Pharmacokinetics of *R*- and *S*-Carvedilol in Routinely Treated Japanese Patients with Heart Failure

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The purpose of this study was to evaluate the pharmacokinetics of *R*- and *S*-carvedilol in routinely treated Japanese patients with heart failure. We measured peak and trough blood concentrations at steady state following repeated oral administration to 24 patients. The blood concentration of *S*-carvedilol with potent β -blocking activity was lower than that of *R*-carvedilol. The mean oral clearance (CL/F) of *R*- and *S*-carvedilol was not altered by *CYP2D610, *UGT2B7**3, and the etiology of heart failure. In addition, the CL/F values of enantiomers were not correlated with age, creatinine clearance, and plasma concentrations of α_1 -acid glycoprotein and brain natriuretic peptide. On the other hand, the mean CL/F values of *R*- and *S*-carvedilol in patients with heart failure were 0.89 and 1.52 l/h/kg, respectively, considerably lower than those estimated previously in healthy subjects. These results suggested that the pharmacokinetics of *R*- and *S*-carvedilol was altered significantly by heart failure.**

Key words carvedilol; pharmacokinetics; heart failure; *CYP2D6**10

Carvedilol is a β -adrenoceptor antagonist, clinically used to treat chronic heart failure as well as hypertension, angina pectoris, and cardiac arrhythmias.¹⁾ 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.²⁾ Carvedilol is metabolized extensively *via* aliphatic side-chain oxidation, aromatic ring oxidation, and conjugation pathways.³⁾ Oldham and Clarke⁴⁾ reported that oxidative activity for carvedilol is observed in cytochrome P450 (*CYP*) 2D6, 2C9, 3A4, and 1A2. In addition, Ohno *et al.*⁵⁾ reported that UDP-glucuronosyltransferase (*UGT*) 2B7, 2B4, and 1A1 are capable of catalyzing the glucuronidation of carvedilol. In the previous study, we examined the effect of *CYP2D6**10, *CYP2C9**3, *CYP2C19**2, *CYP2C19**3, *CYP3A5**3, *UGT2B7**2, *UGT2B7**3, and the *C3435T* mutation of *MDR1* on the pharmacokinetics of carvedilol in 54 Japanese volunteers.^{2,6)} The oral clearance (CL/F) and also volume of distribution (V/F) of *R*- and *S*-carvedilol were significantly lower in subjects with the *CYP2D6**10 allele than those with *CYP2D6**1/*1, *1/*2, or *2/*2 genotype, indicating that the systemic clearance (CL) and/or bioavailability (F) of both enantiomers is significantly altered in Japanese with the *CYP2D6**10 allele. On the other hand, *CYP2C9**3, *CYP2C19**2, *CYP2C19**3, *CYP3A5**3, *UGT2B7**2, *UGT2B7**3, and the *C3435T* mutation of *MDR1* did not affect the pharmacokinetics of *R*- and *S*-carvedilol in healthy Japanese.^{2,6)}

Several pharmacokinetic studies have suggested that hepatic elimination of certain drugs *via* oxidative metabolism is impaired in patients with heart failure (HF)^{7–13)}; that is, the CL/F value of prazosin after oral administration in HF patients was 46% of that in healthy subjects.⁷⁾ It was also reported that the CL/F value of aminopyrine after oral administration in HF patients in the aminopyrine breath test was 24% of that in control subjects.⁸⁾ In addition, CL values of midazolam and quinidine (*CYP3A4* substrates) after intravenous

administration were decreased by 32% and 33% in HF patients, respectively.^{9,10)} The CL value of theophylline (*CYP1A2* substrate) after intravenous administration was markedly decreased in HF patients.^{11,12)} Recently, population pharmacokinetic analysis has revealed that the CL/F value of mexiletine, which is mainly metabolized by *CYP1A2* and *CYP2D6*, is reduced significantly in HF patients as compared with non-HF patients.¹³⁾ However, it is still unclear whether the pharmacokinetics of *R*- and/or *S*-carvedilol is altered by HF. In the present study, therefore, we investigated the pharmacokinetics of *R*- and *S*-carvedilol in routinely treated Japanese patients with HF.

