*10* was 2.4±0.8 l/kg. The (*V*/*F*)/*WT* value of *S*-carvedilol in subjects with *CYP2D6*1/*1* or **1/*2* was 9.2±1.7 l/kg, while that in the subjects with *CYP2D6*10* was 7.0±1.9 l/kg. These findings suggested that the systemic clearance (*CL*) and/or the bioavailability (*F*) of *R*- and *S*-carvedilol is significantly altered in Japanese with the *CYP2D6*10* allele.

DISCUSSION

In the present study, we investigated the effect of genetic polymorphisms of CYP2D6 on the pharmacokinetics of *R*- and *S*-carvedilol in healthy Japanese volunteers. The (*CL*/*F*)/*WT* value was highly correlated with the (*V*/*F*)/*WT* value among the subjects (Table 1, Fig. 2), suggesting that the interindividual difference in bioavailability (*F*) was largely responsible for the pharmacokinetic variability of carvedilol. The (*CL*/*F*)/*WT* and (*V*/*F*)/*WT* values of *R*- and *S*-carvedilol were significantly lower in healthy volunteers with at least one *CYP2D6*10* allele than those with the *CYP2D6*1/*1* and **1/*2* genotype (Figs. 3, 4). The result suggested that the systemic and/or pre-systemic metabolism of *R*- and *S*-carvedilol in the liver is significantly decreased in Japanese subjects with the *CYP2D6*10* allele.

Fujimaki *et al*. investigated the mechanisms responsible for the stereoselective pharmacokinetics of carvedilol in rats and humans.[6,18] In the rat, the mean absolute bioavailability of *S*-enantiomer (13%) was also lower than that of *R*-enantiomer (22%). The unbound fraction of *S*-carvedilol in blood was 1.65-fold greater than that of *R*-carvedilol; however, there was no difference in intrinsic clearance for the unbound drug in the liver between the *R*- and *S*-enantiomer. The finding indicated that the difference in bioavailability after oral administration to rats between two enantiomers is ascribed to the lower plasma protein binding of *S*-carvedilol.[18] In humans, the unbound fraction of *S*-carvedilol in blood (0.47%) is also greater than that of *R*-carvedilol (0.30%).[6] On the other hand, Fujimaki *et al*. reported that the ratio of hepatic intrinsic clearance for the *R*-enantiomer to the *S*-enantiomer was estimated to be 0.46, assuming that the liver was the principal site of pre-systemic and systemic metabolism of the

absorbed drug from the intestine.[6] They ascribed the difference in bioavailability between the two enantiomers in humans to less plasma protein binding of the *S*-enantiomer, and to the greater intrinsic clearance of the *S*-enantiomer than *R*-enantiomer in the liver.[6] It has been reported that carvedilol is metabolized extensively *via* aromatic ring oxidation, aliphatic side-chain oxidation, and conjugation pathways.[6] The *R*-carvedilol glucronide concentration in plasma is approximately 2.5-fold higher than the *S*-carvedilol glucronide concentration in plasma.[19] Therefore, Fujimaki *et al*. suggested that the conjugation reaction with glucuronic acid may not be responsible for the higher plasma concentration of *R*-carvedilol after oral administration of the racemic drug.[19] On the other hand, at present, it is unclear whether any oxidative pathway relates to the stereoselective metabolism of carvedilol.[6,12] Zhou *et al*. reported that the mean *CL*/*F* value of *R*-carvedilol is 67% lower in poor metabolizers than in extensive metabolizers, and that the mean *CL*/*F* value of *S*-carvedilol in the poor metabolizers is 24% lower than that in the extensive metabolizers, suggesting that CYP2D6 is more responsible for the metabolism of *R*-carvedilol.[12] The present findings that the *CYP2D6*10* allele produced a greater change in (*CL*/*F*)/*WT* and (*V*/*F*)/*WT* for *R*-carvedilol as compared with that for *S*-carvedilol (Figs. 3, 4) are in line with those reported by Zhou *et al*.

Oldham *et al*. reported that considerable metabolic activity for carvedilol is observed in a CYP2D6 poor metabolizer liver, and that CYP3A4 may be involved in the production of metabolites of the drug.[8] More than 30 single nucleotide polymorphisms have been identified in the CYP3A4 gene.[20] For the most common variant *CYP3A4*1B*, increased transcription was demonstrated *in vitro*, which may theoretically result in higher enzymatic activity *in vivo*.[21,22] However, the allele frequency ranges from 0% (Chinese and Japanese) to 45% (African-Americans).[20] We, therefore, could not investigate the effect of the polymorphism of CYP3A4 on the stereoselective disposition of carvedilol in Japanese subjects. On the other hand, many drugs undergo substantial metabolism in the intestine as well as the liver, after absorption from the gut lumen.[21] CYP3A4 and P-glycoprotein are jointly expressed in the intestine, and it is believed that the low oral bioavailability of some drugs results largely from the actions of these two enzymes.[23] At present, however, it is unclear whether carvedilol is significantly metabolized in the intestinal tissue, and whether the intestinal metabolism of the drug is stereoselective for the *S*-enantiomer. On the other hand, Giessmann *et al*. reported that 36.8% of the dose (unchanged carvedilol, 13.4%; its oxidative metabolites, 23.4%) is excreted into the feces following intravenous administration of 5 mg of carvedilol to healthy subjects, and that the cumulative fecal excretion of carvedilol after the intravenous dose is highly correlated with the mRNA expression of P-glycoprotein and multidrug resistance protein 2.[24] It should be noted that these intestinal drug transporters may play a significant role in the intestinal ab(ex)sorption and/or fecal excretion of carvedilol following oral administration.[6,24]

In conclusion, though poor metabolism through CYP2D6 is found in only less than 1% of Asians, the bioavailability of carvedilol is significantly increased in Japanese subjects with a common allele, *CYP2D6*10*. The finding may provide new insight into the interindividual variability of bioavailability and the interethnic difference in the pharmacokinetics of carvedilol. Further studies are needed to clarify whether the optimum dose of carvedilol in Japanese patients with the *CYP2D6*10* allele is different from that in Japanese and/or Caucasian extensive metabolizers.

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