Note

**UGT2B7*3 did not Affect the Pharmacokinetics of R- and S-Carvedilol in Healthy Japanese**

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**Summary:** We previously investigated the pharmacokinetics of R- and S-carvedilol in 54 healthy Japanese subjects, and reported that the oral clearance (CL/F) and apparent volume of distribution (V/F) of both enantiomers in subjects with the CYP2D6*10 allele were significantly lower than those in subjects without the CYP2D6*10 allele. In the present study, we examined the genotype of UGT2B7 in these 54 subjects, and investigated the effect of UGT2B7*3 on the pharmacokinetics of R- and S-carvedilol. Forty-three subjects did not have the UGT2B7*3 allele, and 11 subjects had one UGT2B7*3 allele. CL/F and V/F values of R- and S-carvedilol in the subjects with one UGT2B7*3 allele were similar to those without the UGT2B7*3 allele, indicating that the UGT2B7*3 allele did not significantly affect the systemic clearance (CL) and bioavailability (F) of the two enantiomers.

**Key words:** carvedilol; pharmacokinetics; healthy Japanese; UGT2B7*3

**Introduction**

Carvedilol is metabolized extensively via aliphatic side-chain oxidation, aromatic ring oxidation, and conjugation pathways. Oldham et al. reported that oxidative activity for carvedilol is observed in CYP2D6, 2C9, 3A4, and 1A2. In addition, Ohno et al. 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, and the C3435T mutation of MDR1 on the pharmacokinetics of carvedilol in 54 Japanese volunteers. The oral clearance (CL/F) and also apparent volume of distribution (V/F) of R- and S-carvedilol were significantly lower in subjects with the CYP2D6*10 allele than those with the 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, and the C3435T mutation of MDR1 did not significantly affect the pharmacokinetics of carvedilol in the Japanese subjects.

Recently, Takekuma et al. investigated the effect of the polymorphisms of CYP2D6 and UGT2B7 on oxidation and glucuronidation ability for carvedilol in 46 Japanese patients with chronic heart failure (New York Heart Association class II-III) or angina pectoris. They defined the metabolic index (MI) as follows: \[ MI = \frac{\text{AUC}_{\text{glucuronized}} \times C_{\text{cr}}}{\text{AUC}_{\text{unchanged}}} \] where AUC_{\text{glucuronized}} and AUC_{\text{unchanged}} are AUC of glucuronized carvedilol and AUC of unchanged carvedilol, respectively, and C_{\text{cr}} is creatinine clearance. Then, 40 patients were classified into low and high level MI groups. The gene frequency of CYP2D6*10 in the low level MI group was two times higher than that in the high level MI group, suggesting that the reduced catalytic activity by CYP2D6*10 leads to an increase in the unchanged carvedilol plasma concentration. In contrast, there was no significant difference between the low and high level MI groups in the allele frequency of UGT2B7*2. On the other hand, Takekuma et al. reported that the fre-
frequency of UGT2B7*3 in the low level MI group (26.1%) was significantly higher than that in the high level MI group (8.8%). This finding suggested that UGT2B7*3 might be responsible for the low level of glucuronidation activity for carvedilol, and that the genotyping of UGT2B7 might be useful and/or necessary for individualized therapy for chronic heart failure with carvedilol. However, because they did not measure plasma R- and S-carvedilol concentration separately, it is still unclear whether the UGT2B7*3 allele affects the pharmacokinetics of S-carvedilol with high β-blocking activity. In the present study, therefore, we examined the UGT2B7 genotype in 54 healthy Japanese subjects who participated in our previous study, and evaluated the effect of UGT2B7*3 on CL/F and V/F of R- and S-carvedilol.

Methods

Pharmacokinetic parameters of carvedilol in 54 Japanese subjects: The values of CL/F and V/F of carvedilol in individual 54 subjects were obtained in the previous study. The subjects were healthy Japanese volunteers consisting of 38 men and 16 women between 22 and 44 years old (mean: 26.5), weighing between 41 and 86 kg (mean: 60.9). We had already determined the polymorphic alleles of CYP2D6 in each subject. No subject had null alleles of CYP2D6 (CYP2D6*5 and *14). Twelve subjects were homozygous for the CYP2D6*1 allele, two were heterozygous for the CYP2D6*1/*2 alleles, and two were homozygous for CYP2D6*2 allele. Twenty subjects were heterozygous for CYP2D6*1/*10 alleles, and 6 were heterozygous for CYP2D6*2/*10 alleles. Twelve subjects were homozygous for the CYP2D6*10 allele. In addition, we had already determined the UGT2B7*2 allele in each subject: 22 were heterozygous for the UGT2B7*2 allele, and 6 were homozygous for the UGT2B7*2 allele. All subjects gave written consent to participate in the clinical trial, which was approved by the ethics committee of University of Toyama.