MATERIALS AND METHODS

Subjects and Study Protocol Twenty-four Japanese patients with HF (16 men and 8 women) participated in this study. Their age was between 45 and 91 years old (70.5 ± 11.3 years), and their body weight was between 36 and 77 kg (58.0 ± 12.0 kg). Fourteen patients had ischemic heart disease, 8 patients had myopathic heart disease, and 2 patients had hypertensive heart disease. No patients had severe hepatic disease. These patients were routinely treated with oral administration of carvedilol (Artist® Tablet, Dai-ichisankyo Co., Ltd., Tokyo, Japan) at doses between 1.25 and 20 mg/d, and the drug was administered once a day in 22 patients and twice a day in 2 patients. Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers were concomitantly administered to 11 and 6 patients, respectively. No patients received any potent inhibitor of *CYP2D6* (e.g. quinidine) concomitantly. Two blood samples were withdrawn from each patient at steady state following repeated dosing for more than one week; that is, blood samples were obtained just before the dose corresponding to trough concentration (C_{trough}), and at 1.9–3.6 h after the dose corresponding to peak concentration (C_{peak}). Two blood samples for C_{trough} and C_{peak} of 20 patients (1 outpatient, 19 inpatients) were withdrawn on the same day, and those of 4 pa-

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tients (3 outpatients, 1 inpatient) were withdrawn on the separate days. All patients gave written informed consent to participate in this study, which was approved by the ethics committee of University of Toyama.

Genotyping of CYP2D6 and UGT2B7 Genomic DNA was isolated from the peripheral blood with an extraction kit (Qiagen, Hilden, Germany), and was stored at -80°C until use. Genotypes of CYP2D6 and UGT2B7 were determined as described previously.^{2,6)} Briefly, *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. *CYP2D6**5 was detected using the two long-PCR methods reported by Steen *et al.* and Johansson *et al.*^{14,15)} In addition, *UGT2B7**1, *2, and *3 were determined by direct sequencing.

Clinical Laboratory Test Plasma α_1 -acid glycoprotein (AGP) concentration was measured using an immunodiffusion kit (Dade Behring Co., Ltd., Tokyo, Japan). In addition, the plasma concentrations of brain natriuretic peptide (BNP) in 17 patients were measured in a hospital laboratory using chemiluminescence enzyme immunoassay. Plasma creatinine concentration was measured with a kit based on the improved Jaffé methods (Wako Pure Chemical Industries, Osaka, Japan). The creatinine clearance (*CLcr*) value (in l/h) was calculated using the Cockcroft–Gault equation as follows¹⁶⁾:

$$CLcr = \frac{(140 - AGE) \cdot WT}{72 \cdot Scr} \cdot 0.85^{SEX} \cdot \frac{60}{1000} \quad (1)$$

where *AGE* is age, *WT* is body weight, *Scr* is serum (plasma) creatinine concentration (in mg/dl), and *SEX* is one for females and zero for males.

Assay of Carvedilol The blood concentration of carvedilol was measured using chiral high performance liquid chromatography (HPLC) as described by Saito *et al.* with minor modifications.^{2,17)} Briefly, whole blood samples (0.2 ml) were mixed with 0.5 ml of distilled water to hemolyze blood cells. After alkalization with 3 ml of 0.1 M Britton–Robinson buffer (pH 8.5), the samples were extracted with 5 ml of diethylether and back-extracted from the organic phase with 0.3 ml of 50 mM H_2SO_4 . The organic layer was discarded, and the remaining aqueous phase was alkalized with 3 ml of 0.1 M Britton–Robinson buffer (pH 8.5). 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 300 μl of mobile phase, and 70 μl was injected onto the column. The HPLC system consisted of Shimadzu LC-10AS (Kyoto, Japan) and Shimadzu RF-10A (Kyoto, Japan). Separation was achieved with a chiral stationary phase column (CHIRALPAC AD-H: 5 μm particle size, 2 mm i.d. $\times$ 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. The whole blood carvedilol concentration in each sample was estimated to be the mean value of duplicate measurements. The coefficient of intra-day 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 intra-day 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 of each enantiomer was 0.05 ng/ml for blood concentration.

Estimation of Oral Clearance in Individual Subjects *CL/F* values of *R*- and *S*-carvedilol in individual 24 patients were estimated by two simple analysis methods, as reported previously.^{18,19)} The approximate *CL/F* (*CL/F*_{approx}) value was estimated by the following equation¹⁸⁾:

$$CL/F_{\text{approx}} = \frac{2 \cdot D / \tau}{C_{\text{peak}} + C_{\text{trough}}} \quad (2)$$

where *D* is the dose, and τ is the dosing interval. On the other hand, the alternative oral clearance value (*CL/F*_{exp}) was estimated by the simple monoexponential model as follows¹⁹⁾:

$$CL/F_{\text{exp}} = \frac{D \cdot \lambda}{C_{\text{peak}} / \exp(-\lambda \cdot t_{\text{max}}) - C_{\text{trough}}} \quad (3)$$

where t_{max} is the sampling time for C_{peak} , and λ is the rate constant of a monoexponential decline, described as follows:

$$\lambda = \frac{\ln(C_{\text{peak}} / C_{\text{trough}})}{\tau - t_{\text{max}}} \quad (4)$$

Statistical Analysis Values are expressed as the mean $\pm$ S.D. Multiple comparisons were performed using Scheffé's test following one-way ANOVA, provided that the variances of groups were similar. If this was not the case, Scheffé-type test was applied following Kruskal–Wallis analysis. $p < 0.05$ was considered statistically significant.

RESULTS

Two blood samples were collected from 24 patients with HF at steady state following once-daily (22 patients) or twice-daily (2 patients) repeated dosing. Blood was sampled just before the dose corresponding to C_{trough} , and at 1.9–3.6 h after the dose corresponding to C_{peak} . Measured blood concentrations of *R*- and *S*-carvedilol enantiomers in 24 patients are shown in Fig. 1A, whereas those divided by weight-corrected dose are shown in Fig. 1B. The peak blood concentration of *R*-carvedilol was higher than that of *S*-carvedilol in all 24 patients. We calculated the *CL/F*_{approx} and *CL/F*_{exp} values of carvedilol in individual patients using Eqs. 2–4. Figure 2 shows the relationship between *CL/F*_{approx} and *CL/F*_{exp} in the 24 patients. *CL/F*_{exp} values were only slightly higher than *CL/F*_{approx} values, and *CL/F*_{approx} correlated fairly well with *CL/F*_{exp}. In subsequent analysis, therefore, we used the mean values of *CL/F*_{approx} and *CL/F*_{exp} as representative *CL/F* values in the 24 patients.

To evaluate the effect of *CYP2D6**10 on individual *CL/F* values of *R*- and *S*-carvedilol, the patients were classified into three groups (G1, G2, G3) on the basis of the *CYP2D6* genotype (Fig. 3A): eight patients were classified into G1: six patients were homozygous for the *CYP2D6**1 allele, one was heterozygous for the *CYP2D6**1/*2 alleles, and one was homozygous for the *CYP2D6**2 allele; twelve patients were classified into G2: nine patients were heterozygous for the *CYP2D6**1/*10 alleles, two were heterozygous for the *CYP2D6**2/*10 alleles, one was heterozygous for the *CYP2D6**1/*5 alleles; four patients were classified into G3:

Fig. 1. Measured Blood Concentrations (A) and Dose-Corrected Blood Concentrations (B) of R- and S-Carvedilol in 24 Patients

Closed and open circles indicate R- and S-carvedilol, respectively.

Fig. 2. Relationship between CL/F_{approx} and CL/F_{exp} of Carvedilol

Closed and open circles indicate R- and S-carvedilol, respectively.

three patients were homozygous for the *CYP2D6**10 allele, and one was heterozygous for the *CYP2D6**5/*10 alleles. As a result, the mean CL/F values of both enantiomers in G2 and G3 were similar to those in G1 (Fig. 3A). In addition, three patients were heterozygous for the *UGT2B7**3 allele. No significant decrease of the CL/F values of patients with *UGT2B7**3 was observed.