Genotyping of UGT2B7: UGT2B7*3 was determined by direct sequencing. First, the entire UGT2B7 gene was amplified from genomic DNA (100 ng) using 2.6 units of AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA) with 5 μM of the first amplification primers. Amplification primers included a sense primer (5′-TTGTCTTTGTTCCATCACA-3′) and an antisense primer (5′-CAGACATGGGATTTTGA-3′). PCR was performed in a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems) using an initial denaturation cycle of 94°C for 5 min, then 30 cycles of 94°C for 60 s, 54°C for 60 s, and 72°C for 60–120 s, followed by a final extension cycle of 72°C for 7 min. The products were directly sequenced with the primers (sense primer: 5′-CTCAGACTGTGATTTTAA-3′, and antisense primer: 5′-TTGTCTTTGTTCCATCACA-3′) using an ABI Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer’s recommended protocol. The eluates were analyzed on an ABI Prism 3100 DNA Analyzer (Applied Biosystems).

Data analysis: Regression analysis was performed using NONMEM software. Equations (1) and (2) were used to evaluate the effects of CYP2D6*10, UGT2B7*2, and UGT2B7*3 on CL/F (in L/h) and V/F (in L) of each enantiomer, respectively.

\[
CL/F = (\theta_1 - \theta_2) \cdot G2CYP2D6 - 2 \cdot \theta_2 \cdot G3CYP2D6 - \theta_3 \cdot G2UGT2B7*2 - 2 \cdot \theta_3 \cdot G3UGT2B7*2 - \theta_4 \cdot G2UGT2B7*3) \cdot WT
\]

\[
V/F = (\theta_1 - \theta_2) \cdot G2CYP2D6 - 2 \cdot \theta_2 \cdot G3CYP2D6 - \theta_3 \cdot G2UGT2B7*2 - 2 \cdot \theta_3 \cdot G3UGT2B7*2 - \theta_4 \cdot G2UGT2B7*3) \cdot WT
\]

where G2CYP2D6 = 0 and G3CYP2D6 = 0 for Group 1 subjects with CYP2D6*1/*1, *1/*2, and *2/*2; G2CYP2D6 = 1 and G3CYP2D6 = 0 for Group 2 subjects with CYP2D6*1/*10 and *2/*10; G2CYP2D6 = 0 and G3CYP2D6 = 1 for Group 3 subjects with CYP2D6*10/*10; G2UGT2B7*2 = 0 for Group 1’ subjects without the UGT2B7*2 allele; G2UGT2B7*2 = 1 for Group 2’ subjects with one UGT2B7*2 allele; G3UGT2B7*2 = 1 for Group 3’ subjects with UGT2B7*2/*2; G2UGT2B7*3 = 0 for Group 1’ subjects without the UGT2B7*3 allele; G2UGT2B7*3 = 1 for Group 2’ subjects with at least one UGT2B7*3 allele. In addition, WT is the body weight of each subject. The statistical significance of the regression model was evaluated with the likelihood ratio test using the minimum value of the objective function (−2 log likelihood) produced by NONMEM. In the present 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 significant (p<0.05).

Results and Discussion

We previously reported that CL/F and V/F values of R- and S-carvedilol were lower in subjects with the CYP2D6*10 allele than in those without the CYP2D6*10 allele, but that UGT2B7*2 did not affect the pharmacokinetics of carvedilol. In the present study, we reanalyzed the genotype of UGT2B7 in the 54 Japanese subjects who participated in our previous study. Fifty-four subjects were classified into two groups, based on the presence of the UGT2B7*3 allele. Forty-three subjects belonged to Group 1 without the UGT2B7*3 allele; that is, 16 subjects were homozygous for the UGT2B7*1 allele, 21 subjects were heterozygous for UGT2B7*1/*2 alleles, and 6 subjects were homozygous for the UGT2B7*2 allele. On the other hand, 11 subjects belonged to Group 2 with the UGT2B7*3 allele; that is, 10 subjects were heterozygous for UGT2B7*3
*served (Model 3).

4) Model 4 was used to evaluate the effect of the polymorphism of

UGT2B7*3 on (CL/F)/WT of R-carvedilol (B).

(C: Group 1" without UGT2B7*3, €: Group 2" with UGT2B7*3)

Horizontal bars represent the mean ± S.D. for each genotype group.

![Figure 1](image)

Fig. 1. Effect of CYP2D6*10 and UGT2B7*3 on (CL/F)/WT of R- (A) and S-carvedilol (B).

(U: Group 1" without UGT2B7*3, €: Group 2" with UGT2B7*3)

Horizontal bars represent the mean ± S.D. for each genotype group.

Table 1. Analysis Models for CL/F of Carvedilol

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameters fixed</th>
<th>( -2 \log \text{likelihood} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( \theta_1 = \theta_2 = \theta_3 = 0 )</td>
<td>503.07(^a) 560.54(^a)</td>
</tr>
<tr>
<td>2</td>
<td>( \theta_1 = \theta_2 = 0 )</td>
<td>475.72(^4) 549.74(^4)</td>
</tr>
<tr>
<td>3</td>
<td>( \theta_1 = \theta_2 = 0 )</td>
<td>501.09(^6) 557.31(^6)</td>
</tr>
<tr>
<td>4</td>
<td>( \theta_1 = \theta_3 = 0 )</td>
<td>503.04 560.47</td>
</tr>
<tr>
<td>5</td>
<td>( \theta_2 = 0 )</td>
<td>474.98(^a) 547.90(^a)</td>
</tr>
<tr>
<td>6</td>
<td>( \theta_3 = 0 )</td>
<td>472.35 548.71</td>
</tr>
<tr>
<td>7</td>
<td>( \theta_2 = 0 )</td>
<td>472.28 547.80</td>
</tr>
</tbody>
</table>

\(^a\)\(^p<0.01\), compared with Model 1.

UGT2B7*1/*3 alleles, and one subject was heterozygous for UGT2B7*2/*3 alleles.

Figure 1 shows the effect of CYP2D6 and UGT2B7 genotypes on the individual (CL/F)/WT values of R- and S-carvedilol. (CL/F)/WT values were decreased along with an increase in the number of CYP2D6*10. On the other hand, UGT2B7*3 did not systematically affect the (CL/F)/WT values of R- and S-carvedilol. We further examined the effect of the polymorphism of UGT2B7 on CL/F values in individual subjects using regression analysis (Table 1). Model 1 was the basic model for CL/F: \( \theta_3 \), \( \theta_1 \), and \( \theta_2 \) values were fixed to zero. The effect of CYP2D6*10 on CL/F of R- and S-carvedilol was statistically significant (Model 2), whereas no significant effect of UGT2B7*2 on CL/F was observed (Model 3). Model 4 was used to evaluate the effect of UGT2B7*3 on CL/F, and the LLD values between Model 1 and Model 4 were 0.03 and 0.07 for R- and S-carvedilol, respectively. Model 6 was used to investigate the effect of UGT2B7*1/*3 and UGT2B7*2 on CL/F, and the LLD values between Model 2 and Model 6 were 3.37 and 1.03 for R- and S-carvedilol, respectively. In addition, Model 7 was used to investigate the effect of CYP2D6*10, UGT2B7*2, and UGT2B7*3 on CL/F, and the LLD values between Model 5 and Model 7 were 2.7 and 0.1 for R- and S-carvedilol, respectively (Table 1). These results indicated that the effect of UGT2B7*3 on systemic clearance (CL) and/or bioavailability (F) of R- and S-carvedilol was not statistically significant.

Figure 2 shows the effect of CYP2D6 and UGT2B7 genotypes on the individual (V/F)/WT values of R- and S-carvedilol. (V/F)/WT values were decreased along with an increase in the number of CYP2D6*10; however, UGT2B7*3 did not systematically affect the (V/F)/WT values of R- and S-carvedilol. We further examined the effect of the polymorphism of UGT2B7 on V/F values in individual subjects using regression analysis (Table 2). Model 1 was the basic model for V/F: \( \theta_6 \), \( \theta_7 \), and \( \theta_8 \) values were fixed to zero. Model 4 was used to evaluate the effect of UGT2B7*3 on V/F, and the LLD values between Model 1 and Model 4 were 0.03 and 0.23 for R- and S-carvedilol, respectively. Model 6 was used to investigate the effect of CYP2D6*10 and UGT2B7*3 on V/F, and the LLD values between Model 2 and Model 6 were 0.91 and 0.05 for R- and S-carvedilol, respectively. In addition, Model 7 was used to investigate the effect of CYP2D6*10, UGT2B7*2, and UGT2B7*3 on V/F, and the LLD values between Model 5 and Model 7 were 0.15 and 0.24 for R- and S-carvedilol, respectively (Table 2). These results indicated that the effect of UGT2B7*3 on F of R- and S-carvedilol was not statistically significant.

On March in 2007, Takekuma et al. 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 heart failure or angina pectoris. A one compartment phar-
Fig. 2. Effect of CYP2D6*10 and UGT2B7*3 on (V/F)/WT of R- (A) and S-carvedilol (B).
(○: Group 1" without UGT2B7*3, ●: Group 2" with UGT2B7*3)
Horizontal bars represent the mean ± S.D. for each genotype group.

Table 2. Analysis Models for V/F of Carvedilol

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameters fixed</th>
<th>−2 log likelihood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>θ₁ = θ₃ = θ₅ = 0</td>
<td>597.87⁴ 689.23⁴</td>
</tr>
<tr>
<td>2</td>
<td>θ₁ = θ₃ = 0</td>
<td>578.72⁴ 680.58⁴</td>
</tr>
<tr>
<td>3</td>
<td>θ₁ = θ₃ = 0</td>
<td>594.16⁴ 687.88⁴</td>
</tr>
<tr>
<td>4</td>
<td>θ₁ = θ₃ = 0</td>
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<tr>
<td>5</td>
<td>θ₃ = 0</td>
<td>576.94⁴ 680.44⁴</td>
</tr>
<tr>
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<td>θ₃ = 0</td>
<td>577.81 680.53</td>
</tr>
<tr>
<td>7</td>
<td>—</td>
<td>576.79 680.20</td>
</tr>
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</table>

*p < 0.01, compared with Model 1.

References