We also evaluated the effect of HF etiology and aging on the individual CL/F values of R- and S-carvedilol (Figs. 3B, C). The etiology of HF in the 24 patients was ischemic (8 patients), myopathic (14 patients), and hypertensive heart disease (2 patients). No significant difference of the CL/F values of R- and S-carvedilol among the three HF etiologies was observed (Fig. 3B). On the other hand, patient age in the present study ranged from 45 to 91 years old (Fig. 3C). The

Fig. 3. Effect of CYP2D6/UGT2B7 Genotypes (A), HF Etiology (B), and Age (C) on the CL/F of R- and S-Carvedilol

Closed and open symbols indicate R- and S-carvedilol, respectively. G1: *CYP2D6**1/*1, *CYP2D6**1/*2, or *CYP2D6**2/*2; G2: *CYP2D6**1/*5, *CYP2D6**1/*10, or *CYP2D6**2/*10; G3: *CYP2D6**5/*10 or *CYP2D6**10/*10. Circles indicate patients without *UGT2B7**3, and diamonds indicate patients with *UGT2B7**3. Horizontal and vertical bars represent the mean and S.D., respectively.

effect of age on the CL/F values of R- and S-carvedilol was not significant. We further evaluated the effect of CL_{cr} and plasma concentrations of AGP and BNP on CL/F values in individual HF patients (Fig. 4). The CL_{cr} values in 24 patients ranged from 29 to 102 ml/h/kg. Plasma concentrations of AGP in 24 patients ranged from 45 to 155 mg/dl. Plasma concentrations of BNP were measured in 17 patients, and ranged from 4 to 977 pg/dl. The effect of CL_{cr} , AGP, and BNP on the CL/F values of R- and S-carvedilol was not significant (Fig. 4).

We have previously reported that CL/F values of R- and S-carvedilol were significantly lower in healthy Japanese subjects with the *CYP2D6**10 allele than those with the *CYP2D6**1/*1, *1/*2, or *2/*2 genotype, indicating that the CL and/or F values of both enantiomers are significantly altered in subjects with the *CYP2D6**10 allele.²⁾ The present finding that there was no significant effect of *CYP2D6**10 on CL/F of R- and S-carvedilol in HF patients (Fig. 3A) suggested that the metabolic activity of CYP2D6 was diminished by cardiac dysfunction and/or subsequent hypoxemia. Figure 5 compares the CL/F values of R- and S-carvedilol in HF patients with those in healthy subjects. The mean CL/F values of R- and S-carvedilol in HF patients were only 29.0% and 25.2%, respectively, of those in healthy volunteers without *CYP2D6**10 allele (G1). Furthermore, mean CL/F values

Fig. 4. Effect of CLcr (A), AGP (B), and BNP (C) on the CL/F of R- and S-Carvedilol

Closed and open circles indicate R- and S-carvedilol, respectively.

Fig. 5. CL/F of R- and S-Carvedilol in Healthy Volunteers and Heart Failure Patients

Closed and open columns indicate R- and S-carvedilol, respectively. G1: CYP2D6*1/*1, CYP2D6*1/*2, or CYP2D6*2/*2; G2: CYP2D6*1/*10 or CYP2D6*2/*10; G3: CYP2D6*10/*10. HF: heart failure patients.

of R- and S-carvedilol in HF patients were significantly lower than those in healthy subjects homozygous for the CYP2D6*10 allele (G3). The findings suggested that not only CYP2D6 activity but also the activities of other carvedilol-metabolizing enzymes were decreased in HF patients.

DISCUSSION

The purpose of the present study was to evaluate the pharmacokinetics of R- and S-carvedilol in routinely treated

Japanese HF patients. Previously, we performed a simulation study of a clinical repeated-dose pharmacokinetic trial, applying a peak-and-trough sampling design to estimate CL/F , and reported that CL/F_{approx} is accurate for drugs with an elimination half-life comparative to or longer than the dosing interval, and that CL/F_{exp} is accurate for drugs with a short elimination half-life relative to the dosing interval.^{18,19} In the present study, therefore, we analyzed peak and trough blood concentration data at steady state following repetitive oral administration to 24 patients with HF. As a result, CL/F_{approx} of R- and S-carvedilol was very close to CL/F_{exp} (Fig. 2). We therefore used the mean value between CL/F_{approx} and CL/F_{exp} as the representative CL/F value in the 24 patients. CL/F values of R- and S-carvedilol were not altered by CYP2D6*10, UGT2B7*3, and the etiology of HF (Fig. 3). In addition, CL/F values of enantiomers were not correlated with age, CLcr, AGP, and BNP (Figs. 3, 4); however, the mean CL/F values of R- and S-carvedilol in HF patients were considerably lower than those in healthy subjects with CYP2D6*10/*10 (Fig. 5), suggesting that not only CYP2D6 activity but also the activities of other carvedilol-metabolizing enzymes were decreased in HF patients.

Several reports have suggested that the pharmacokinetic change associated with HF and/or subsequent hypoxemia was ascribed to diminished activity of drug-metabolizing enzymes, including CYP and UGT.^{20–22} Lambert *et al.*²⁰ reported that the total hepatic CYP content declined 41% in dogs with congestive HF. Fradette *et al.*²¹ evaluated the effect of hypoxemia on P450 expression in rabbit hepatocytes, and reported that the expression of CYP1A1 and 1A2 in rabbits with hypoxia was 63% and 60%, respectively, of that in control rabbits. They also reported that serum mediators such as interferon- α , interleukin (IL)-1 β , and IL-2 contributed to the change in the expression of several CYP enzymes.²¹ In addition, Monshouwer *et al.*²² reported that not only CYP but also UGT activity of pig hepatocytes was attenuated by recombinant human IL-1 α , IL-6, and tumor necrosis factor- α .

It is possible, however, that impaired presystemic and systemic hepatic drug clearance in patients with HF is not primarily accounted for by changes in the hepatic content and activity of drug-metabolizing enzymes.^{23,24} Ng *et al.* investigated the effect of HF on propranolol elimination in rats with right ventricular failure (RVF) induced by pulmonary artery constriction²³; that is, they compared propranolol clearance in isolated perfused livers from RVF rats with that from control rats, and demonstrated a 97% reduction in propranolol intrinsic clearance in RVF livers.²³ In RVF livers, total hepatic CYP expression was reduced by 19% as compared with controls, whereas cytochrome P450 isoenzymes 1A1/2 and 2D1 were reduced by 41 and 26%, respectively. Despite the 97% reduction in propranolol intrinsic clearance in perfused RVF liver, intrinsic clearance in microsomal preparations from the same livers was reduced by only 48% compared with controls.²³ Ng *et al.* also studied the effect of HF on hepatic elimination of *p*-nitrophenol (PNP) using isolated perfused livers from rats with RVF due to pulmonary artery constriction.²⁴ Hepatic clearance of the drug was found to be significantly impaired in RVF as compared with the control group. The impairment of PNP clearance in RVF occurred in parallel with a significant reduction in metabolic formation

clearance of *p*-nitrophenyl- β -D-glucuronide, the major metabolite of PNP; however, there was no significant difference between control and RVF groups in either the content or activity of UGT. Ng *et al.* concluded that RVF impairs hepatic elimination of PNP, and that this appears to be independent of changes in hepatic perfusion and oxygenation or alterations in hepatic content and activity of UGT.²⁴ They suspected that the reduced clearance of drugs in RVF is due to the depletion of carbohydrate reserves and cofactor supply within the liver. They also speculated that sinusoidal congestion in RVF livers leads to disturbed microcirculation and impaired access of drugs to functional hepatocytes.^{23,24}

Takekuma *et al.*²⁵ reported the population pharmacokinetic parameters of carvedilol in Japanese patients with cardiac diseases. The population analysis was performed using 373 plasma carvedilol concentrations from 41 patients with chronic HF or angina pectoris. A one compartment pharmacokinetic model with first-order absorption was used to describe the concentration–time data for carvedilol. They reported that the *CL/F* value in the patients who have intermediate activity of CYP2D6 was decreased by 39%. They also reported that *UGT2B7*3* decreased the *CL/F* value by 37%, but that *UGT2B7*2* did not show any effect.²⁵ However, it is possible that the pharmacokinetics of carvedilol in patients with angina pectoris is different from that in patients with chronic HF, but is rather similar to that in healthy subjects. Accordingly, the study design and/or population may be a crucial factor to investigate the effect of genotypes of drug metabolizing enzymes on the pharmacokinetics of carvedilol in patients with cardiac diseases. Further studies are needed to clarify the pharmacokinetics of *R*- and *S*-carvedilol in Japanese patients with angina pectoris.

In conclusion, the present study showed that *CYP2D6*10* was not a major factor affecting the *CL/F* value of *R*- and *S*-carvedilol in HF patients, and that the *CL/F* values of *R*- and *S*-carvedilol were considerably lower in patients with HF than in healthy subjects. Our findings suggested that metabolic clearance of carvedilol *via* not only CYP2D6 but also other metabolizing enzymes is diminished in patients with HF. The elimination of a wide range of drugs may be impaired in HF, increasing the risk of drug accumulation and toxicity.²⁴

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